Gokhale Memorial Girls' College

Date: 17/04/2023

To whom it may concern

Subject: Completion of Dissertation by CEMA students of Semester VI in 2021-22

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DISSERTATION: REVIEW REPORT

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THIS IS TO CERTIFY THAT THIS DISSERTATION REVIEW ENTITLED COPPER BASED CATECHOL OXIDASE: BIOMIMETIC FUNCTIONAL MODEL AND MECHANISTIC PATHWAY SUBMITTED BY NAJNIN MANDAL (CU REG. NO: 013-1215-0233-19), DEPARTMENT OF CHEMISTRY OF GOKHALE MEMORIAL GIRL'S COLLEGE IS RECORD OF AN ORIGINAL AND INDEPENDENT STUDY CARRIED OUT BY HER UNDER THE SUPERVISION AND GUIDANCE OF **DR. ANANGAMOHAN PANJA** , GOKHALE MEMORIAL GIRL'S COLLEGE , AND THAT NEITHER THIS REVIEW NOR ANY PART OF IT HAS BEEN SUBMITTED FOR EITHER ANY DEGREE / DIPLOMA OR ANY ACADEMIC AWARD ANYWHERE BEFORE.

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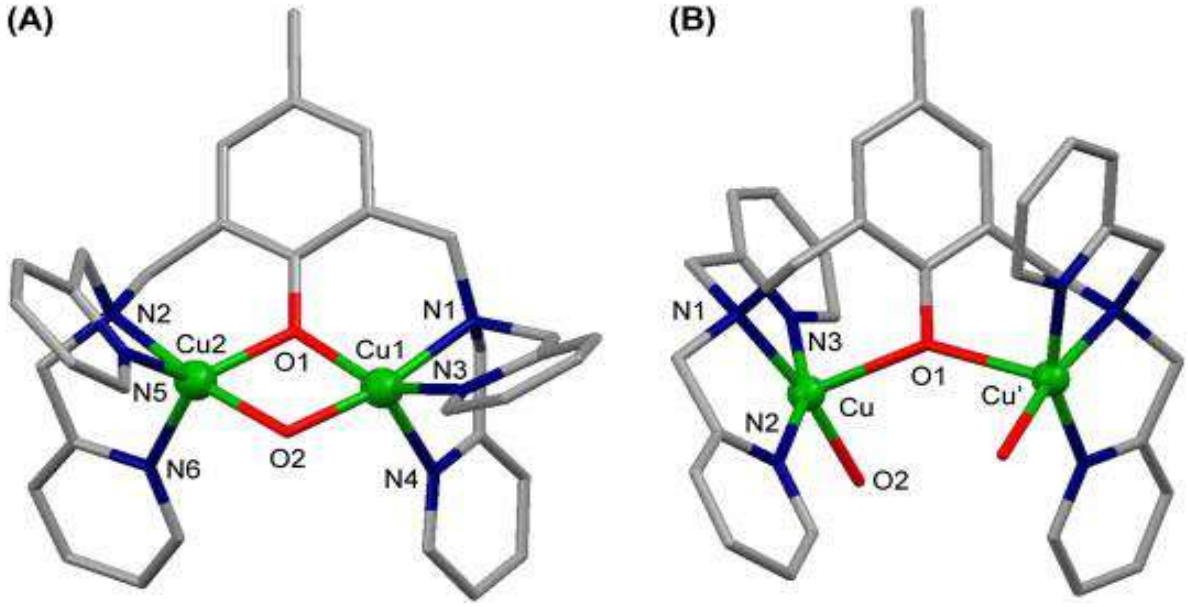
REVIEW ON

COPPER BASED CATECHOL OXIDASE : BIOMIMETIC FUNCTIONAL AND MECHANISTIC STUDIES



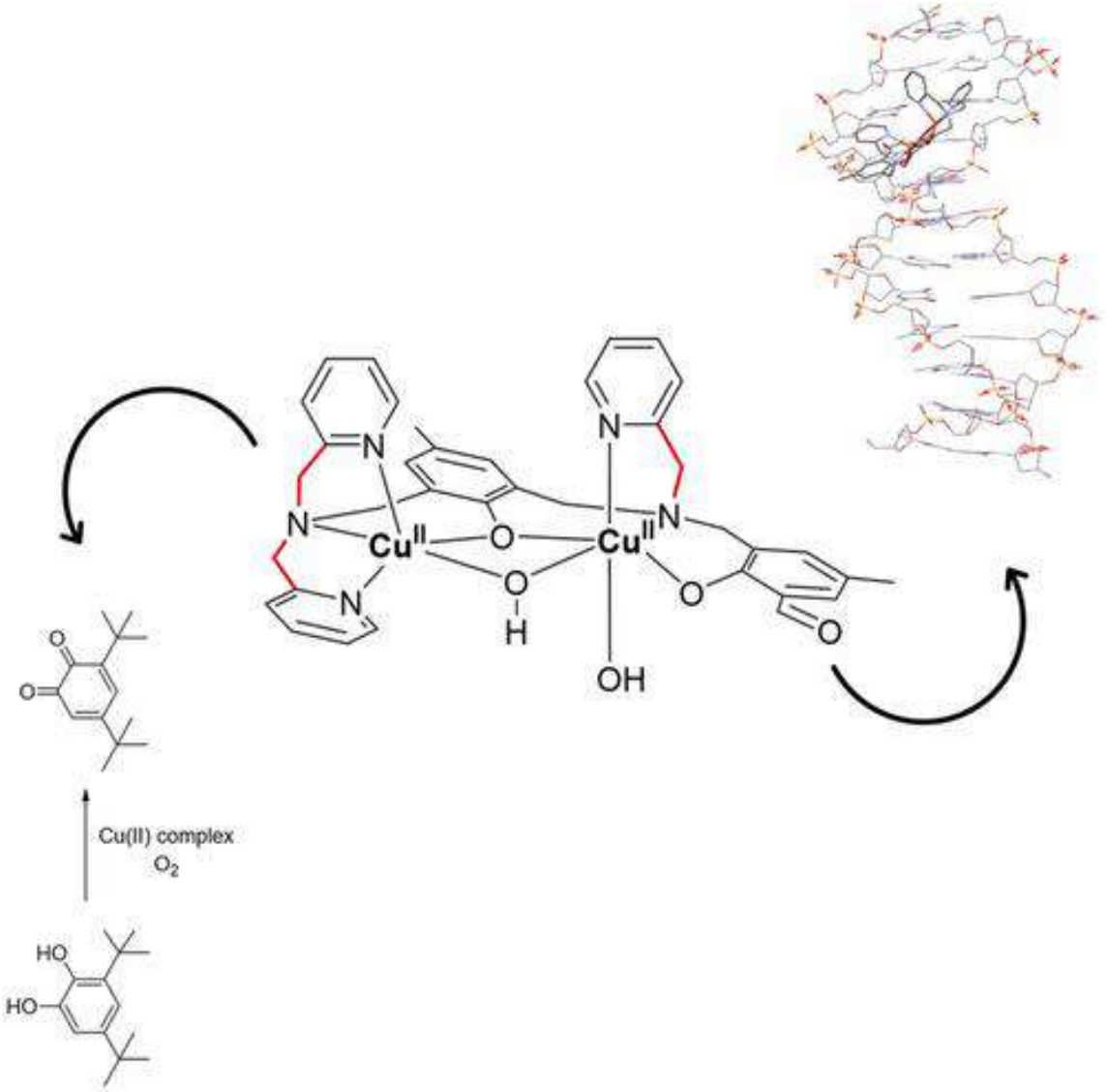
INDEX

ABSTRACT :

This present review deals with the impetus to modelling of enzyme active sites comes from their potential to provide insight to the mechanistic pathways of the native enzymes, establish the role of that particular metal in the active site and to design better catalysts inspired by nature. Most of the metalloenzymes are capable of activating molecular oxygen due to the presence of the metal ions. In this review we discuss the progress made so far in the area of catechol oxidase modelling on copper complexes. The review shows how CO enzyme have been widely attempted for modelling the active site.

As the native enzyme contains copper centres at the active site, several copper-based model complexes were found to be excellent functional models for CO enzyme. It is inserting to observed that other first row transition metal complexes were also quite efficient to mimic the function of the metalloenzymes. In this review we discuss the progress made so far in the area of catechol oxidase modeling. Emphasis will be given mostly on copper-based metal complexes those showed such activity. Assembly of those results in a single review helps the scientists to understand the influence of nuclearity, coordination environment around the metal centers as well as oxidation state of the cobalt complexes on the catalytic efficiency and that in turn helps us to develop better catalysts.

Three copper binuclear complexes that differ by the number of chelate member rings of the ligand were synthesized and characterized. The effect of these modifications on the enzyme mimicking (catecholase and DNAse) was evaluated, revealing that changes in the soft side contributed positively to the activity.



2. INTRODUCTION :

Copper Oxidase is a catechol oxidase present in a variety of species of plants and fungi including *Ipomoea batatas* (sweet potato) and *Camellia sinensis* (Indian tea leaf). In plants, catechol oxidase plays a key role in enzymatic browning by catalyzing the oxidation of catechol to o-quinone in the presence of oxygen (Scheme 1), which can rapidly polymerize to form the melanin that grants damaged fruits their dark brown coloration. When plant tissue is damaged, the chloroplast may rupture and release catechol oxidase into the plant cytoplasm, and vacuoles may also rupture, releasing

stored catechol into the cytoplasm. The tissue damage also allows oxygen to penetrate into the cell. Thus, tissue damage facilitates the interaction of catechol oxidase with its substrate to produce o-benzoquinone, which can polymerize non-enzymatically to yield melanin that form an insoluble barrier for wound protection.

2.1 ACTIVATION OF MOLECULAR OXYGEN :

Molecular oxygen is an ideal oxidant because of its availability directly from air. Due to its kinetically inert nature, the activation of molecular oxygen for its use in oxidation reactions is challenging. It is generally activated through heavy metals, but stoichiometric quantities of inorganic oxidants are toxic and enrich the environment with pollution. That is why oxidations using catalytic amount of activator which can activate molecular oxygen with minimum chemical waste is inspiring.

The reactivity of dioxygen is not easily controlled and often lead to low selectivity and over – oxidation. This kinetic barrier is overcome by using transition metal incorporated in proteins, known as 'metalloenzyme'. In metalloenzymes, the metal ion is bound to the protein with one labile coordination site. The metal ion is usually located in a pocket whose shape fits the substrate. The metal ion catalyses reactions that are difficult to achieve in organic chemistry.

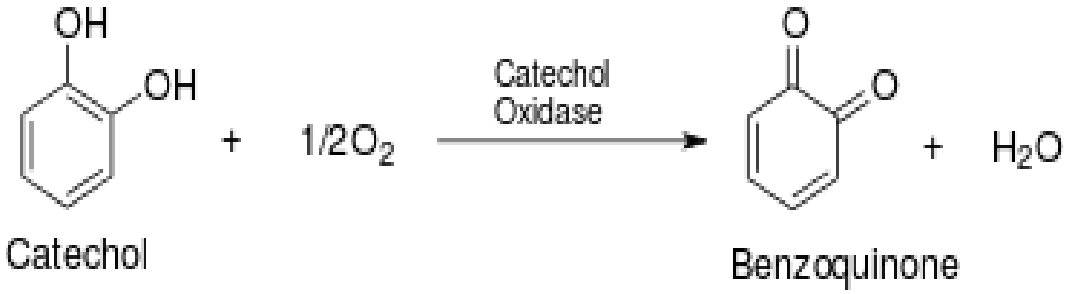
Inorganic chemists have designed several oxygen activation catalysts which act as small molecule mimics of metalloenzymes and helps to understand the mechanistic pathways. Considering the redox potential, electronic factors and coordination chemistry, the enzyme donor sites are modelled with small molecule called ligands, which then binds with metal to form complexes that

are probed as structural and functional models. The catalytic activity of the complexes will vary on metal to metal. Many complexes may not be considered efficient due to their inability to perform the relevant oxidative transformation. But such complexes are very useful in providing us with insight about the important mechanistic aspect of metalloenzymes.

2.2 Nature's Choice and Role of Biomimetics

Nature uses several metalloenzymes to catalyse the controlled and selective oxidation of organic compounds. The geometry and structural feature of enzyme active sites and the choice of incorporated metals are very diverse and fully optimized to the function of the proteins or enzymes. In addition, it also takes into account the availability of the metal ion in environment. Establishing the correlation of the geometric and electronic structure with function is one of the main objectives of the bioinorganic chemists. The activation of dioxygen on metal sites requires the availability of different accessible redox states. Metalloenzymes capable of dioxygen activation consist mainly of enzymes with copper, iron or manganese active sites. A wide variety of different mono- or multinuclear iron and copper enzymes has been discovered and catalyzes major biological transformations. The primary goal in designing mimics of metalloenzymes is to understand the structures of active sites and reactive intermediates and the mechanistic details of dioxygen activation and oxygenation reactions occurring at the active sites. Metalloenzymes use diverse active sites such as heme iron sites, mono-and dinuclear nonheme iron sites, mono-and dinuclear copper sites, a heteronuclear heme iron–copper site, and other metal sites to activate dioxygen. Progress made so far in terms of understanding the mechanistic pathway of oxidative enzymes has been very

encouraging [24]. The huge library of complexes available to us as model complexes of various enzymes shows that in spite of sincere efforts, we have not obtained turnovers close to native enzymes but the dissemination of mechanistic pathways and introduction to alternative pathways has been possible. This review is on CU-based model complexes of catechol oxidase (CO). The works in this area that have appeared in the literature over the past few decades shows the impetus to understand the chemistry of these metalloenzymes. I have tried to take into account all the reports that deal with mechanistic pathway of CO. The catechol oxidase enzyme has a type-3 active site and catalyses the oxidation of dioxolenes (catechols) to the corresponding o-quinones (Scheme 1). However, there are other metal based model complexes of catechol oxidase. Assembly of those results especially CU-based model complexes in this review article may help the readers about the progress made so far with this enzyme and further help them to develop this field.



Scheme 1 . Function of catechol

3. CATECHOL OXIDASE :

3.1 STRUCTURE AND FUNCTION :

It is a dinuclear Cu(II) containing enzyme with a type-3 active site, is responsible for catechol oxidation in higher plant which oxidises catechol to o-quinone. The crystal structure of the met form of the enzyme was determined in 1998. This revealed that the active site consists of a hydroxo bridged dicopper(II) centre in which each copper(II) centre is coordinated by three histidine nitrogen atoms and adopts an almost trigonal pyramidal environment with one nitrogen at the apical site (Fig. 1). The crystal structure of catechol oxidase from sweet potatoes (Ipomoea batatas) was reported by Krebs and co-workers in three different catalytic states: the native met (Cu^{II}/Cu^{II}) state, the reduced deoxy (Cu^{I}/Cu^{I}) form, and in complexation with the inhibitor phenylthiourea. The Cu· · ·Cu distances are 2.9 Å and the hydroxide oxygen bridges two Cu^{II} ions with a distance 1.8 Å from each of them. In the reduced or deoxy state both Cu^{II} converts to Cu^{I} and the Cu^{I}· · ·Cu^{I} distance increases to 4.4 Å. The coordination number of Cu_A and Cu_B become 4 and 3, respectively with the geometry changing to trigonal pyramidal and square planar. Since the enzyme is a dicopper enzyme it is not surprising that the highest

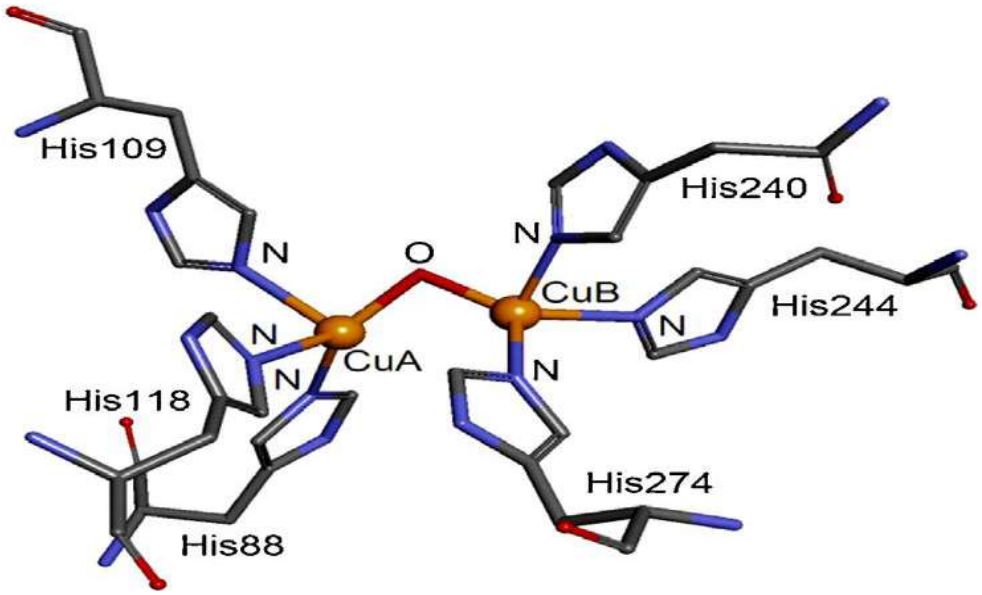


Fig.1. Coordination sphere of the dinuclear copper(II) centre of catechol oxidase from sweet potato in the met state (PDB ID: 1BT3).

Model complexes contain Cu^{II} ion. Several dicopper(II)complexes with similar ligand environment have been designed to mimic the enzyme and probe its mechanism. Here we briefly discussed about Cu-based model system. However, many copper complexes oxidise DTBC through an alternate pathway which involves production of H_2O_2 along with o-quinone and involvement of peroxo species. One of the major drawbacks of the model complexes are that the activity (k_{cat}) of the mimics are quite low when compared with the enzymes (table 1). Hence the structure-property correlation still provides scope for newer designs to build structural and functional model systems with better activity close to the enzyme for potential in industry. An advantage of the model systems compared to the native enzyme is the ability of the model complexes to function in organic solvents unlike the natural enzymes.

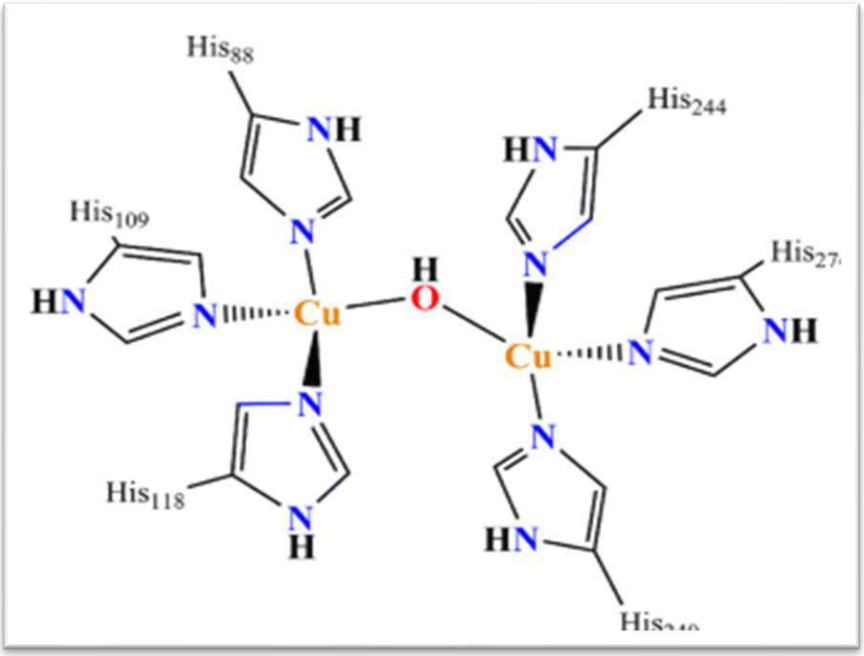


Table 1: Kinetic parameters of catechol oxidation by metalloenzymes.

Enzyme	Substrate	k_{cat} (h−1)	K_M (M)	k_{cat}/K_M (mM^{-1} s^{-1})
Catechol oxidase [a]	Catechol	5.7×10^5	0.005	31.67
Catechol oxidase [b]	Catechol	8.25×10^6	0.0025	916.67
GriF [c]	Catechol	4.0×10^4	0.0025	4.44
Mushroom tyrosinase	Catechol	3.15×10^6	0.00016	5463.13

[a] Catechol oxidase from Lycopus europaeus; [b] Catechol oxidase from Ipomoea batatas (sweet potatoes); [c] A tyrosynase homolog.

3.2 MODEL SYSTEM OF CATECHOL OXIDASE :

The structure of the enzyme shows dicopper(II) moiety at the active site (Fig.1.2) hence, several dicopper(II) complexes with similar ligand environment have been designed to mimic the enzyme and probe its mechanism. But besides them lots of other transition metal mimics also have been reported over the years out of which the catalytic activity of many are comparable with the copper based mimics. Catecholase activity of metal complexes are controlled by many different factors. A general structure–activity correlation (Scheme 2) is difficult to establish. Many research groups have attempted to correlate the catecholase activity with metal-metal separation, electrochemical properties of the complexes, influence of ligand structure or exogenous bridging ligand which is the best that could be done so far. The variation in the activity with subtle changes in the electronic factors suggests that the synergism of the ligand and the metal renders a strong effect on the affinity for substrate and in addition the change in metal also changes oxygen affinity.

Nature has designed catechol oxidase to ligate Cu^{II} ion in the required geometry and with optimum redox potential to oxidise catechol through switching

between Cu^{II} and Cu^{I} redox states with necessary stability. Hence, to probe catecholase activity with a different metal ion, the coordination environment may need a change, for better reactivity. The geometry rendered by the ligand used and the redox potential of the resultant complex also is important and so should be the presence of accessible labile site(s) on the complex for possible substrate/oxygen binding. Thus, in order to probe the activity of $Co^{II/III}$ or $Mn^{II/III}$ or any other metal instead of Cu^{II}, there may not be a necessity to mimic the similar coordination environment with nitrogen donors for achieving enzymatic activity but rather it may be disadvantageous because of the inherent electronic differences of the two metals. In fact, relevant literature in this area shows that replacement of the native metal of an enzyme by a non-native one may degrade performance drastically. Different types of ligand system were also designed to make dinuclear as well as mononuclear complexes of transition metal such as Mn, Co, Ni, Zn, Fe along with Cu to modulate the activity and get a better structure property correlation.

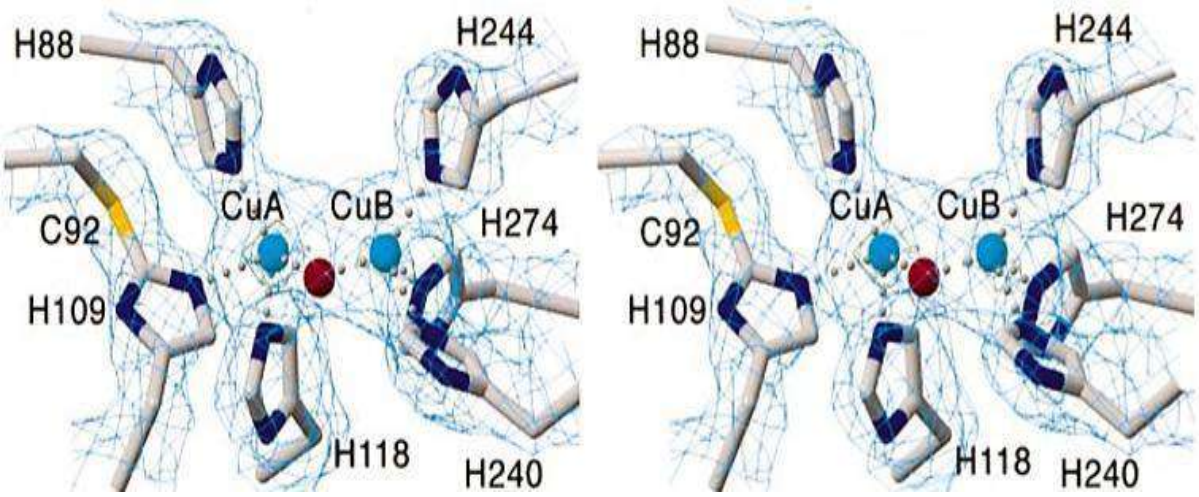
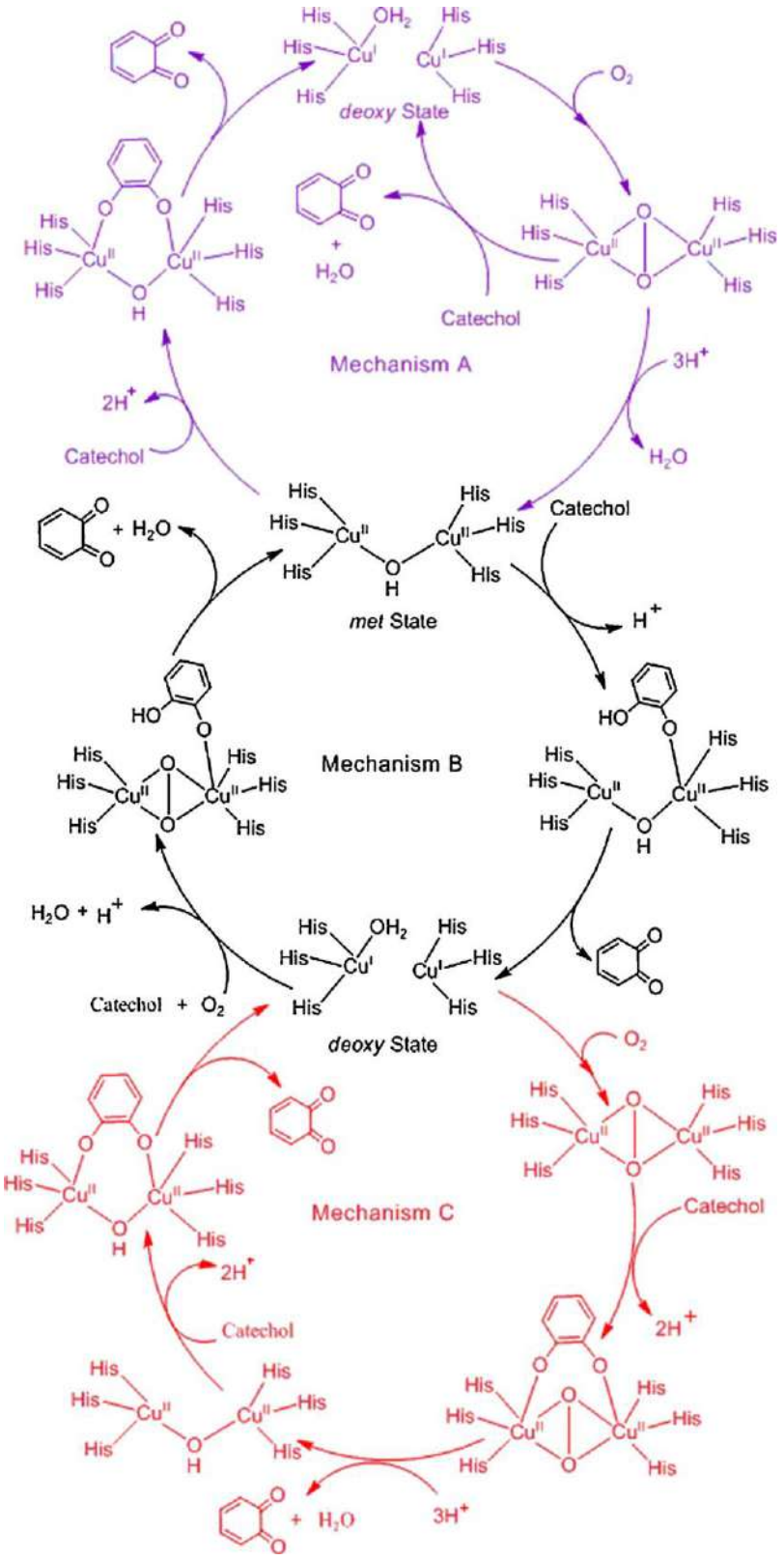


Fig.1.2. Structure of active site of Catechol Oxidase


deoxy State
Catechol
Mechanism A
met State
Mechanism B
deoxy State
Mechanism C

Scheme 2: Mechanistic pathways of Catechol Oxidase enzyme

4. COPPER BASED MODEL :

Copper (II) being the native metal for the enzyme catechol oxidase (CO) a large number of copper (II) model complexes have been studied as models for CO in attempts to disseminate the mechanism and generate efficient mimics. Different di-nuclear and mononuclear copper complexes have been synthesized and used as catalyst for the oxidation of the widely used substrate DTBC.

When one tries to correlate the parameters of catalysis, it shows that the best models involve ligands which have phenoxide bridges and aromatic imines (L1–L30) with the imine nitrogen (Figs. 2 and 3) being one of the donors. In some cases, along with a phenoxo, a hydroxo also bridges two copper (II) ions. There is another type of complexes showing high activity which have bis-phenoxo bridges . Although this is unlike the CO enzyme active site which has a single hydroxo bridge but the ligand design shows better results with bis-phenoxo since there is increase in efficiency in spite of the rigidity of the ligand binding the two copper (II) ions. One reason may be that after reduction to Cu(I) during the catalytic cycle the metal centers might still remain in the same place because of bonding with either of the phenoxo bridges and the aliphatic sp^2 hybridized N-donors (Fig. 3) which would prohibit the dissociation of CuI centers which are neither too soft nor hard.

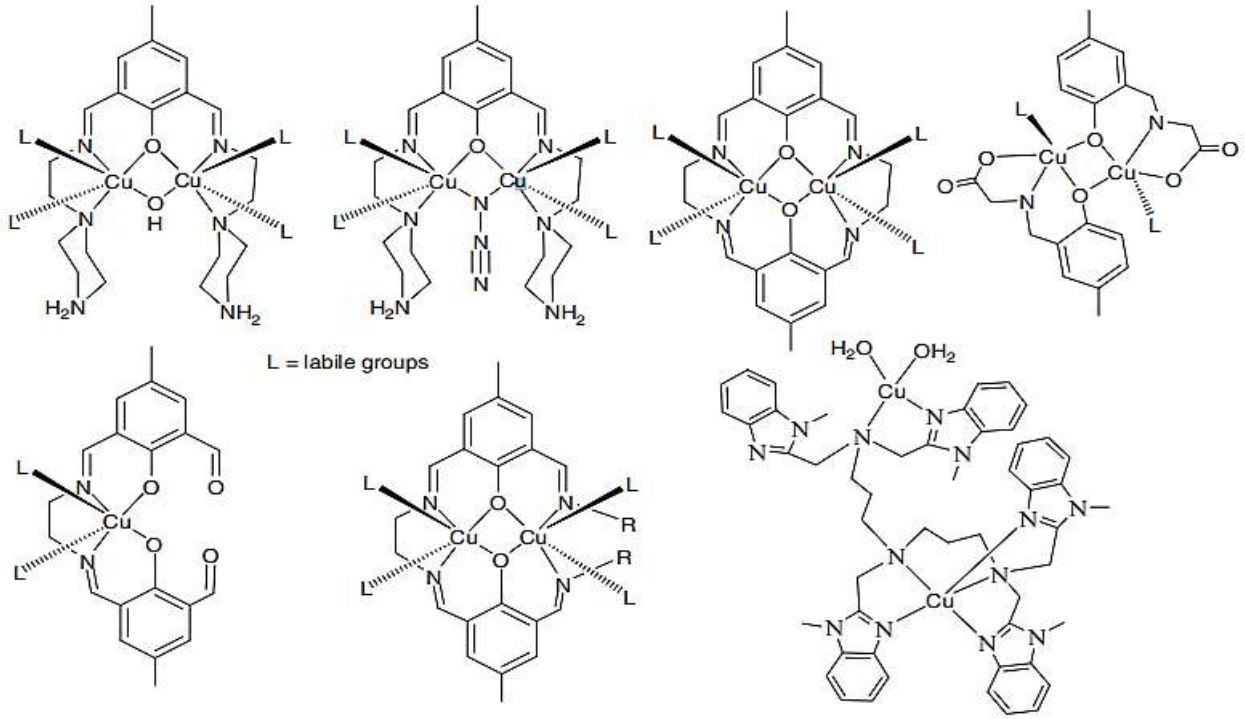
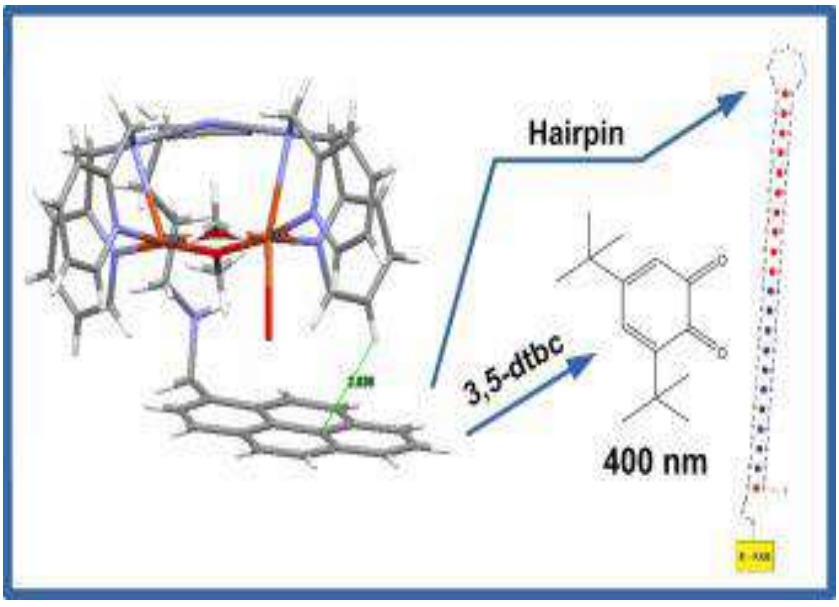


Fig 2. Schematic drawing of metal environment of the most active copper (II)

The catechol oxidase active site Cu centers are neither tetrahedral nor square planar, this is to minimize the geometry change during the CuII ↔ CuI transformation which makes the active site stable and efficient. If we look at the best active CuII model complexes we find that the phenoxo bridged CuII complexes are less flexible and mostly square planar and they are the ones showing the best catalytic activity (kcat = 1.08 − 3.24 × 104 h−1). Hence as mentioned earlier the higher activity for these complexes might be due to the intrinsic nature of the ligand which would help holding the two CuII centers together even after reduction (Fig. 2). The ligand leads to the di-nuclear center being held in close proximity for formation of the phenoxo bridge during oxidation. This is further supported when we closely look at the

complexes designed using L1–L30 and find that the ligand design involves macrocyclic type N-donor ligands, where the aliphatic chains holding the two nitrogen atoms has been varied in length from 2 to 4 carbon atoms but that does not drastically affect the activity. Even instead of the imine nitrogen donor if there is an aldehyde O-donor the catalytic turnover number is more or less similar. Hence the capability of the single ligand to hold the two CuII centres and the stability of the phenoxide anion and its donor ability to the Cu centres are important in rendering structures that stabilize both the oxidized and the reduced state providing better catalytic activity. In addition, this may also have a strong role in rendering adequate redox potential to the metal center. The presence of a relatively rigid ligand frame work in (L1–L30) and the phenoxo oxygen for bonding enables the Cu· · ·Cu distance to be ca. ∼2.9A˚ which is appropriate for the catalysis . Hence, these complexes also seem to have promising activity (kcat = 0.50 − 0.95 × 104 h−1). The presence of the phenoxo bridge with aromatic imine donor-based ligand are so far the best copper (II) based mimics in exhibiting catechol oxidase activity with catalytic turnover of the order of 104 h−1 [14 – 17].



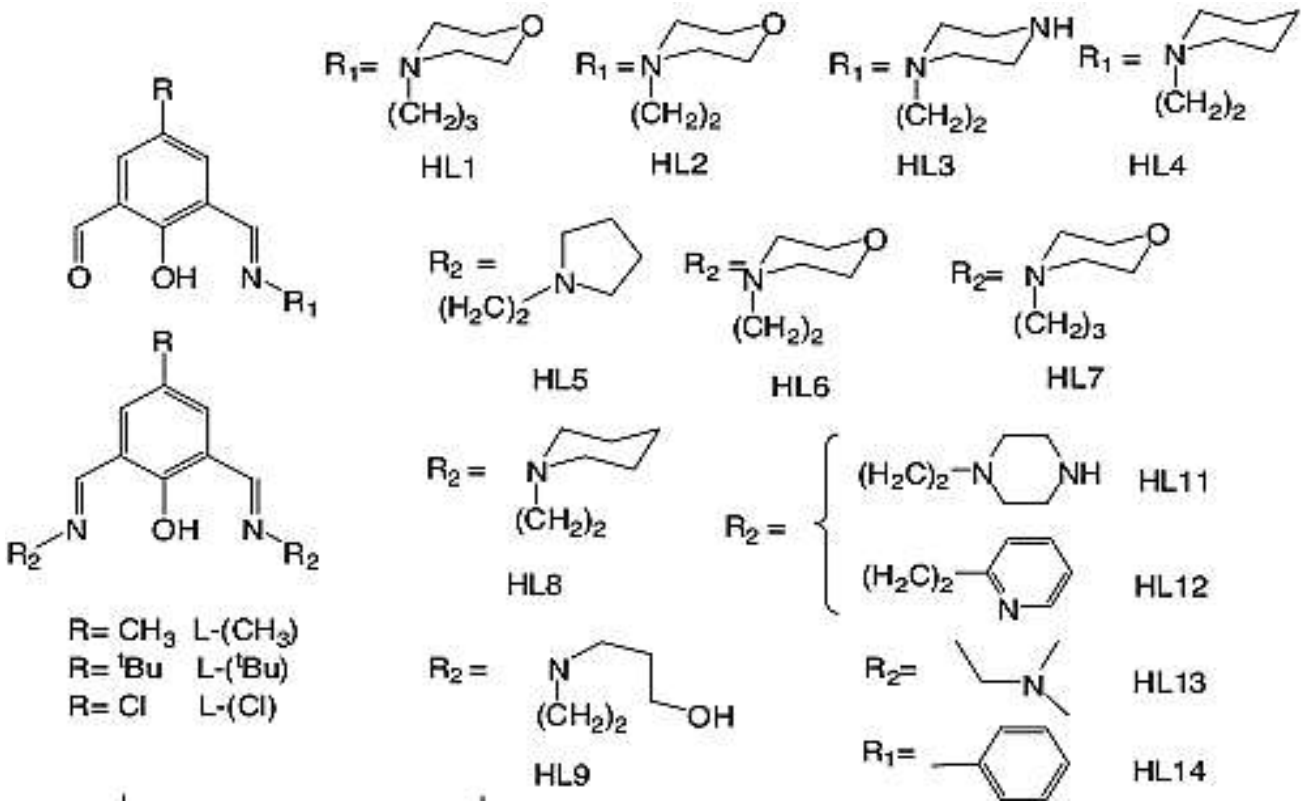


Fig 3. Different NO donor ligand systems.

If we closely look at the structure of those active complexes apart from the presence of a phenoxo bridge there are also labile groups which create unsaturated metal environment suitable for substrate coordination. Di-nuclear Cu complexes reported by Das et al. with ligand L1–L14 always have the phenoxo bridge structural motif with additional phenoxo or hydoxo or azido bridge at the other side. The geometry around the metal's centres is distorted octahedral or square pyramidal or square planar with available coordination sites for substrate or oxygen binding and these complexes are mostly very efficient. They have extended their study with similar Robson type macrocyclic ligands with variable alkyl groups between the imine nitrogens to synthesize di-phenoxo bridge complexes having labile groups

with each metal centres and all of them show significant catecholase activity Similar phenoxo bridged di-nuclear copper (II) complexes were also synthesized by Vittal and co-workers using reduced Schiff bases of salicylaldehyde and amino acids (L100–L118). Nitrogen based ligands were also designed and used to prepare di-nuclear complexes by different research groups especially by Reedijk and co-workers and by Casella et al. , it should be noted here that different functional groups at the proximity of bimetallic active site can modulate the mechanistic pathway. Meyer et al. have found that certain copper (II) complexes of similar ligands (HL37 and HL38, where only the side arm varies) show different mechanistic pathways for oxidation of DTBC which includes formation of hydrogen in one case and water for the other. The mechanistic pathways of the model copper (II/I) complexes have been investigated in details in many cases. Different approaches have been used by different research groups to investigate the mechanistic pathway of DTBC oxidation by the mode copper (II/I) complexes. Since the oxidation mostly follows first order kinetics with respect to the substrate it is always very important to know the binding modes of the substrate with the catalyst.

5. SOME MODEL SYSTEM REVIEW :

In 2016, Kelly A.D.F.Castro et al. reported the synthesis strategy, characterization, and catalytic activity for new copper-based porphyrins (**CuP2** and **CuP3**) isolated by metallation of the corresponding free base derivatives, **H_2P2** and **H_2P3**. The solid **CuP2S** was obtained from the reaction of **H_2P_2** with copper(II) acetate (10 equiv.). These complexes have been used in homogeneous (**CuP2** and **CuP3**) and

heterogeneous (**CuP2S**) oxidative catalysis of 3,5-di-*tert*-butylcatechol and catechol, in the presence of air, with or without hydrogen peroxide. The obtained results show that the new copper porphyrins **CuP2** and **CuP3** and the **CuP2S** material are able to efficiently mimic the activity of catechol oxidase, the latter being easily reused and maintaining its activity for more than three catalytic cycles[18,19].

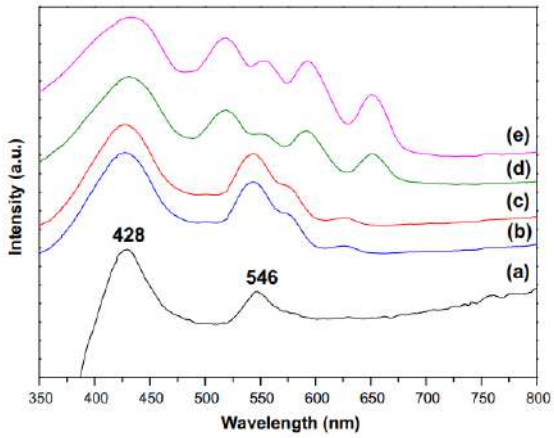


Fig: 4

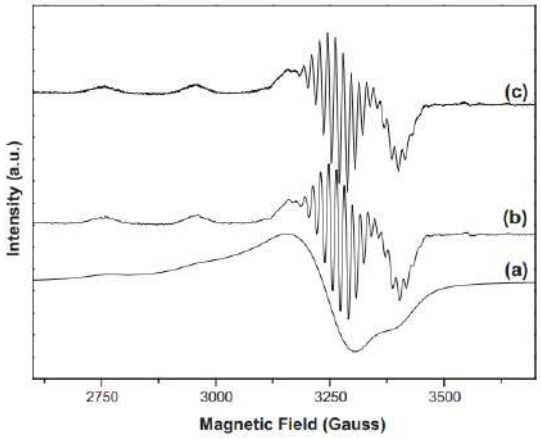


Fig: 5

Fig. 4: UV–Vis spectra of the solid sample: (a) CuP2S, (b) CuP2, (c) CuP3, (d) H2P2, and (e) H2P3.

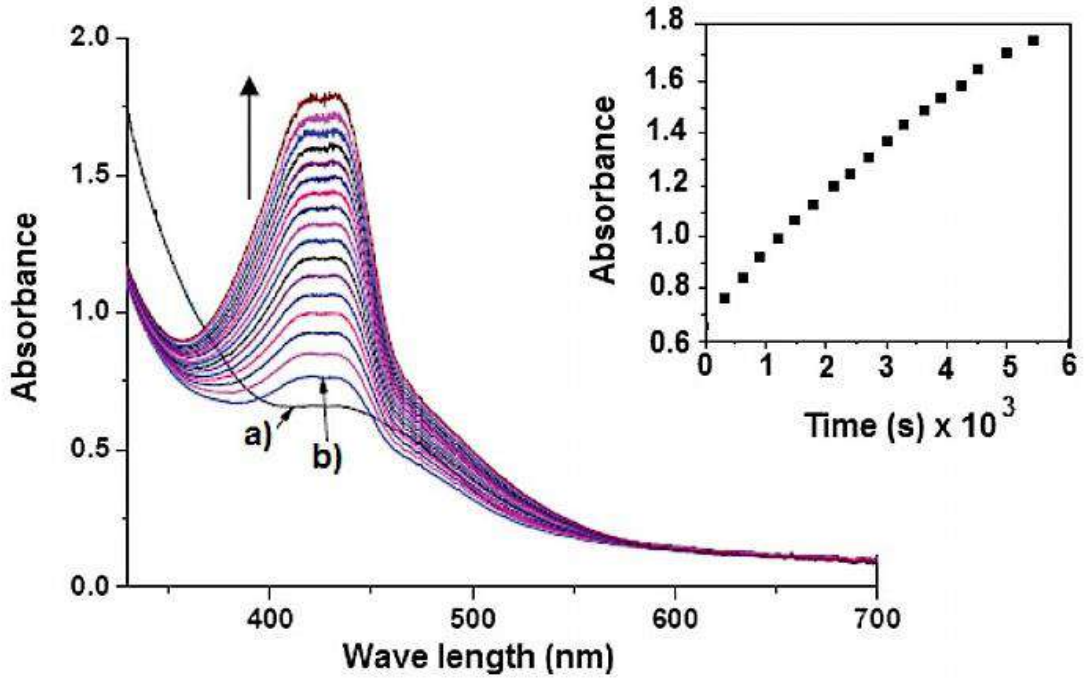
Fig. 5. EPR spectra: (a) solid sample CuP2S at 77 K, (b) CuP2S dissolved in hydrochloric acid solution, dried, and redissolved in acetone (frozen solution EPR spectrum at 77 K), and (c) CuP2 in acetone solution at 77 K.

In 2017, a new series of copper (II) complexes have been synthesized with macrocyclic ligands L^1 and L^2 having N_3S_2-donating atoms in the 12-membered macrocyclic ring. The structure characterization of these newly synthesized copper (II) complexes was achieved by various physicochemical techniques. It has been shown that the stereochemistry of complexes is dependent on the type of counter anions incorporated in the complex molecule. Mimicking copper oxidase enzymes, namely catechol oxidase, was investigated and the results obtained demonstrated that there is a correlation between the structural properties of these copper (II) complexes and the oxidase biomimetic catalytic

activities. Kinetic measurements revealed second-order dependence on the catalyst concentration for 3,5-DTBCH_2 and first order in the case of OAPH. On the other hand, for the substrate concentration dependence, a saturation-type behavior was detected for both 3,5-DTBCH_2 and OAPH. Addition of Lewis base, triethylamine, in both systems exhibits dramatic effect on the rate of these catalytic aerobic oxidation reactions. The probable mechanistic implications of both catalytic systems are discussed [20-22].

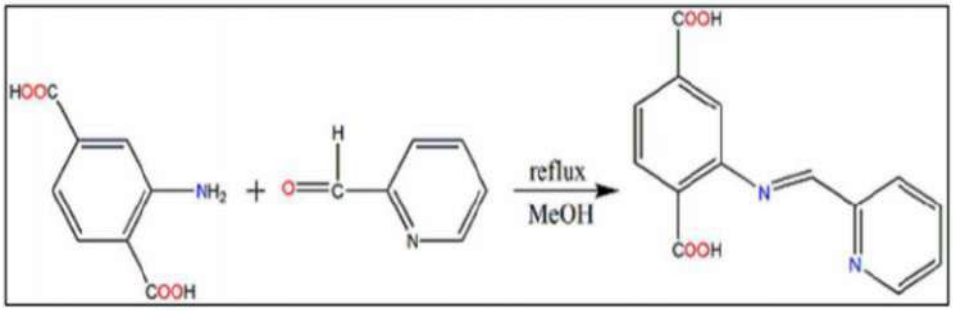
From variations of the reaction rates, saturation kinetics was found for the reaction rate constant kobs versus the OAPH concentrations as shown in the representative Figure 6. This behavior indicates that an intermediate complex–substrate adduct forms in a preequilibrium process and that the irreversible substrate oxidation is the rate-determining step of the catalytic cycle similar to that above reported for the catechol oxidation. However, all the copper (II) complexes showed a saturation kinetic at higher concentration.

Fig 6. UV/Vis spectral changes recorded for the reaction of complex 3 with o-aminophenol (0.5 £ 10¡1 M) in methanol at 296 K; (a) spectrum before the reaction; (b) spectrum obtained several milliseconds after mixing of the reactants in the stopped-flow; inset is the kinetic trace at 433 nm. The solvent used was CH3OH.



In 2018, Sandeepta Saha et al. synthesized two new copper (II) polymeric complexes, {[Cu(H*Pymat*)(H_2O)](NO_3)}$_n$ (**1**) and [Cu_2(*Pymat*)$_2$(H_2O)$_3$]$_n$ (**2**), using the Schiff base ligand H_2*Pymat* [H_2*Pymat* = (*E*)-2-(1-(pyridin-2-yl)-methyleneamino)terephthalic acid]. Complex **1** is a cationic 1D polymer, whereas complex **2** is a two-dimensional polymer. Both complexes were crystallographically, spectroscopically and magnetically characterized. Theoretical studies were performed and the catecholase activity of the complexes was also examined. Complex **1** is a ferromagnetically coupled complex with J = 2.8 cm^{-1} and **2** shows antiferromagnetic coupling with J = −1.6 cm^{-1}. Both complexes show notable features in the EPR study[23,24]. They show rhombic spectra at 77 K in the solid state, but by varying the temperature or solvents the nature of the spectra can be changed or inverted. This behaviour indicates a change of the ground state from $d_{x^2-y^2}$ to d_{z^2} orbitals. Theoretical calculations of **1** focus on the evaluation and characterization of interesting anion–π–anion assemblies that are formed in the solid state. In **2** we have

analysed the unconventional chelate ring···chelate ring π-stacking interactions that govern its solid-state architecture. Both complexes act as functional models and show catechol oxidase activity with a k_{cat} value of the order of 10^3 h^{-1}.



Scheme 2: Representation for synthesis of ligand H_2Pymat.

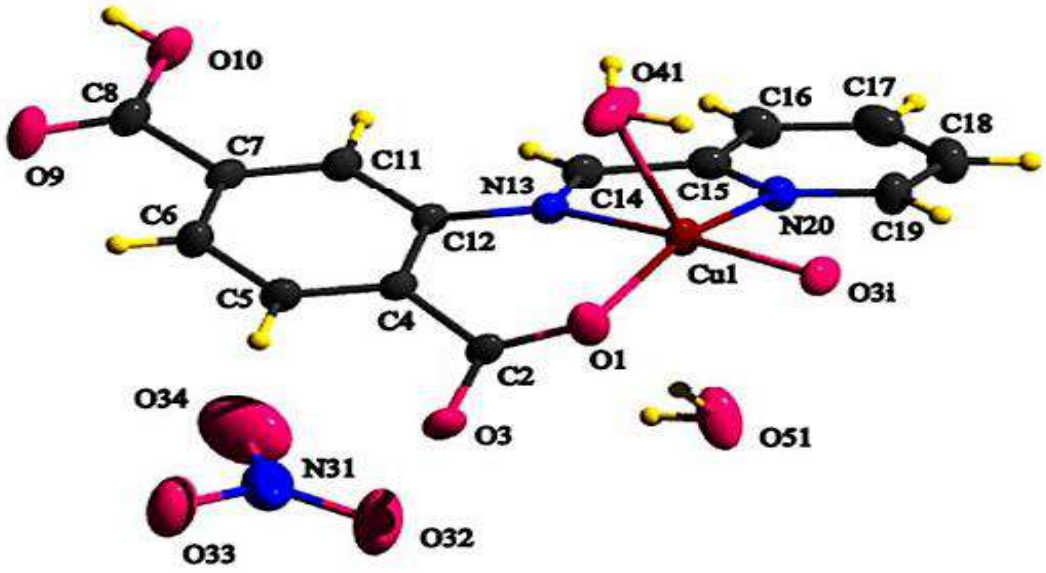


Fig. 7 View of asymmetric unit of1with atom numbering scheme.

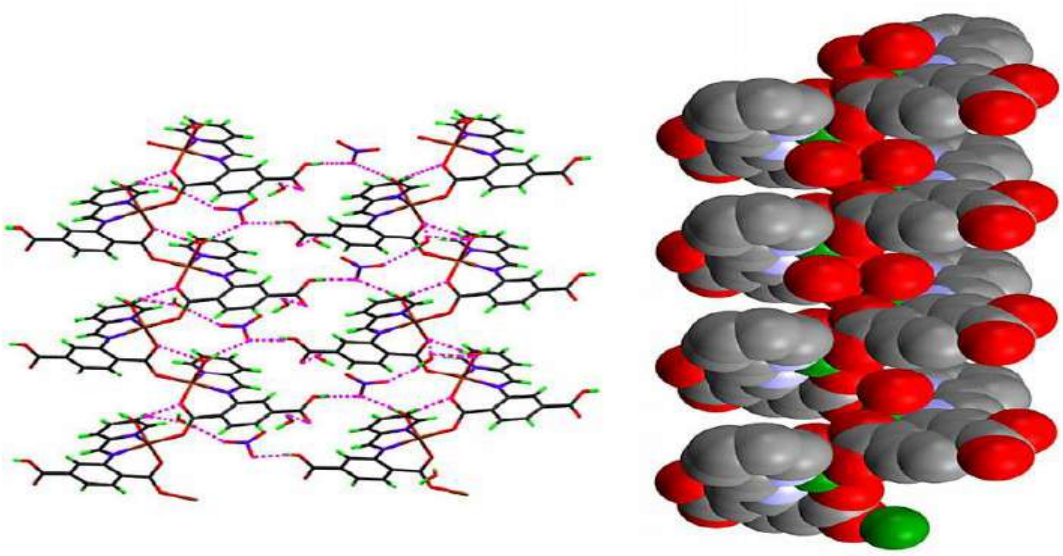


Fig. 8: a) H-bonds (shown by dotted lines) between adjacent 1D helical chains along with intramolecular H-bonds in the complex 1. b) The helical chain of complex 1 (Cu centre is shown in green colour).

In 2019, T. Ben Hadda et al. investigated the *in situ* copper(I/II) complexes of pyrazole based on ligand: 5,5'-diphenyl-1H,1'H-3,3'-bipyrazole (**H_2L**) and Its corresponding [$C_{36}H_{28}CuN_8$] (**CuL**). Copper(I/II) complexes were tested for their reactivity towards the oxidation of catechol to o-quinine with the atmospheric dioxygen by following the appearance of quinone spectrophotometrically at ambient conditions in order to demonstrate the structural parameters essential to the reactivity of the enzyme and to understand the mechanism of action. The reaction rate depends on three parameters: the nature of counter anion, the nature of solvent and the concentration of ligand. We compared the potentialities of the complexes formed *in situ* as well as the synthesized complex [25], as a catalyst of the oxidation reaction of catechol to o-quinone.The highest rate activity given by the complex resulting from two equivalents of ligand **H_2L** and one equivalent of [$Cu(NO_3)_2$] in MeOH is to 3.10 µmol.L^{-1}.min^{-1}.

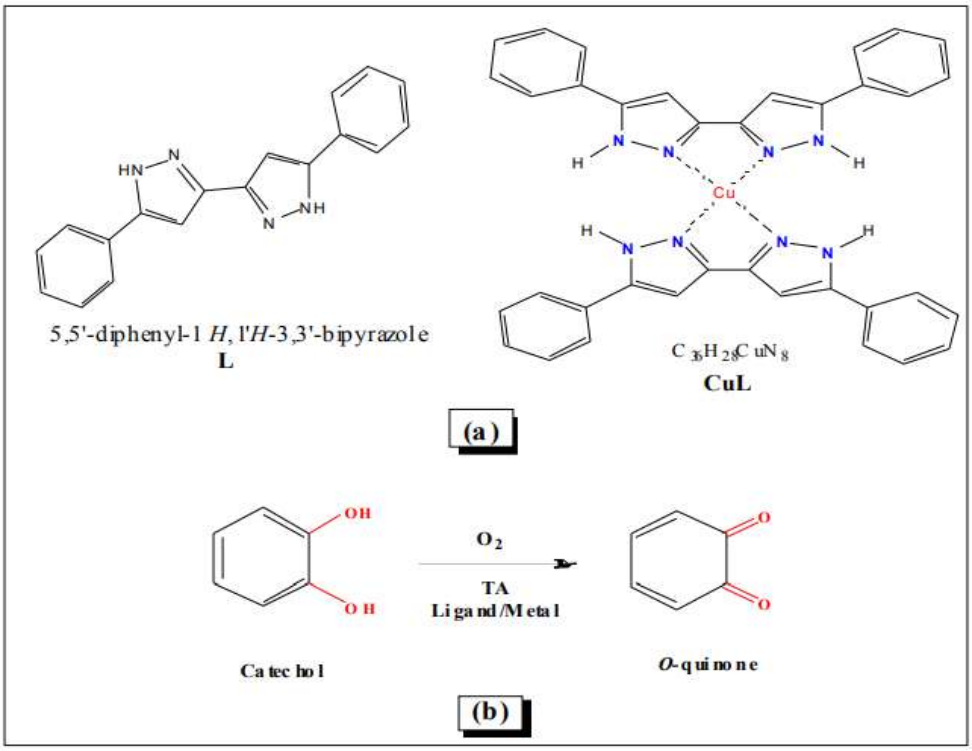


Fig 9: : (a) Tested compounds, (b) Reaction model (catechol oxidation).

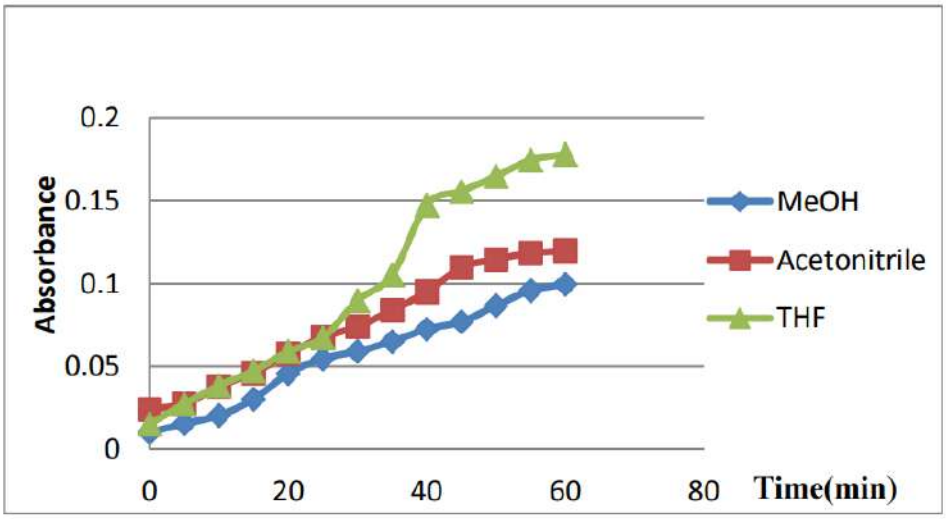


Fig 10: Catechol oxidation in the presence of synthetic copper complex in different solvents.

In 2020, Two ternary copper(II) complexes **1** [$(Cu_2Me_4en)_4(EDTA)$] and **2** [$(CuMe_4en)_2(MIDA)$] containing the mixed ligand system of 1,1′,4,4′-tetramethylethylenediamine (Me_4en) (L) and *N*-methyliminodiacetic ($MIDAH_2$) (L′) or ethylenediaminetetraacetic acid ($EDTAH_4$) (L′) were synthesized. Complete structural elucidation was achieved by many spectroscopic, electrochemical, and magnetic measurements. In addition, the spectral data of PXRD with Expo 2014's structural solution software were utilized for the structural illustration of the homobinuclear complex **1**. Furthermore, the structural formulation of complex **2** was affirmed by the structural analysis of single-crystal X-ray. Square pyramidal geometry was suggested for both the homobinuclear and mononuclear complexes **1** and **2**. The oxidase catalytic activities of complexes **1** and **2** were tested towards a series of catechols and *o*-aminophenol and the complexes were found to be promising functional mimics of catechol oxidase and phenoxazinone synthase. The tendency of the studied phenols to oxidize and transform to oxidation products is correlated with their binding affinity to the current complexes and the structure of the substrates. The driving force (λ) or the free energy change, $-\Delta G°$, of the studied oxidation processes was computed from the electrochemical results [26]. The plausible catalytic reaction pathways were suggested in light of the spectral, electrochemical, and stopped-follow kinetic measurements.

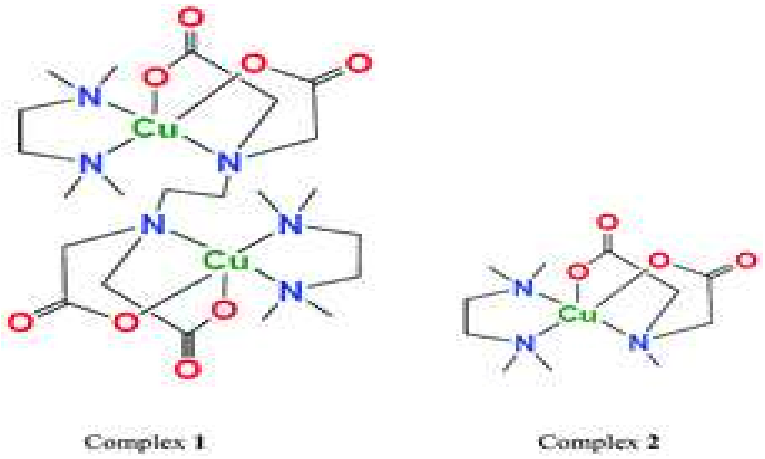
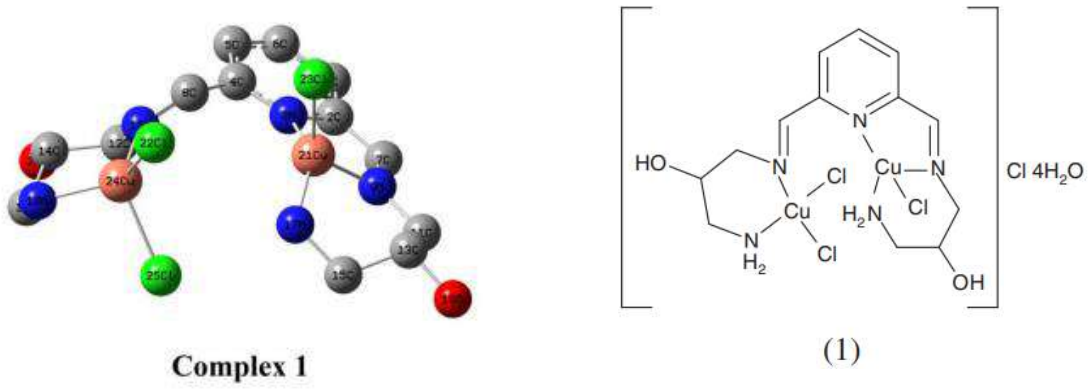


Fig 11. Complexes discussed in the article.

In 2019, Mohammed M. Ibrahim *et al.* articulated a template (26) Schiff condensation of 2,6-pyridine dicarbaldehyde or 2,6-diformyl-4- bromophenol and 1,3–diamino-2-hydroxy propane or 3,4-diaminotoluene in the presence of copper(II) salts (CuX_2) (X = Cl, Br, CH_3COO, or ClO_4) affords different types of copper(II) complexes. Depending on the employed molar ratio of the dicarbonyl compounds and diamines, different types of copper(II) complexes formed during the template condensation reaction. Structural formulation of the complexes was confirmed by elemental analysis (C, H, N, and M), physical measurements such as thermal analysis (TAG & DTG), molar conductivity, and magnetic moments in addition to spectral studies (UV–Vis, IR, and ESR). Homobinuclear in a four-coordinate square planar and five-coordinate square pyramidal and trigonal bipyramidal in monomeric structures are proposed. A mononuclear hexa-coordinate in an octahedral geometry is suggested as well. Oxidase biomimetic catalytic activity of these newly synthesized copper(II) complexes was examined toward the aerobic oxidation of 4-*tert*-butylcatechol (4-$TBCH_2$) and *o*-aminophenol under catalytic conditions. Both catalytic and kinetic investigations demonstrate promising oxidase catalytic activity and based on the kinetic results, probable mechanistic catalytic implications are discussed. Geometrical structures of representative copper(II) complexes were determined by optimizing their bond lengths, bond angles, dihedral angles, and the structural index (τ).



Complex 1

Fig 12. The hydrogen atoms are omitted for clarification.

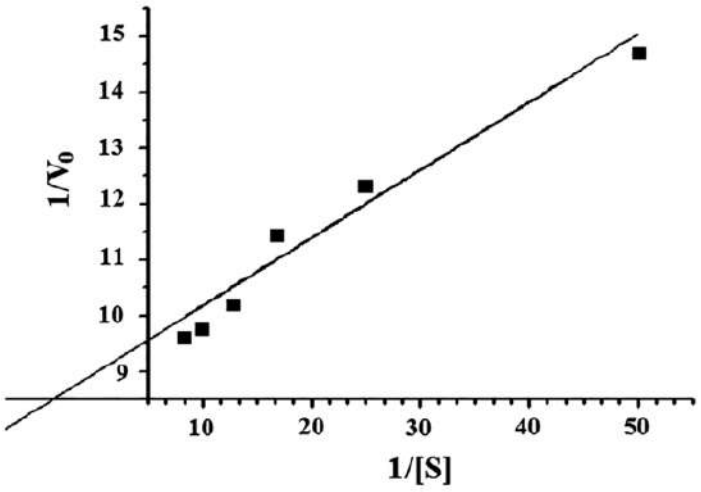


Fig 13 Lineweaver–Burk plot of 1/V0 versus 1/[S] concentrations in the range 0.02–0.12 M for determining KM and Vmax values for complex 1

6.CONCLUSION :

I have presented the biomimetic model complexes of copper based CO , discussed about their activity, collated the known mechanistic pathways involved during the catalysis and disseminated them based on the reports. It appears that the model complexes act by binding to the substrate and then oxidizing it while the catalyst gets reduced; this step is followed by oxidation of the catalyst by molecular oxygen. The first step in the substrate oxidation, leads to formation of radicals and the next step completes the product formation while the catalyst goes back to its oxidised state. Except for a few cases, most model systems show that the oxidation of catechol involves reactive oxygen species and H2O2 is formed as a by-product. In contrast, the enzymatic pathway produces water instead of hydrogen peroxide, involving a four electron reduction of oxygen. Hence, most model complexes are capable of only performing a two electron reduction of dioxygen while oxidizing catechol to o-quinone. The enzyme clearly stands out here since it is not only a very efficient

catalyst for the oxidation of catechol but also the by-products water instead of hydrogen peroxide. To argue in favour of hydrogen peroxide production by the model complexes, it may be said that a major source of industrial production of hydrogen peroxide involves

palladium catalyst using anthraquinone. However, if the efficiency of generation of hydrogen peroxide can be made better by tuning the CO model complexes and the o-quinone be converted back to the o-di-oxolene for reuse in an industrially efficient way, then production of hydrogen peroxide might be done at room temperature unlike the palladium process and using a cheaper metal source. The knowledge of the oxidation mechanism of catechol learned through studies on the enzyme and design of biomimetic model complexes led to the

hypotheses that di-nuclear Cu II complexes must have a higher reaction rate then their mononuclear analogues (Table 2). The literature data strongly suggests that metal ions would exhibit redox properties with same ligands due to differences in their electronic properties. Hence the change in metal ion requires tuning of ligand if the redox potential of the metal centre is to be kept in range for a certain catalytic process. This might be a possible explanation as to why the replacement of the metals in the same protein may not give efficient catalytic activity. Many metal complexes have been designed to activate molecular oxygen with the objectives to develop bioinspired catalysts for oxidation reactions but in most of the cases the turn over numbers is low compared to enzyme.

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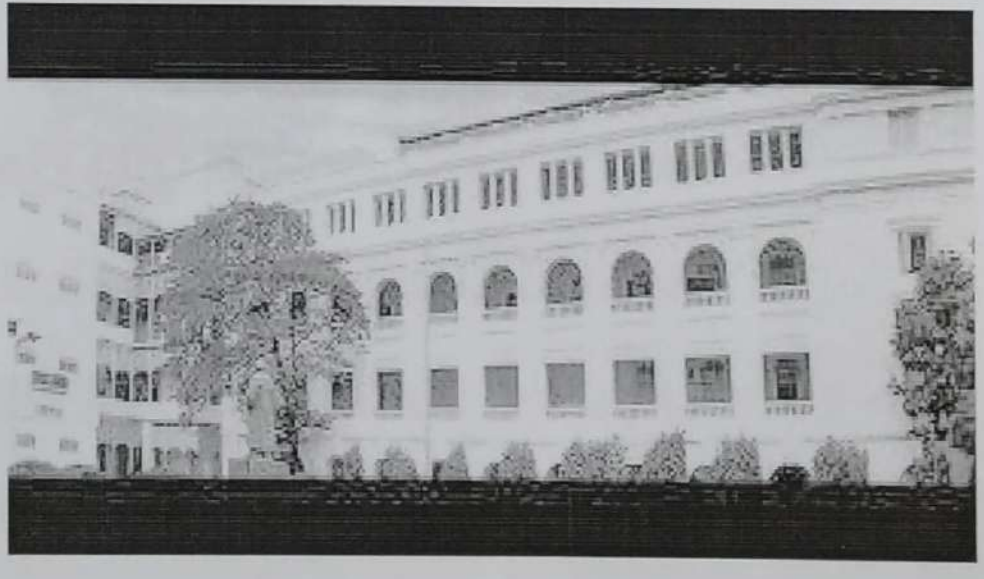

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REVIEW ARTICLES

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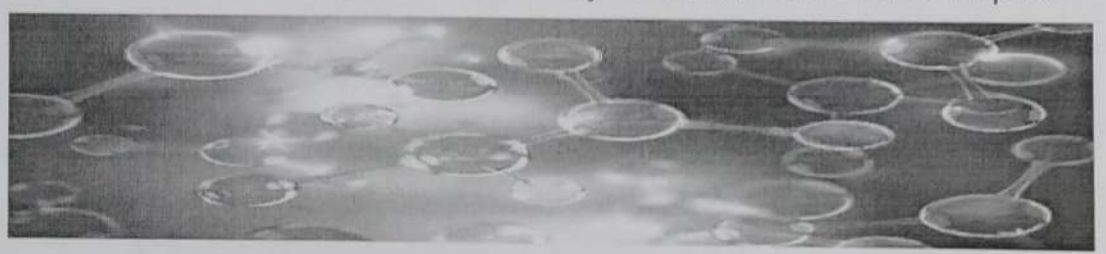
DEPARTMENT OF CHEMISTRY

TOPIC

Phenoxazinone sythase activity of a Mn based complex



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CERTIFICATE

This is to certify that this dissertation project entitled `Mn based model complexes on phenoxazinone sythase`submitted by Ms.Sridipa Mandal (Reg no.013-1214-0232-19),Department of Chemistry,Gokhale Memorial Girls' College is record of an original and independent study carried out by her under supervision and guidance of Dr.Anangamohan Panja,Department of chemistry,Gokhale Memorial Girls' College.Neither this review nor any part of it has been submitted for either any degree/diploma or any academic award anywhere before.

All help received by her from various sources have been duly acknowledged.

28-02-22

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CONTENT

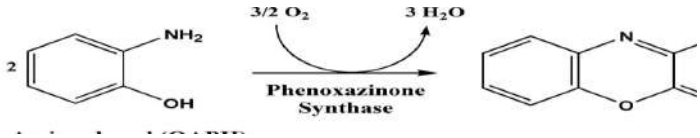
ABSTRACT:

This review describes recent progress in modeling the active sites Phenoxazinone Synthase that activate dioxygen to carry out several key reaction in nature.This review(upto 2021)is the continuation of the review done (upto2015) by S.K.Dey and A.Mukherjee (Coord.chem.Rev.310(2016),80-115.The impetus to modeling of enzyme active sites comes from their potential to provide insight to the mechanistic pathways of the native enzymes,establish the role of that particular metal in the active site and to design better catalysts inspired by nature.Most of the metallo enzymes are capable of activating molecular oxygen due to the presence of the metal ions.The name phenoxazinone synthase (PHS,2-amiophenol:Oxygen oxidoreductase) is used for the enzyme catalysing the oxidative coupling of substituted o-aminophenols to produce phenoxazines.

In this review we discuss the progress made so far in the area of phenoxazinone synthase modeling.The review shows that PHS has been widely attempted for modeling the active site.The biommimetic studies strongly suggest that among the various metal ions probed for modeling the catalytic activity of PHS .MnII/III based systems are so far the most promising candidates apart from the nature's choice of Cu(II)ion.PHS like activity suggests the potential of such mimics may extend beyond the biological modeling and provide insight to the various possible mechanistic pathways that may be adapted by a model complex.

INTRODUCTION:

Phenoxazinone synthase:This topic is taken from bioinorganic chemistry.It is a metalloenzyme containg copper.It acts as catalyst.Eg.it catalyzes the coupling of 2-aminophenols to form the 2-aminophenoxazinone chromophore during the synthesis of actinomycin D.



1.ACTIVATION OF MOLECULAR OXYGEN

Oxidation reaction is a fundamentally important component of organic synthesis and plays an important role in rendering the desired functionality to the intermediates of valuable compounds such as pharmaceuticals, agrochemicals, and other fine chemicals. Molecular oxygen is an ideal oxidant because of its availability directly from air rendering it inexpensive and environmentally benign. The challenges faced to activate molecular oxygen for its use in oxidation reactions is due to its kinetically inert nature. A major problem while using dioxygen in chemical transformation is that its reactivity is not easily controlled and often may lead to low selectivity and over-oxidation . Nature has evolved an elegant solution to overcome the kinetic barrier of dioxygen activation by using transition metal incorporated in proteins, the so called 'metalloenzyme'. Inorganic chemists have largely exploited the concept of nature by designing oxygen activation catalysts which act as small molecule mimics of the metalloenzymes and help to understand the mechanistic pathways. Using the knowledge of co-ordination chemistry, redox potential and electronic factors, the enzymes donor sites are modeled with small molecule called ligands, which are then incorporated with metals to form complexes that are probed as structural and functional models[1].

2.NATURE'S CHOICE AND ROLE OF BIOMIMETICS

Nature uses several metalloenzymes to catalyze the controlled and selective oxidation of organic compounds (Fig. 1). The geometry and structural feature of enzyme active sites and the choice of incorporated metals are very diverse and fully optimized to the function of the proteins or enzymes.In addition,it also takes into account the availability of the metal ion in environment. The activation of dioxygen on metal sites requires the availability of different accessible redox states. Metalloenzymes capable of dioxygen activation consist mainly of enzymes with copper,iron or manganese active sites.The primary goal in designingmimics of metalloenzymes is to understand the structures of active sites and reactive intermediates and the mechanistic details of dioxygen activation and oxygenation reactions occurring at the active sites. Progress made so far in terms of understanding the mechanistic pathway of oxidative enzymes has been very encouraging. In the progress made so far, disseminationof mechanistic pathways and introduction to alternative pathways has been possible. This reviewis based on model complexes of phenoxazinone synthase(PHS).The works in this area that have appeared in the literature over the past few decades shows the impetus to understand the chemistry of these metalloenzymes. We have tried to take into account all the reports that deal with mechanistic pathwayof PHS and also many others.However,it is not possible always to provide an exhaustive account of all related previous works.To keep the focus,we have rather discussed the mechanistic pathways of the model systems with literature examples and whenever available tabulated them with most important kinetic parameters. Phenoxazinone synthase is a multicopper oxidase that catalyzes the last step in the biosynthesis of the antibiotic actionmycin D (Fig. 2) by Streptomycesantibioticus and the catalytic reaction is the oxidative condensation of two molecules of 3-hydroxy-4-methylanthranilic acid pentapeptide lactone to form the phenoxazinone chromophore.There are more than 250 structural/functional mimics known for this.The scope of this review include esthe collection of manganese-based model complexes that displayed phenoxazinone synthase activity.

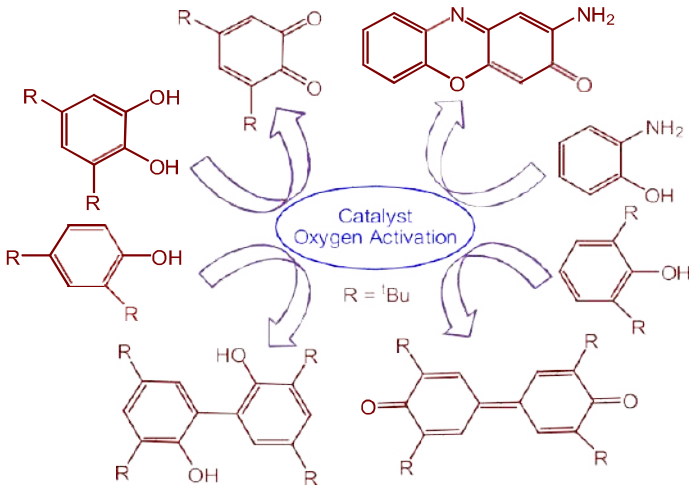


Fig:1 A representative chart of the oxidative transformations that commonly carried out by various research group[1]

3.PHENOXAZINONE SYNTHASE (PHS):ACTIVE SITE STRUCTURE AND FUNCTION

Phenoxazinone synthase (PHS) is a pentanuclear copper containing enzyme that catalyzes the formation of the phenoxazinone chromophore during synthesis of Actinomycin D. It is an aromatic heterocyclic natural product in which the 2-aminophenoxazinone chromophore is linked to two cyclic pentapeptides. These classes of compounds are potent antineoplastic agents. Their clinical use, however, are limited to the treatment of choriocarcinoma,Wilms tumors, rhabdomyosarcoma, and Kaposi's sarcoma due to their high toxicity. It has been shown that actinomycin binds to DNA by intercalation of the phenoxazinone chromophore and that the cyclic pentapeptide lactone confers sequence specificity to adjacent GC base pairs. This interaction results in highly specific inhibition of DNA-dependent RNA synthesis. In addition to the actinomycins, both xanthommatin and cinnabarin contain the phenoxazinone chromophore. During biosynthesis of actinomycin, 3-hydroxyanthranilic acid is formed from tryptophan in a multistep process. A pentapeptide lactone is then attached to COOH unit of 3-hydroxyanthranilic acid forming the substituted 2-aminophenol unit. The resulting 2-aminophenols then undergo oxidation followed by coupling to form the phenoxazinone chromophore thus completing the synthesis of Actinomycin. This last step, the oxidative coupling of 2- aminophenol to phenoxazinone is carried out by PHS. It should be noted that 2-aminophenol-1,6-dioxygenase(ADP) is a ring-cleaving enzyme which have the ability to cleave the C-C bond of 2-aminophenol in presence of molecular oxygen to yield picolinic acid and it is an alternative reaction that may happen in presence of molecular oxygen.

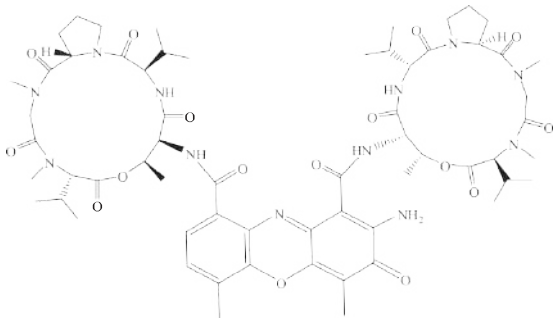


Fig: 2 Structure of actinomycine D[1]

In 1962, Katz and Weissback [2] first isolated the enzyme, phenoxazinone synthase from Streptomyces antibiotus. Later it was cloned and overproduced in Streptomyces liuidans and isolated in 100 mg quantities. The subunit molecular weight is 88000 daltons. The enzyme PHS, had also been isolated from S. Antibioticus. However, structure was unknown until in 2006, James P. Allen and Wilson A. Francisco reported the crystal structure of PHS from Streptomyces antibioticus[3]. The structure was solved at a resolution limit of 2.3A˚ which reveals thatit existin two oligomeric forms with distinct catalytic activities:low activity dimers and high activity hexamers. The structure of PHS can be divided into three domains. Out of which two domains mostly form barrel structure and the third domain contains two short helical segments and multiple strands folding into a common cupredoxin-like topology. The structural data showed that each subunit of the hexamer contains five copper atoms and it confirms the presence of all three-type copper-binding motifs, as usually known for multicopper oxidases: one type 1 (blue), two type 2 (normal), and one binuclear type 3 centres. The fifth copper centre which is a type 2 copper, is located at a distance of 25A˚ from the blue copper and the other normal type 2 copper, and the requirement of five copper atoms for maxim activity

suggest that the fifth copper atom is not merely advantageously bound but has a structural role as well. The hexameric form has been reported as the most active form of PHS . The high activity of the hexameric form is likely due to a number of factors viz. stabilization of hexameric form of the protein relative to the dimer, accessibility of the active site, geometry of the copper centres, and the availability of proper solvent channel. The above factors play important role in the regulation of PHS activity. These copper atoms form one mononuclear type 1, one mononuclear type 2 and one binuclear type 3 copper centre, which is similar to that observed for other multicopper oxidases. The fifth metal atom is in the loop connecting domains 2 and 3 and represents a new cofactor not previously described in any other multicopper oxidases. On the basis of the coordination of the fifth copper, it was expected to be a type 2 centre. The type 1 copper centre, identified as Cu1, has four ligands. The geometry around the Cu1 centre is distorted bipyramidal with a missing axial ligand which is common to type 1 copper centre structure of actinomycin-D. Among the residues shown(Fig3 and 4), only the axial methionine ligand is not conserved among the multicopper oxidases. Although this ligand is methionine in most multicopper oxidases, in some laccases, this residue can also be leucine or phenylalanine. The Cu-ligand bond distances in PHS are similar to that observed in other multicopper oxidases. The type 1 copper centre is 12.5A˚ away from Cu2 of the type 3 centre and is connected to the type 3 centre through a central bridging ligand that facilitates the transfer of electrons from the type 1 to the type 3 centres(Figs 3 and 4) . Before the structure was solved, spectroscopic evidences suggested that there is no type-3 copper centre in PHS. Although conserved histidine residues were there in the primary sequence. However when the structure was solved the electron density provided a clear evidence for two copper atoms with a bridging ligand. The bridging ligand however though modeled as OH, other ligands such as water or various anions would also be compatible with the electron density. Hence the type of bridging ligand is not unambiguous. The 3.88A˚ distance between the two Type-3 copper atoms, which are identified as Cu2 and Cu3, and the Cu2-X-Cu3 bond angle of 153.14◦ are comparable with other multicopper oxidases. The copper atom of the type 2 centre, identified as Cu4, is 3.63 and 3.86A˚ away from the Type-3 Cu2 and Cu3 copper atoms respectively. The two histidines (His161 and His527) coordinated to the Type-2 Cu is contributed by domain 1 and 3, respectively. The third ligand to the Cu4 is a water molecule [1].

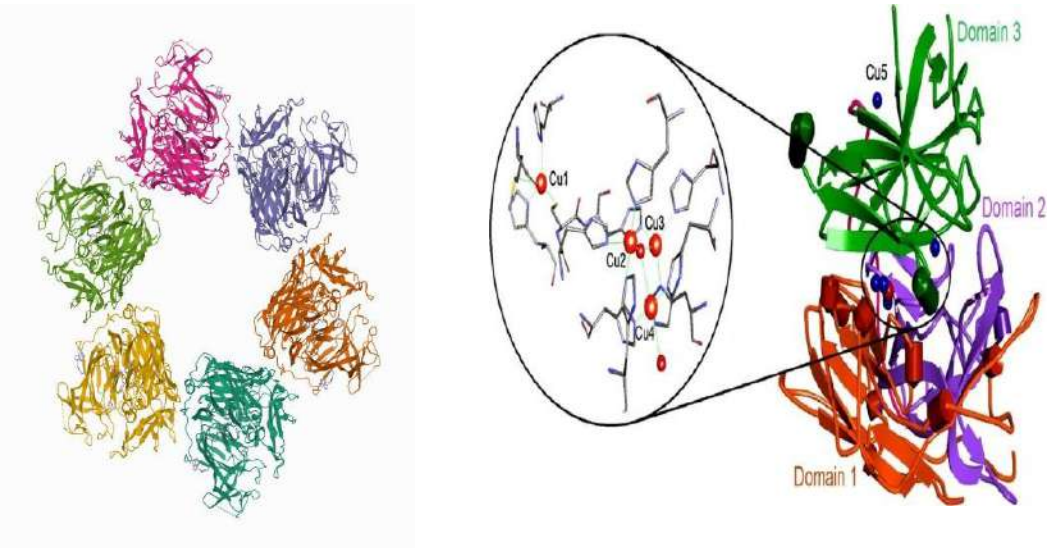


Fig:3 Phenoxazinone synthase 3D structure(left) and active site structure of phenoxazinone synthase(right)[1]

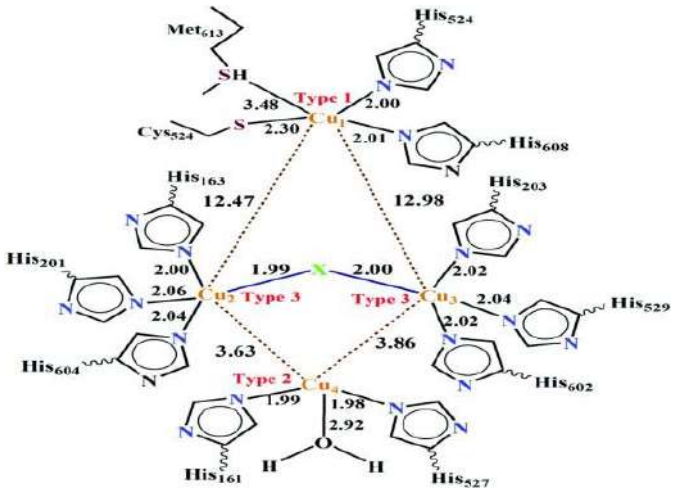


Fig:4 Schematic representation of active site[1]

4.MECHANISM OF THE OXIDATION OF OAP BY PHENOXYZINONE SYNTHASE(PHS)

After the discovery of PHS in 1962 although the structure of the enzyme was unknown the enzymatic catalysis and mechanistic studies of the phenoxazinone synthase (PHS) was initiated by Tadhg P.Begley and co-workers[4]where they concluded that among four possible mechanistic pathways phenoxazinone synthesis proceeds via a quinone imine intermediate that undergoes a conjugate addition, at the active site, with a second molecule of OAP (Fig. 4). The resulting intermediate then undergoes a 2-electronoxidation to the p-quinone imine. This reaction occurs,inpart,at the active site.A second conjugate addition followed by a fina l2- electron oxidation gives the phenoxazinone chromophore. They proposed that both of these steps occur outside the active site, and the phenoxazinone synthesis occurs via a cascade of three consecutive 2-electron aminophenol oxidations in which the 2-aminophenol (OAP) functionality is regenerated, after each conjugate addition, by a facile tautomerization reaction. In 1993, Villafranca et al. [5]showed that the enzyme requires 4- 5 copper atoms/monomer for full catalytic activity and additional copper inhibits the enzyme activity. Their spectroscopic studies indicate the presence of three functional copper atoms that can accept electrons from substrate and two additional copper atoms with unidentified functional behavior. It is strange to find that although the enzyme was isolated by more than one research group, no detailed kinetic study to determine the turn over number of the enzyme have been performed.

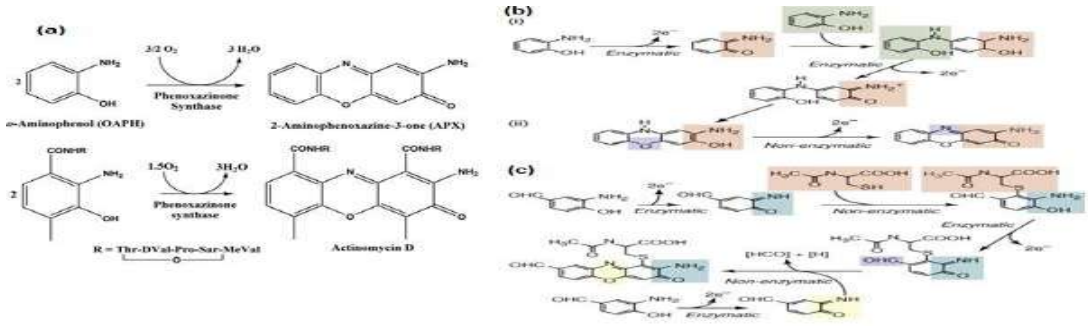


Fig:5 Oxidation of Ortho amino phenol derivative by phenoxazinone synthase[1]

5.MODEL SYSTEMS OF PHENOXAZINONE SYNTHASE(PHS)

25years after the discovery of the enzyme, use of transition metal and complexes as synthetic model of PHS started although the structure of the enzyme was not yet known In recent decades the modelling of PHS activity has been performed also by manganese complexes.

5.1 Mn-based model

In 2006, Simandi et al.[6] first reported a dimeric dioximatomanganese(II) complex which in methanol dissociates to monomeric form and behaves as functional model of PHS.However the kinetic parameters were not reported. A mononuclear manganese(II) complex was demonstrated as an active catalyst for OAP oxidation by Speier et al. Kinetic study was performed in details for the complex with kcat = 0.81×10−3 s−1 where use of triethylamine as base enhances the rate of oxidation. According to their proposed mechanism the reaction proceeds through a hydroperoxo intermediate of the OAPO2 − formed by the reaction of deprotonated OAP with molecular oxygen. Since triethylamine enhances the deprotonation hence oxidation rate was also increased in presence of triethylamine[1]. Manganese(III) complex of the type Mn(III) TPPS, was also an active catalyst for OAP oxidation in presence of H2O2. Recent reports by Panja et al. demonstrated that manganese complexes of N-donor rich ligands are also active for such oxidation[1]. Apart from the metal based model complexes,different enzymes other than PHS were also used for catalytic oxidation of OAP or other substituted aminophenols. Both tyrosinase and laccase from different sources were widely used to synthesise phenoxazinonechromophore[1] which is quite efficient with kcat values in 106 h−1 order. Though different transition metal complexes are reported to catalyse OAP to APX conversion,but simple organic oxidants are also active for OAP oxidation. However, such processes are not catalytic. Speier et al. in 2002 reported a significant observation that TEMPO can initiate the OAP oxidation in absence of any metal salt or complex. Using equimolar quantity of OAP and TEMPO in methanol after stirring at 50 ◦C for 10 h they got APX with 54% yield[1]. Previous reports on the plausible mechanism of oxidationof OAP to APX described the involvement of organic radical intermediates in their mechanistic pathways[1]. However, detection of an organic radical in PHS mimics is less, since only in a couple of cases organic radical could be detected[1]. Mechanistic studies of OAP model systems show that the formation of benzoquinone monoamine (BQMI)is the most crucial step of the oxidation process. Formation of BQMI from OAP was possible through various reactive oxygen species(ROS). However in most cases a hydroperoxo or superoxo species are shown as the plausible intermediate.Hence the dissemination of the mechanistic pathway for the oxidation of OAP to APX is still an open area of investigation that needs attention. However, the oxidation of OAP has been elusive interms of trapping the intermediate.An obvious conclusion that can be made so far is that although nature has designed a complex enzyme with pentanuclear Cu(II) active site, the conversion of OAP to APX chromophore can also be carried out catalytically with significant efficiency using simple metal complexes and metal salts. As evident from the literature most of the model complexes rather have N-donor rich coordination environment[Fig.5] while O-donor rich coordination complexes have never been probed so far for such oxidation reactions. We have probed O-donor based systems of various metals for oxidation of OAP and found them capable of APX formation.In addition most of these are mainly commercially available metal acetates that perform the conversion. Mn(II)/(III) acetates is the best one so far among all reported mimics which suggests that catalytically the reaction may be performed using other metal complexes. The turnover number for Mn(III) acetate obtained for OAP to APX conversion is 0.078 s^{-1}[1].

In 2015, S.K. Dey et al. [ref]have done a research in investigation of 3d-transition metal acetates to establish a trend in reactivity for catalytic conversions similar to Phenoxazinone Synthase. They found that Mn is the best 3d transition metal for similar catalysis with K_{cat} value 111(2)h^{-1}.

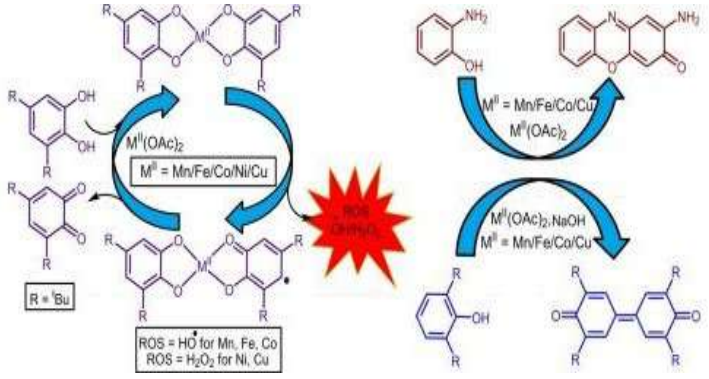


Fig:6 Representive diagram[2]

In 2016, our research group reported two new Mn(III) complexes derived from redox 'noninnocent' bromo-substituted catecholate ligands. Both the complexes were characterized by spectroscopic analysis, single crystal X-ray crystallography, cyclic voltametricstudies.It is the first example of valence tautomerism induced nucleophilicip so substitution by a nitrogen containing ligand. The complexes showed Phenoxazinone Synthase like catalytic activities[3].

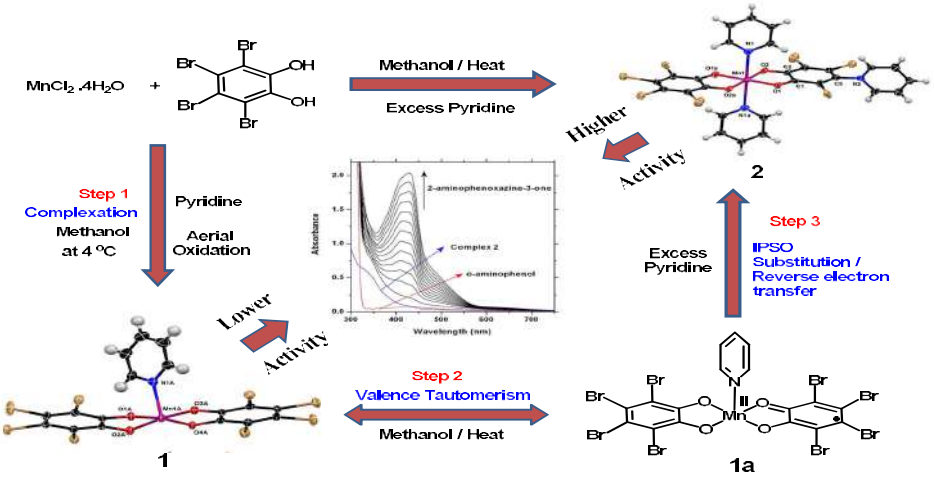


Fig:7 Representative Diagram

In 2016, J. Adhikary and his co-workers synthesized three new mononuclear manganese(II) complexes of an end-off compartmental Schiffbase ligand with binding sites.The complexes were characterized by EPR and cyclic voltametric studies. The complexes showed Phenoxazinone Synthase like biomimetic catalytic activities[4].

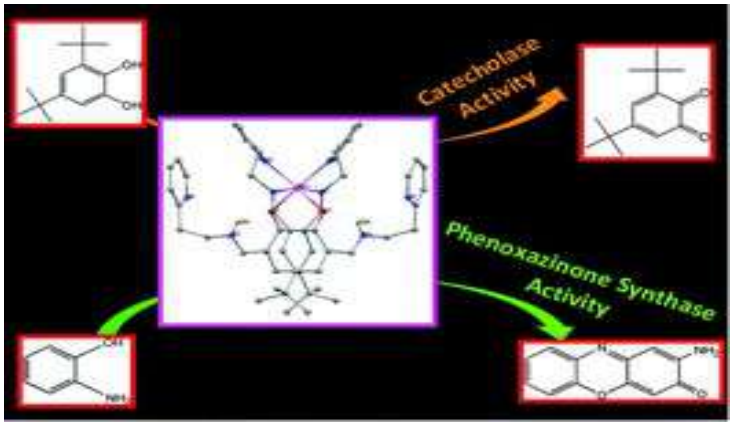


Fig:8 Representative diagram[4]

In 2016, N Sarkar et al. prepairedtwo new mononuclear manganese(III) complexes and characterized them by elemental analysis, IR, UV–Vis spectroscopy and single crystal X- ray diffraction studies. Manganese(III) in each complex assumes distorted octahedral geometry. Supramolecular interactions in both complexes were explored. Both complexes show phenoxazinone synthase mimicking activity[5].

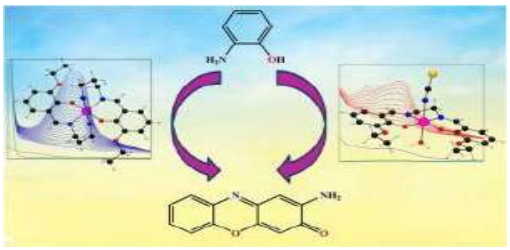


Fig:9 Representative diagram

In 2017, she and her group synthesized ionic coordination complex of manganese(III) with Schiff base ligands . Elemental analysis, single crystal X-ray diffraction studies, Hirshfeld surface analysis, fingerprint plot analysis and spectroscopic techniques have been used to characterize the complex. The complex was found to show efficient catalytic activity with *K*cat value 215.58 h^{-1}[6].

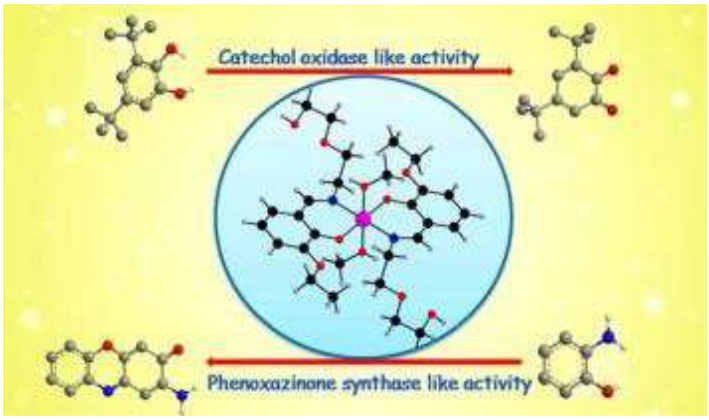


Fig:10 Representative diagram[6]

In the same year,the same group with an additional member also prepaired and characterized two new octahedral manganese(IV) complexes.. The energetic features of these interactions have been studied by DFT calculations and characterized using Bader's theory of atoms in molecules in both complexes. Both complexes exhibit phenoxazinone synthase like activities[7].

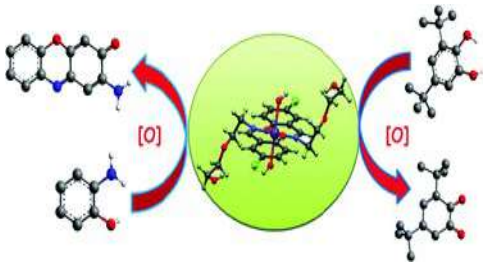


Fig:11 Representative diagram[7]

In 2017, S.C. Kumar and his research group found structurally characterized mononuclear Mn(II) complex which shows phenoxazinone synthase activities in MeOH at room temperature.Each of the reactions is found to be of first order with turnover numbers 3.15×10^{2} h^{-1}.Catalyst-substrate adduct as intermediate is trapped by mass spectrometry[8].

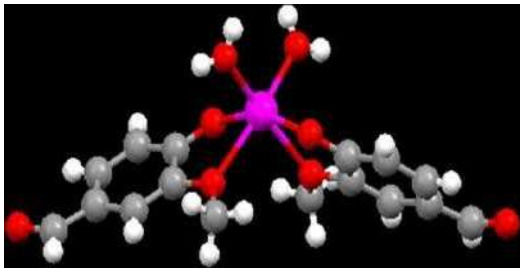


Fig:12 Representative diagram[8]

In 2017, our group described the synthesis and structural characteristics of six new manganese(III) complexes. X-ray crystal structure analysis revealed the geometry of manganese(III) centres. All the complexes are active toward the phenoxazinone synthase like activity and the detailed kinetic analysis was performed to get better insight into their catalytic efficiency. EPR spectroscopy and theoretical study were further helpful to get insight into origin of the catalytic activity in these compound

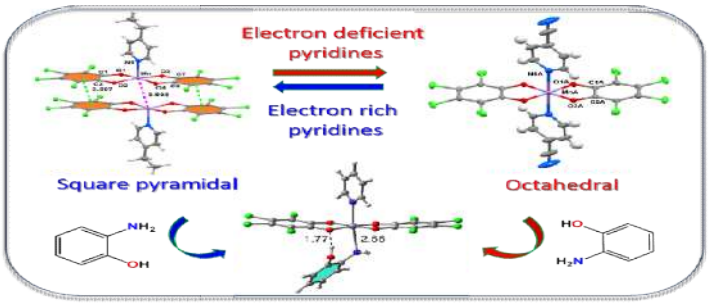


Fig:13 Representative diagram [9]

In 2017, our group synthesized a novel mononuclear Mn(III) complex and a tetranuclear Zn(II)–Mn(II) complex involving azo Schiff base ligands. The solid-state structures were determined by single crystal X-ray crystallography. The phenoxazinone synthase-like activity of both complexes has been examined and a detailed investigation of the structure– property correlation has been performed. This work highlights the importance of higher oxidation states of manganese over nuclearity for the development of better catalysts [10].

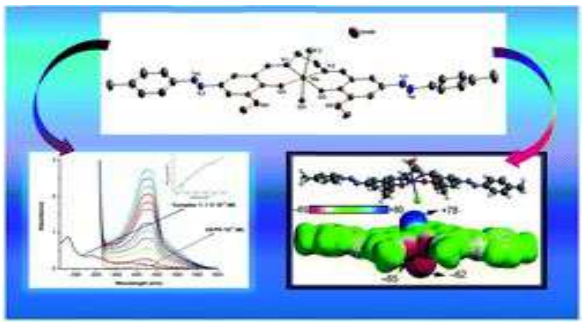


Fig:14 representative diagram[10]

In 2017, P. Mahapatra et al. prepaired two new trinuclear Cu-Mn complexes using a Cu(II) metalloligand. The presence of of a labile H2O coligand makes one of the complexes catalylically active in mimicking Phenoxazinone Synthase.The turnover numbers(Kcat)are 1118 and 6581 hour inverse respectively. The mechanism of these biomimetic oxidase reactions are proposed on the basis of mass spectral analysis, EPR spectroscopy and cyclic voltammetry[11].

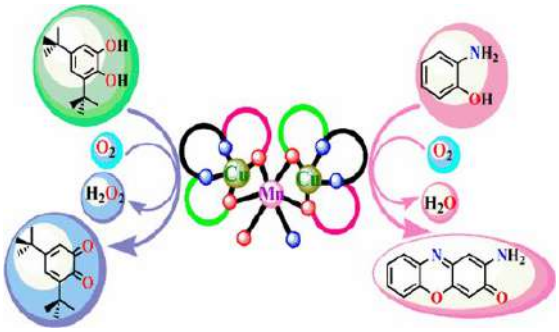


Fig:15 Representative diagram[11]

In 2017, P. Mahapatra and another group of researchers have synthesized three new heterometallic Cu(II)-Mn(II) complexes using a Cu(II)-metalloligand of an asymmetrically dicondensed Schiff base ligand. All the complexes show the bimimetic Phenoxazinone Synthase like activity for oxidation of o-amionophenol to amino phenoxazinone. The turnover number(Kcat) for the pocess are 4966, 2021, 1107 hour inverse respectively [12].

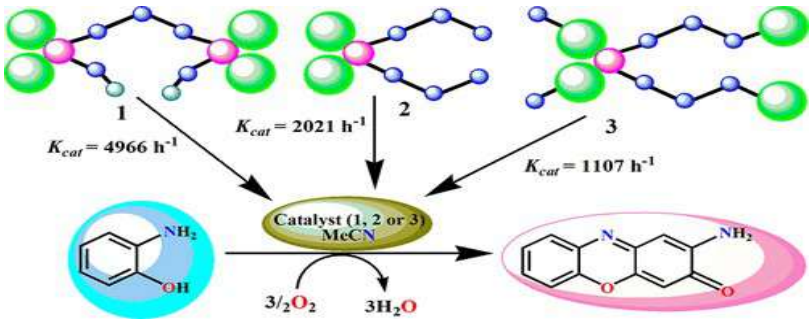


Fig:16 Representative diagram[12]

In 2018, the same group synthesized three novel heterometallic Ni(II)-Mn(II) complexes. The compounds were prepaired using a new mononuclear Ni(II) complexe of an unsyetrically dicondensed N2O3 donor ligand. All complexes showed biomimetic catalytic oxidase activities. For Phenoxazinone Synthase like activities, the turnover numbers are 3240,3360 and 13248 hour inverse, respectively.Mass spectral analysis, single crystal structure analysis, magnetic analysis and cyclic voltammetry analysis have also been performed [13].

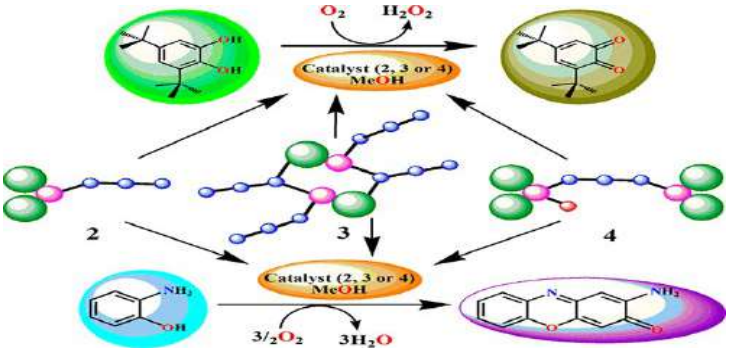


Fig:17 Representative diagram[13]

In 2018, the same group also prepared three new heterometallic Ni(II)-Mn(II) complex, using a new mononuclear Ni(II) complex of an unsymetrically dicondensed N2O2 ligand. Two of them showed biomimetic catalytic activity. For Phenoxazinone Synthase like activities, the turnover numbers are 6351 and 10545 hour inverse, respectively [14].

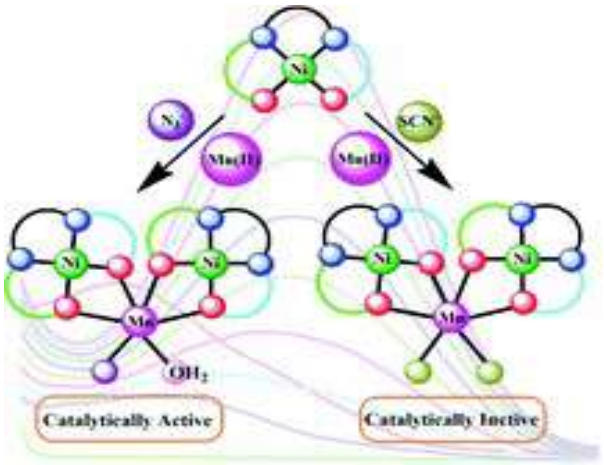


Fig:18 Representative diagram[14]

In 2018, S.Ganguly et al. have synthesized an unprecedented one-dimensional mixed-valence chain complex of Mn(II)-Mn(III). The complex has been characterized by IR spectroscopy, single-crystal X-ray diffraction analysis, and variable-temperature magnetic measurements. The phenoxazinone synthase-like activity of the complex have been studied. The turnover numbers (*k*cat) for these oxidase reaction have been calculated to be 738 h^{-1}

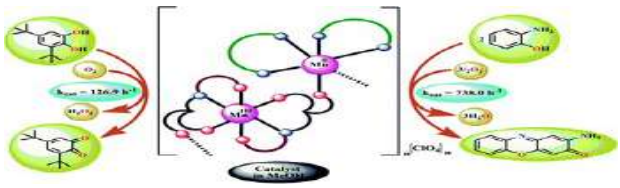


Fig:19 Representative diagram[15]

In 2019,T.Chakraborty and his group synthesized one Cu(II)-Mn(II)and oneNi(II)-Mn(II) heterometallic schiff base compounds using N2O2 donor ligand and a dicyanamide spacer.Between the two,only the Cu(II)-Mn(II) complex is catalytically active in mimicking Phenoxazinone Synthase. The turnover number for aerial oxidation is 5129 h^{-1}. Single crystal X-ray structural analysis, magnetic studies, ESI-mass spectrometry, EPR measurements and cyclic voltammetry analysis have also been performed[16].

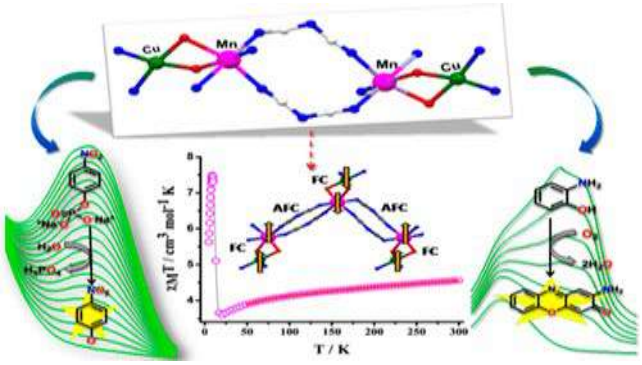


Fig:20 Representative diagram[16]

In 2019, A. Das et al. studied that reaction of Mn(II) salts with a flexidentate Mannich base ligandn the presence of chloride or azide ions yielded two new tetranuclear Mn(II) complexes. Single crystal X-ray structural analyses reveal that these two discrete tetranuclear Mn(II)complexes possessd effective dicubanecores. Both the complexes exhibit phenoxazinone synthase-like activity under ambient conditions.The turnover numbers(*k*cat) for the aerobic oxidation of *o*-aminophenol are 2265.5 and 2132.2 h^{-1}. Mass spectral analysis, magnetic studies were also performed [17].

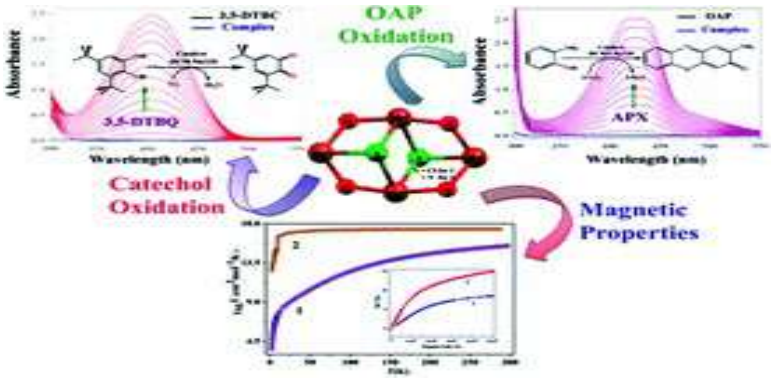


Fig:21 Representative diagram[17]

In 2019, Guvenc et al. synthesized Mn(II) complexe with a novel amine containing ketooxime ligand. Structural characterization was carried out by elemental analysis, ICP- OES, ^{1}H and ^{13}C NMR, UV–Vis, FT-IR, XRD, TG-DTG, magnetic susceptibility, Elemental analysis, stoichiometric analysis and molar conductivity measurements. HOMO and LUMO analyses and molecular electrostatic potential (MEP) properties of the synthesized molecule have been calculated. The complex showed Phenoxazinone Synthase like activity[18].

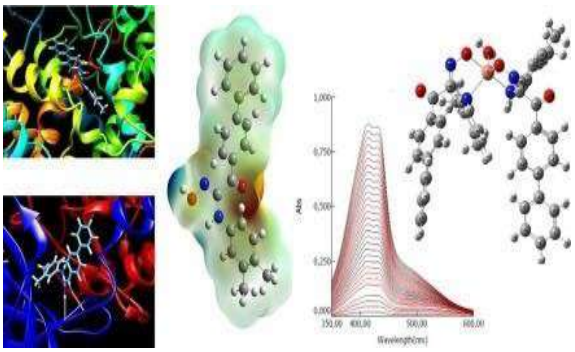


Fig:22 Representative diagram[18]

In 2019, our group synthesized a new flattened tetrahedral high spin Mn(II) complex using N_2O_4 donor Schiff base ligand. The complex was characterized by X-ray diffraction, DFT calculation,electro chemical studies and mass spectroscopy.The complex exhibits excellent catalytic property towards oxidation of *o*-aminophenols in aerobic condition[19].

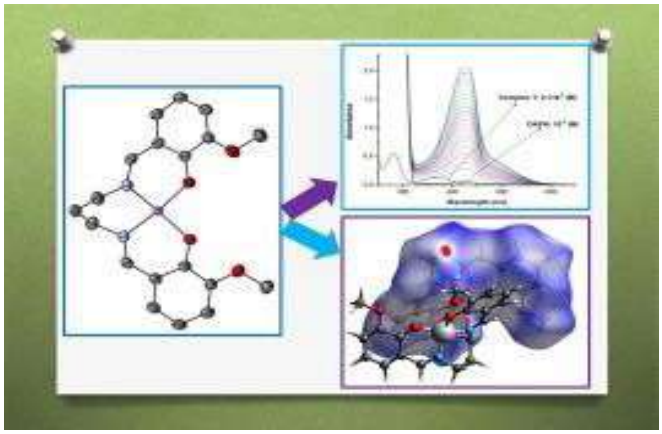


Fig:23 Representative diagram[19]

In 2020,S.Dutta and her research group synthesized two new Cu(II)-Mn(II) complexes with nicotinate ions. The complex was characterized by single-crystal-X-ray crystallography, ESI-mass spectroscopy and magnetic susceptibility measurements.Both the complexes showed Phenoxazinone Synthase like catalytic activities with turnover number (Kcat) 429 and 398 h^{-1}[20].

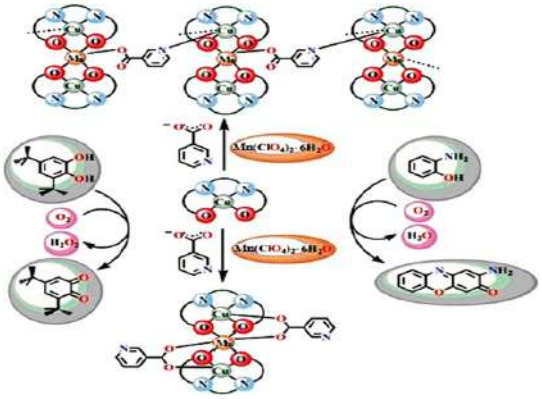


Fig:24 Representative diagram[20]

In2020,S.Dutta and another research group synthesized four new Cu(II)-Mn(II)complexes using a O3 donor Cu(II) metalloligand. The complexes waschracterized by single crystal structural analysis, cyclic voltammetry anlysis, mass spectroscopy and magnetic suseptibility measurements. One of the complex is catalytically very active towards mimicking the Phenoxazinone Synthase like oxidation reactions with turnover number (Kcat) value 230 h^{-1}[21].

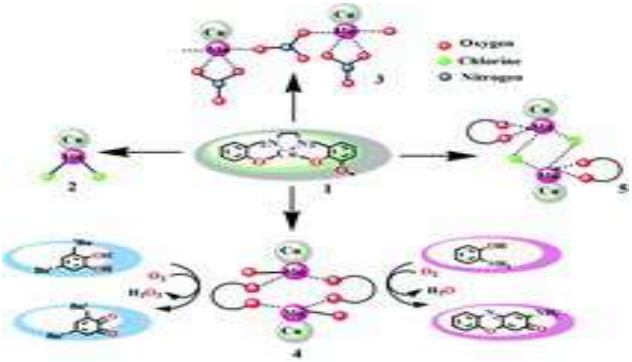


Fig:25 Representative diagram[21]

In 2020, A. Mandal et al. synthesized three mononuclear and one hexanuclear manganese(III) complexes using a Schiff-base ligand and characterized by EPR and single crystal XRD. Moreover, Mn_3O_4 nanoparticles have been synthesized using these complexes by calcination, with the aim to prepare nanozymes. These two synthesized nanoparticles were also able to show phenoxazinone synthase like activity and thus the complexes **c**an be claimed as precursors of nanozymes[22].

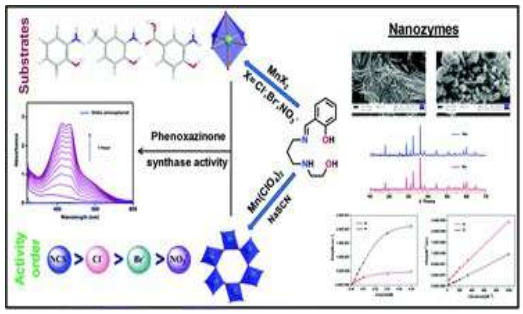


Fig:26 Representative diagram[22]

In 2020, our group described the synthesis and structural characterization of four new manganese(III) complexes derived from N3O donor Schiffbase ligands and their biomimetic catalytic activities related to phenoxazinone synthase. X-ray crystallography was done to describe the structure. This report was a bit unique because direct participation of the secondary coordination sphere particularly in modelling phenoxazinone synthase, has not been observed to date[23].

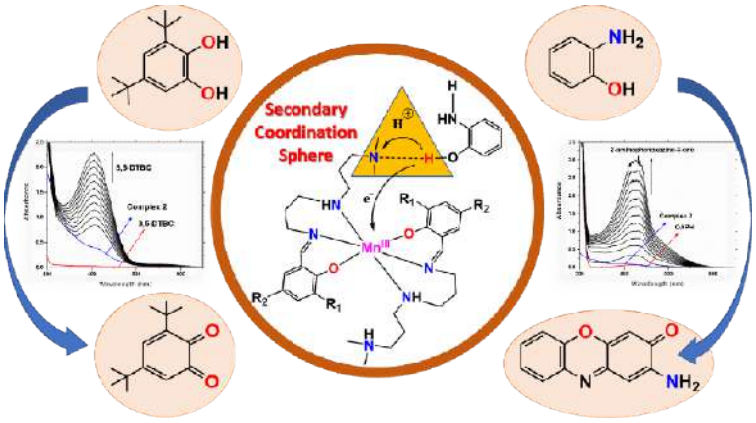


Fig:27 Representative diagram[23]

In 2021, S. Barışezginet al. synthesized a Mn(II) complex with coumarin based bidented ligand (HPYC). The compound was characterized by elemental analysis, stoichiometric analysis, spectral analysis, DFT/B3LYP, cyclic voltammetric analysis, ICP-OES, FT-IR, XRD, thermal analysis, magnetic susceptibility and molar conductivity measurements. The complex showed Phenoxazinone synthase like cataytic activities [24].

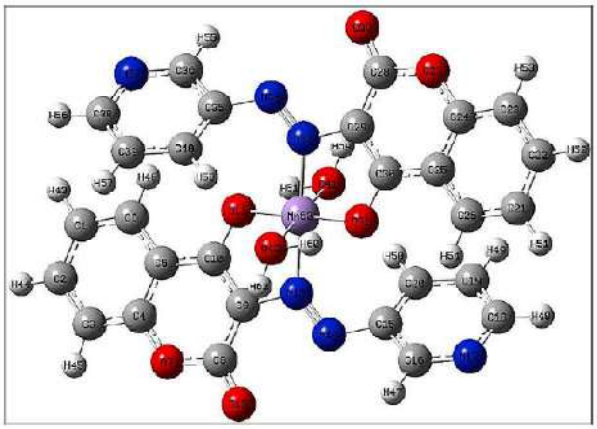


Fig :28 Representative diagram[24]

In 2021, Kumbhakar et al. reported two mononuclear manganese(II) complexes with a N_4 donor tetradentate tripodal ligand(Fig.29). Both the ligand and its metal complexes had been successfully synthesized and thoroughly characterized by different spectroscopic and analytical techniques such as FT-IR, 1 H NMR, UV-vis spectroscopy, EPR spectroscopy and ESI mass spectroscopy. Under ambient conditions, both complexes showed excellent phenoxazinone synthase activity as both are very susceptible to oxidize *o*-aminophenol to phenoxazinone. The turnover numbers (k_{cat}value) of these two complexes found to be extremely high (440 h^{-1} and 234 h^{-1}). The reported work evidently showed better performance of the synthesized Mn(II) complexes than all the predecessors. The plausible mechanism has been reiterated based on the experimental data via ESI-MS spectra and considering the concepts from the previously reported mechanisms involved in the formation of

hydrogen peroxide (H_2O_2) as an intermediate substrate is fairly indicating the involvement of molecular oxygen in the catalytic cycle[25].

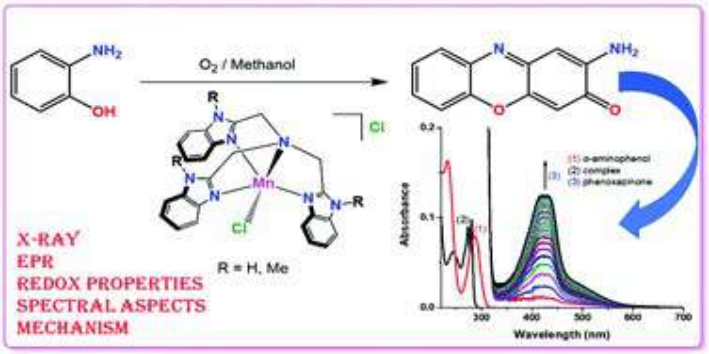


Fig:29 Representative diagram[25]

6.CATALYTIC PROMISCUITY OF PHS MIMICS:

Catalytic promiscuity is the ability of a single active site to catalyse more than one distinctly different chemical transformation. Such transformations may differ in terms of functional groups involved, the types of bond formed or cleaved during the reaction and hence the mechanistic pathway. To cite, an oxidase model complex may also act as a hydrolase mimic viz. phosphatase, nucleases and metallo-β-lactamases. Over the last few years the literature on biomimetics has shown evidences of such catalytic promiscuity. Hence, it may be said that catalytic promiscuity exists not just among many enzymes but also in certain small molecule enzyme mimics. A quick review of the literature on model systems of PHS shows that there are many small molecule PHS mimics which also exhibit hydrolase and nuclease activity. Detailed discussions on these are beyond the scope of this review since they are individually vast areas. However, for the benefit of the reader it is worth citing a few selected examples which are known not only as PHS mimics but also have been successful in performing catalytic transformations similar to hydrolases. I expect that increased knowledge on the catalytic promiscuity of such model complexes will enrich the understanding of the various possible mechanistic pathways that may be followed by a single biomimetic complex to catalyze multiple reactions of high significance to industrially relevant organic syntheses [1].

7.CONCLUSION:

We have presented the biomimetic Mn-based model complexes of PHS, discussed about their activity, collated the known mechanistic pathways involved during the catalysis anddisseminated them based on the reports. It appears that almost all model complexes act by binding to the substrate and then oxidizing it while the catalyst gets reduced; this step is followed by oxidation of the catalyst by molecular oxygen. The first step in the substrate oxidation, leads to formation of radicals and the next step completes the product formation while the catalyst goes back to its oxidised state. The literature data strongly suggests that many manganese based complexes show quite higher activity.Infact the activity of Mn(II)/(III) acetate itself is better than most Cu(II),Co(II)/Co(III),Fe(III),Ni(II) based model complexes known. Many metal complexes have been designed to activate molecular oxygen with the objectives to develop bioinspired catalysts for oxidation reactions but in most of the cases the turn over numbers are low compared to enzyme. It should be borne in mind that nature

has multiple constraints while designing a metalloenzyme viz. the designed enzyme has to function in cellular environment,it should have a metal that is available for uptake,it should not participate in undesirable reactions, the geometry of the protein active site hosting the metal should be such that the desired redox chemistry is feasible.In contrast in the laboratory while designing a catalyst we preferably are performing the reaction in a pot which does not have many of the above constraints faced by nature and hence we have more liberty to choose the metal and ligand with the basis, that the designed catalysts should function efficiently. In addition probing the catalytic promiscuity based on the designed complex and the available literature knowledge also provides insight to the various possible mechanistic pathways that may be adapted by a model complex. Hence, designing the metal complex similar to the active site of the metalloenzyme may not always be needed if ones objective is only to carry out efficient transformation rather than following a pathway same as that of, or similar to the metalloenzyme for the desired reaction. Thus to design efficient functional model systems of phenoxazinone synthase (PHS), Mn(II)/(III) might be the metal of choice for laboratory or industrial purposes based on the activity of Mn(II)/(III) catalysts in literature.

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ZINC BASED CATECHOL OXIDASE: BIOMIMETIC FUNCTIONAL MODEL AND MECHANISTIC PATHWAY

BSc Chemistry (Honours) Semester – VI (Under CBCS) Examination,2022

Course: CEMA DSE – B4 (Dissertation)

CU Roll No. – 193013-11-0004

CU Registration No. – 013-1212-0241-19

Gokhale Memorial Girls' College Kolkata

Authenticated.

Principal
Gokhale Memorial Girls' College

CERTIFICATE

This is to certify that this project of dissertation entitled '**Zinc based models on Catechol Oxidase'** submitted by Ms. Arunima Mondal (Reg no. – 013-1212-0241-19) , Department of Chemistry of Gokhale Memorial Girls' College is record of an original and independent study carried out by her under supervision and guidance of **Dr. Anangamohon Panja,** Gokhale Memorial Girls' College and that neither this review nor any part of it has been submitted for either any degree/diploma or any academic award anywhere before.

All help received by her from various sources have been duly acknowledged.

28-07-22

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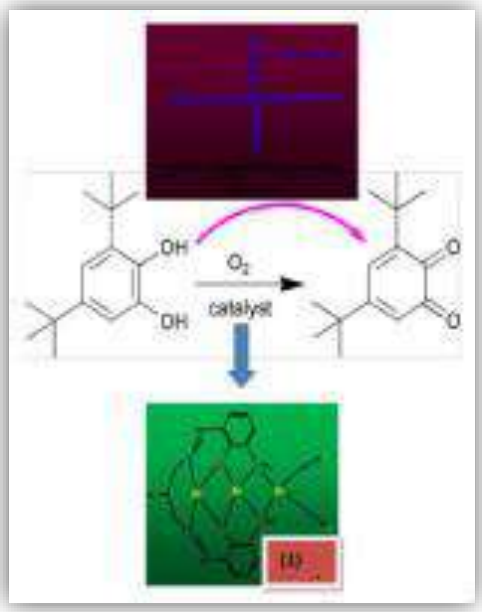
ABSTRACT

A new trinuclear zinc (II) complex, [Zn3(**L**)(NCS)2] (NO3)2·CH3OH·H2O (**1**), of a (N, O)-donor compartmental Schiff base ligand (H2**L** = N, N′-bis(3-methoxysalicylidene)-1,3-diamino-2-propanol), has been synthesized in crystalline phase. The zinc (II) complex has been characterized by elemental analysis, IR spectroscopy, UV–Vis spectroscopy, powder X-ray diffraction study (PXRD), 1H NMR, EI mass spectrometry and thermogravimetric analysis. PXRD revealed that **1** crystallizes in $P-1$ space group with a = 9.218 Å, b = 10.849 Å, c = 18.339 Å, with unit cell volume is 2179.713 (Å)3. Fluorescence spectra in methanolic solution reflect that intensity of emission for **1** is much higher compared to H2**L** and both the compounds exhibit good fluorescence properties. The complex **1** exhibit significant catalytic activities of biological relevance, viz. catechol oxidase. In methanol, it efficiently catalyses the oxidation of 3,5-di-*tert*-butylcatechol (3,5-DTBC) to corresponding quinone via formation of a Di nuclear species as [Zn2(**L**)(3,5-DTBC)]. Electron Paramagnetic Resonance (EPR) experiment suggests generation of radicals in the presence of 3,5-DTBC and it may be proposed that the radical pathway is probably responsible for conversion of 3,5-DTBC to 3,5-DTBQ promoted by complex of redox-innocent Zn (II) ion.

GRAPHICAL ABSTRACT:

A trinuclear zinc (II)–Schiff base complex has been employed to mimic catechol oxidase activity. This zinc–Schiff base complex exhibits significant catechol oxidation in methanol through ligand centred pathway, which is the rare example among the redox innocent zinc complexes till date.



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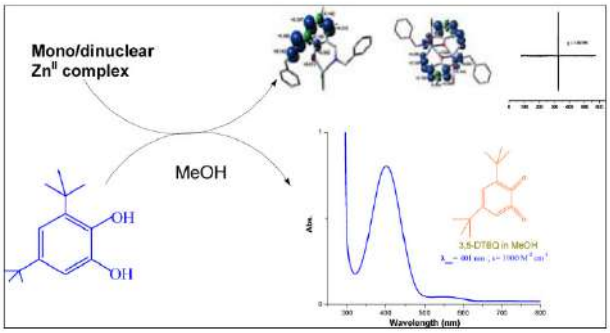
INTRODUCTION

Four binuclear and three mononuclear ZnII complexes of phenol-based compartmental ligands (HL1–HL7) have been synthesized with the aim to investigate the viability of a radical pathway in catecholase activity. The complexes have been characterized by routine physicochemical studies as well as X-ray single-crystal structure analysis: [Zn2(H2L1)(OH)(H2O)(NO3)](NO3)3 (1), [Zn2L2Cl3] (2), [Zn2L3Cl3] (3), [Zn2(L4)2(CH3COO)2] (4), [Zn(HL5)Cl2] (5), [Zn(HL6)Cl2] (6), and [Zn(HL7)Cl2] (7) [L1–L3 and L5–L7 = 2,6-bis(R-iminomethyl)-4-methylphenolato, where R= N-ethyl piperazine for L1, R = 2-(N-ethyl)pyridine for L2, R = N-ethyl pyrrolidine for L3, R = N-methylbenzene for L5, R = 2-(N-methyl)thiophene for L6, R = 2-(N-ethyl)thiophene for L7, and L4 = 2-formyl-4-methyl-6-N-methylbenzene-iminomethyl-phenolato]. Catecholase-like activity of the complexes has been investigated in methanol medium by UV–vis spectrophotometric study using 3,5-di-tert-butylcatechol as model substrate. All complexes are highly active in catalyzing the aerobic oxidation of 3,5-di-tert-butylcatechol (3,5-DTBC) to 3,5-di-tert-butylbenzoquinone (3,5-DTBQ). Conversion of 3,5-DTBC to 3,5-DTBQ catalyzed by mononuclear complexes (5–7) is observed to proceed via formation of two enzyme–substrate adducts, ES1 and ES2, detected spectroscopically, a finding reported for the first time in any ZnII complex catalyzed oxidation of catechol. On the other hand, no such enzyme–substrate adduct has been identified, and 3,5-DTBC to 3,5-DTBQ conversion is observed to be catalyzed by the dinuclear complexes (1–4) very smoothly. EPR experiment suggests generation of radicals in the presence of 3,5-DTBC, and that finding has been strengthened by cyclic voltametric study. Thus, it may be proposed that the radical pathway is probably responsible for conversion of 3,5-DTBC to 3,5-DTBQ promoted by complexes of redox-innocent ZnII ion. The ligand-centered radical generation has further been verified by density functional theory calculation.

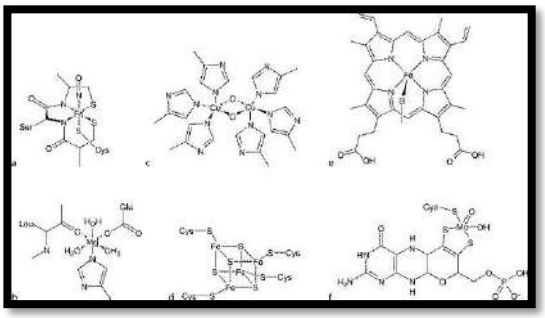
Background

Oxidation reactions are fundamentally important component of organic synthesis and play an important role in rendering the desired functionality to the intermediates of valuable compounds such as pharmaceuticals, agrochemicals, and other fine chemicals. For economic and environmental reasons, the oxidation processes of bulk chemical industries predominantly involve the use of molecular oxygen as the primary oxidant. Molecular oxygen is an ideal oxidant because of its availability directly from air rendering it inexpensive and environmentally benign. However, the application of oxidation reactions in scaled-up synthesis is limited due to the use of heavy metals, thermal hazards, and moderate chemo selectivity for highly functionalized compounds in most oxidation reactions. Classical oxidation methods with stoichiometric quantities of inorganic oxidants are toxic and enrich the environmental pollution. That is why oxidations using catalytic amount of activator which can activate molecular oxygen with minimum chemical waste is inspiring. The challenges faced to activate molecular oxygen for its use in oxidation reactions is due to its kinetically inert nature. The reaction of molecular oxygen with organic substrates do not take place under ambient conditions as typical organic molecules in general posses singlet ground state and their reaction with oxygen is spin forbidden. However, if the organic substrate gets converted to a radical then its reaction with oxygen is a spin-allowed process. Among the other possibilities, the orbital overlap of oxygen with a suitable metal ion may help its activation through electron transfer from the metal. Electron transfer may also happen through orbital overlap with potent organic electron transfer agents, viz. flavins or pterins, which may render reduced oxygen species. Such organic co-factors have been reviewed elsewhere and are beyond the scope of this review. A major problem while using dioxygen in chemical transformation is that its reactivity is not easily controlled and often may lead to low selectivity and over-oxidation. Nature has evolved an elegant solution to overcome the kinetic barrier of dioxygen activation by using transition metal incorporated in proteins, the so called ‘metalloenzymes’. Among various types of oxidative transformations, the oxidation of alcohols to carbonyl compounds occupies an important place in both laboratory and industry.



Inorganic chemists have largely exploited the concept of nature by designing oxygen activation catalysts which act as small molecule mimics of the metalloenzymes and help to understand the mechanistic pathways. Using the knowledge of co-ordination chemistry, redox potential and electronic factors, the enzymes donor sites are modeled with small molecule called ligands, which are then incorporated with metals to form complexes that are probed as structural and functional models. In general, the catalytic activity of most of the metal complexes is highly encouraging. However, a fair share of these complexes may not be considered very efficient as catalysts due to their inability to efficiently perform the relevant organic oxidative trans-formations. Many of the complexes may seem not efficient but they undoubtedly are very useful in providing us with useful insight about the important mechanistic aspects of the metalloenzymes. Development of catalytic reactions with the perspective of understanding and mimicking the enzymatic functions of metalloenzymes has been the focus of bio-inorganic chemists during the past few decades. Other than understanding the possible mechanistic pathway of the enzyme through such mimics, a long-term idea is to develop complexes which would be very useful as catalysts.

METALLOENZYMES:- Metalloenzymes are a broad group of enzymes that use a metal cation as a cofactor in the enzyme active site. The enzymes promote a diverse range of reactions including hydrolytic processes and oxidation/reductions.

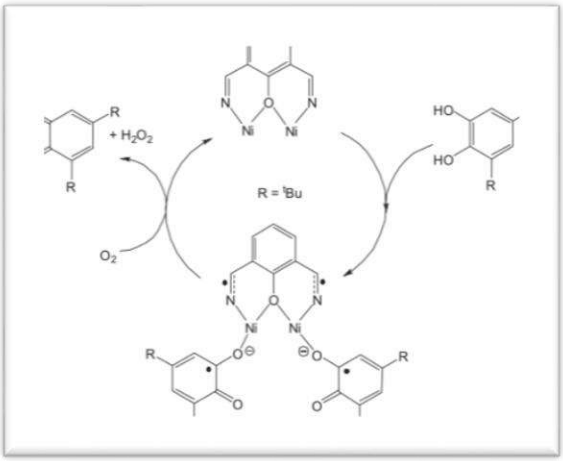


Most of the metalloenzymes are capable of activating molecular oxygen due to presence of metallions. Among the various metalloenzymes , in this project , I will discuss about Catechol Oxidase which shows interest in oxidation to generate o-quinone.

NATURE'S CHOICE AND ROLL OF BIOMIMETICS

Nature uses several metalloenzymes to catalyze the controlled and selective oxidation of organic compounds. The geometry and structural feature of enzyme active sites and the choice of incorporated metals are very diverse and fully optimized to the function of the proteins or enzymes. In addition, it also takes into account the availability of the metal ion in environment. establishing the correlation of the geometric and electronic structure with function is one of the main objectives of the bioinorganic chemists. The primary goal in designing mimics of

metalloenzymes is to understand the structures of active sites and reactive intermediates and the mechanistic details of dioxygen activation and oxygenation reactions occurring at the active sites. Progress made so far in terms of understanding the mechanistic pathway of oxidative enzymes has been very encouraging. The huge library of complexes available to us as model complexes of various enzymes shows that in spite of sincere efforts, we have not obtained turn overs close to native enzymes but the dissemination of mechanistic pathways and introduction to alternative pathways has been possible. This review is on zinc model complexes of catechol oxidase (CO). The works in this area that have appeared in the literature over the past few decades shows the impetus to understand the chemistry of these metalloenzymes. We have tried to take into account all the reports that deal with mechanistic pathway of CO. However, it is not possible always to provide an exhaustive account of all related previous works. To keep the focus, we have rather discussed the mechanistic pathways of the model systems with literature examples and whenever available tabulated them with most important kinetic parameters. The catechol oxidase enzyme has a type-3 active site and catalyzes the oxidation of dioxolanes (catechol) to the corresponding o-quinones. However, there are other metal based model complexes of catechol oxidase. The tabulation of the known kinetic parameters of the model complexes and the native enzymes would help the perception of the readers in regard to the progress made so far with this enzyme. Finally, many of the reported mimics of CO showed efficiency to catalyze the C-C bond coupling in certain sterically hindered phenols.

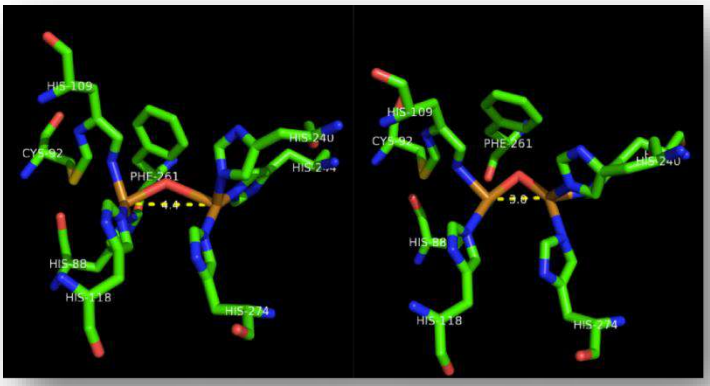


Catechol oxidase

Catechol oxidase is a copper oxidase that contains a type 3 di-copper cofactor and catalyzes the oxidation of ortho-diphenols into ortho-quinones coupled with the reduction of molecular oxygen to water. It is present in a variety of species of plants and fungi including Ipomoea batatas (sweet potato)and Camellia sinensis (Indian tea leaf).Metalloenzymes with type 3 copper centers are characterized by their ability to reversibly bind dioxygen at ambient conditions.[3] In plants, catechol oxidase plays a key role in enzymatic browning by catalyzing

the oxidation of catechol to o-quinone in the presence of oxygen, which can rapidly polymerize to form the melanin that grants damaged fruits their dark brown coloration.

STRUCTURE:- The crystal structure of catechol oxidase purified from Ipomoea batatas has been resolved in its active form in both the oxidized Cu (II)-Cu (II) state and the reduced Cu(I)-Cu(I) state. It is a globular, single domain, monomeric enzyme that is approximately 55 by 45 by 45 Å in size and ellipsoid in shape. A four α-helix bundle comprises the enzyme core, which girds the active site containing the Di copper center. The nitrogen on the imidazole side chains of His88, His109, and His118 coordinate with the first catalytic copper while the nitrogen on the imidazole side chains on His240, His244 and His274 coordinate with the second catalytic copper ion. In the oxidized Cu (II)-Cu (II) state, each copper ion possesses a four-coordinate trigonal pyramidal geometry, with the three histidine residues and a bridging hydroxide molecule forming the four ligands on each copper ion. Comparing the reduced (Cu(I)-Cu(I)) state with the native (Cu (II)-Cu (II)) state of the enzyme, the key difference is the distance between the two copper centers. In the oxidized Cu (II)-Cu (II) state, the Cu-Cu distance is 3.3 Å while in the reduced Cu(I)-Cu(I) state, the distance increases to 4.4 Å.



Reduced Cu(I)—Cu(I) state Native Cu(II)—Cu(II) state

While the active site of both tyrosinase and catechol oxidase contain the di-copper center, variations in each enzyme's respective structure result in differing activity. In catechol oxidase, a phenylalanine side-chain (Phe261) is above one of the copper centers and prevents the substrate from coordinating with both copper ions in the active site. This precludes the bidentate coordination complex necessary for di-phenolate hydroxylation characteristic of tyrosinase but absent in catechol oxidase. Furthermore, His109 bound to one of the copper centers is also covalently linked with Cys192 through a thioether bridge. This cysteine-histidine cross-linking may further restrain the enzyme active site from assuming the bidentate coordination complex readily formed in tyrosinase.

FUNCTIONS:-

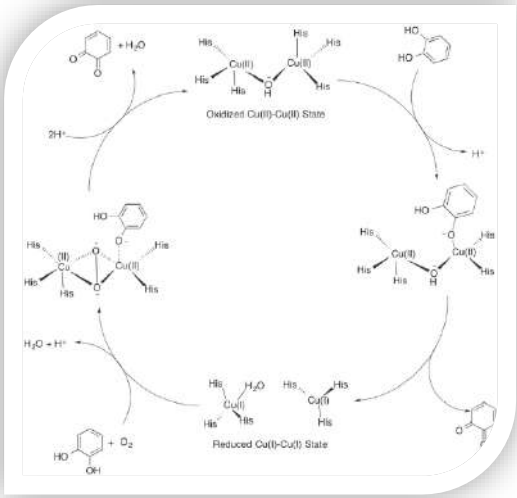
Polyphenol oxidases are a family of di-copper metalloenzymes that include tyrosinase and catechol oxidase. In plants, both enzymes can catalyze the oxidation of ortho-diphenols substrates into their corresponding ortho-quinones. The key difference between the two related enzymes is that tyrosinase can catalyze the hydroxylation of monophenols to diphenols (monophenolase activity) as well as the oxidation of the o-diphenol to the o-quinone (diphenolase activity) whereas catechol oxidase only possesses diphenolase activity.

[Overall reaction catalyzed by catechol oxidase: production of two o-quinones and 2 molecules of water from two molecules of catechol and one molecule of dioxygen.]

When plant tissue is damaged, the chloroplast may rupture and release catechol oxidase into the plant cytoplasm, and vacuoles may also rupture, releasing stored catechol into the cytoplasm. The tissue damage also allows oxygen to penetrate into the cell. Thus, tissue damage facilitates the interaction of catechol oxidase with its substrate to produce o-benzoquinone, which can polymerize non-enzymatically to yield melanin that form an insoluble barrier for wound protection.

CATALYTIC ACTIVITY AND MECHANISM: -

Proposed catalytic cycle of catechol oxidase purified from Ipomoea batata Although a crystal structure of catechol oxidase has been solved, questions concerning the exact mechanism of the reaction remain. One mechanism proposed by Eicken et al. is based on the crystal structure of catechol oxidase purified from Ipomoea batatas. The catalytic cycle begins with the catechol oxidase in its native oxidized Cu (II)-Cu (II) state with a coordinated hydroxide ion bridging the two copper centers. As catechol enters the active site, a proton is abstracted from one of the alcohols. The catechol coordinates with a Cu (II) center in a monodentate fashion, displacing one of the coordinating histidine residues. The coordinated hydroxide ion abstracts another proton from catechol to form



water, and the catechol is oxidized to o-quinone. The two resulting electrons reduce both copper centers to their Cu(I)-Cu(I) state. Dioxygen then binds one copper center, displacing the coordinated water molecule, and another molecule of catechol binds to the other copper center, displacing another histidine residue. This forms a complex in which one copper center has a tetragonal planar coordination with His240, His244 and the dioxygen molecule. The other copper center retains its initial tetragonal pyramidal geometry with dioxygen, His88 and His118 in the equatorial positions, and His109 in an axial position. In this state, the enzyme active site is in a ternary catechol oxidase–O22−–catechol complex. Two electrons are transferred from the substrate to the dioxygen, followed by cleavage of the O–O bond. Water is released, and the second o-quinone product is formed together with the restoration of the initial Cu (II)-Cu (II) state to complete the catalytic cycle.

This proposed catalytic cycle is supported by the experimental observation that stoichiometric amounts of o-quinone form after catechol addition to the enzyme, even when dioxygen is absent. Furthermore, both the oxidized Cu (II)-Cu (II) state and the reduced Cu(I)-Cu(I) state were the two states identified by the crystal structure of Ipomoea batatas. The monodentate binding of catechol to the copper center was supported by the crystal structure of catechol oxidase bound with the bound-substrate analogue inhibitor phenylthiourea, which also binds to the copper center in a monodentate fashion. However, one issue with this catalytic cycle is that the charge of the active site changes during the catalytic cycle from +1 to +3. This necessitates the presence of nearby bases that can store the protons; however, the X-ray crystal structure does not indicate the presence of any such bases as the histidine residues are coordinated with the copper centers. Other catalytic cycles elucidated with DFT calculations and crystal structures have been proposed which maintain the same charge in the active site throughout the cycle and thus do not require nearby bases. However, certain intermediates in the proposed cycle are not consistent with experimental findings such as that stoichiometric amounts of o-quinone can form after catechol addition in the absence of oxygen.

Scheme:1

CATECHOL OXIDASE ACTIVITY:-

Table 1: *Kinetic parameters of catechol oxidation by metalloenzymes*

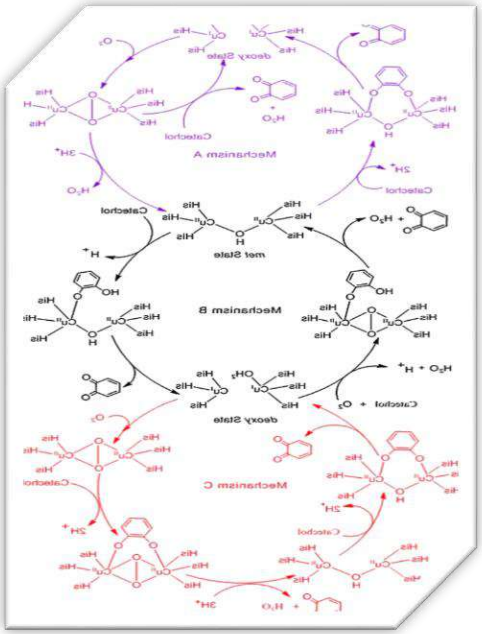
ENZYMES	SUBSTRATE	$K_{cat(h-1)}$	K_M(M)	Kcat/K_M($Mm^{-1}s^{-1}$)
Catechol Oxidase[a]	Catechol	5.7×10^5	0.005	31.67

Catechol Oxidase[b]	Catechol	8.25×10^{6}	0.0025	916.67
GriF[C]	Catechol	4.0×10^{4}	0.0025	4.44
Mushroom tyrosinase	Catechol	3.15×10^{6}	0.00016	5463.13

[a] Catechol oxidase from Lycopuseuropaeus; [b] Catechol oxidase from Ipomoca batatas (sweet potatoes) ; [c] A tyrosinase homolog.

MODEL SYSTEMS OF CATECHOL OXIDASE:-

The structure of the enzyme shows di copper (II) moiety at the active site hence, several di copper (II) complexes with similar ligand environment have been designed to mimic the enzyme and probe its mechanism. But besides them lots of other transition metal mimics also have been reported over the years out of which the catalytic activity of many is comparable with the copper-based mimics. Catecholase activity of metal complexes are controlled by many different factors. A general structure–activity correlation is difficult to establish. Many research groups have attempted to correlate the catecholase activity with metal-metal separation, electrochemical properties of the complexes, influence of ligand structure or exogenous bridging ligand which is the best that could be done so far. The variation in the activity with subtle changes in the electronic factors suggests that the synergism of the ligand and the metal renders a strong effect on the affinity for substrate and in addition the change in metal also changes oxygen affinity.



Nature has designed catechol oxidase to ligate CuII ion in the required geometry and with optimum redox potential to oxidize catechol through switching between CuIIand CuIredox states with necessary stability. Hence, to probe catecholase activity with a different metal ion, the coordination environment may need a change, for better reactivity. The geometry rendered by the ligand used and the redox potential of the resultant complex also is important and so should be the presence of accessible labile site(s)on the complex for possible substrate/oxygen binding. Thus, in order to probe the activity of CoII/IIIor MnII/III or any other metal instead of CuII, there may not be a necessity to mimic the similar coordination environment with

nitrogen donors for achieving enzymatic activity but rather it may be disadvantageous because of the inherent electronic differences of the two metals. In fact, relevant literature in this area shows that replacement of the native metal of an enzyme by a non-native one may

degrade the performance drastically. Different types of ligand system were also designed to make dinuclear as well as mononuclear complexes of transition metal such as Mn, Co, Ni, Zn along with Cu to modulate the activity and get a better structure property correlation.

Advantages of Model Complex:

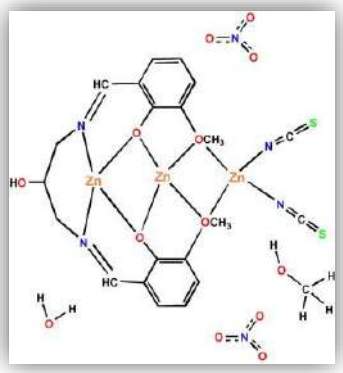
An advantage of model complex compared to the native enzymes is the ability of the model complexes to function in organic solvents unlike the natural enzymes.

Disadvantages of Model Complex:

The activity (kcat) of most of the mimics are quite low when compared with the enzymes. Hence the structure property correlation still provides scope for newer designs to build structural and functional model systems with better activity close to the enzyme for potential in industry.

ZINC-BASED MODELS

Zinc (II) being a d10metal ion, one would in general not be interested to investigate Zn II for catecholase activity. However, although scarce, Zn II complexes do participate in catecholase activity (Table 2). The redox non-innocent ligands act via a radical generation pathway in presence of ZnII. Recently some research groups have tried to use dinuclear zinc complexes to probe the role of ligand and involvement of radical intermediate. In 2012, Das et al. reported few dinuclear and mononuclear ZnII complexes of redox non-innocent ligands that showed catecholase activity. Their aim was to investigate the viability of radical pathway in catecholase activity. Based on the spectroscopic studies and density functional theory calculation they were able to propose that the oxidation of DTBC occurred through an alternative pathway which involve generation of ligand centered radical species. In 2013, Biswas et al. reported a trinuclear Zn-Schiff base complex which again showed catecholase activity through generation of ligand Centre radical. The turnover numbers for most of these complexes show that they are quite efficient in the catalytic oxidation. However, the ligands of the metal complexes alone are not investigated for the catecholase activity. So in absence of such evidences, the only role of Zn II maybe the binding of the substrate DTBC. It is also not clear if the Lewis acidic character of Zn II has a role in such catalysis. Besides those there are few reports of FeII/III complexes discussion of which are not included in this review since there has not been much attempts to use FeII/IIIbased complexes as CO mimics and they are rather a better choice for catechol dioxygenase (which leads to intradiol or extradiol cleavage) based on the literature data. However, all the mechanistic studies performed on various metal complexes suggests that the oxidation proceeds through semiquinone intermediate and the side product generated during the process is mostly H2O2. There are some other zinc-based model complexes as mentioned below.



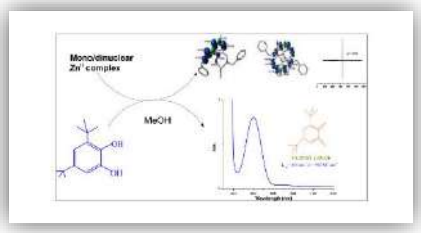


Table 2: *Reported zinc (II) complexes showing catechol oxidase activity with important kinetic parameters*

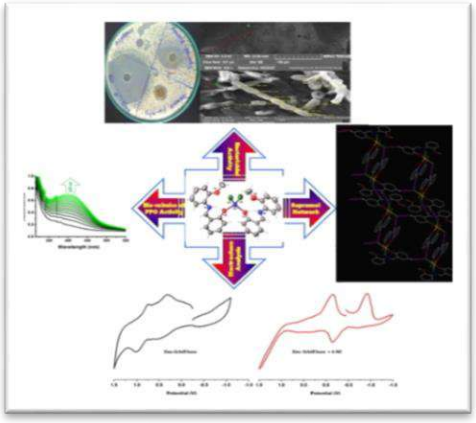
[b] CH3OH used as solvent

- **Subrata Das, Amrita Sahu and their coworkers** in 2018 reportedthe synthesis and structural characterization of a new Zn(II)-Schiff base complex, [Zn(L)(H2O)], [L=N,N′-bis(3-methoxysalicylidene)-1,3-diamino-2-propanol]. Single crystal X-ray structural analysis reveals that the compound crystallizes in monoclinic system with P21/c space group. The compound shows good photo-luminescence property in methanol medium. This Zn(II) complex has been evaluated as a catalytic system in the catalytic oxidation of 3,5-di-tert-butylcatechol (DTBC) in methanol. The Zn(II) complex displays good catecholase like activity with significant turn over, kcat(h–1)=7.99×102 in methanol under aerobic condition.

- **Sukanta Pal and his coworkers** in 2015 synthesized a new trinuclear zinc(II) complex, [Zn3(L)(NCS)2](NO3)2·CH3OH·H2O with a (N,O)-donor compartmental Schiff base ligand (H2L = N,N′-bis(3-methoxysalicylidene)-1,3-diamino-2-propanol). The zinc(II) complex has been characterized by elemental analysis, IR spectroscopy and X-ray crystallography. Electron Paramagnetic Resonance (EPR) experiment suggests generation of radicals in the presence of 3,5-DTBC and it may be proposed that the radical pathway is probably responsible for conversion of 3,5-DTBC to 3,5-DTBQ promoted by complex of redox-innocent Zn(II) ion.

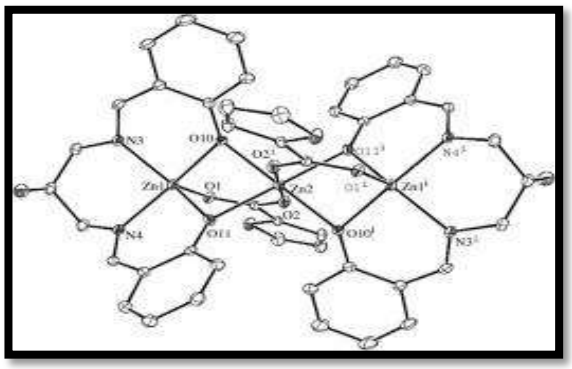
Zn – complexes[a]	**Solvent**	**K_{cat}(h-1)**	**K_m(M)**	**V_{max}(Ms^{-1})**
[Zn2(H2L-(CH3)11)(OH)(H2O)(NO3)]3+	[b]	1.06×104	6.71×10^{-3}	2.96×10^{-3}
[Zn2(L-(CH3)12)CI3]	[b]	0.88×104	2.62×10^{-3}	2.45×10^{-3}
[Zn2(L-(CH3)14)2(CH3COO)2]	[b]	3.52×103	1.05×10^{-3}	9.78×10^{-4}
[Zn2(L-(CH3)13) Cl3]	[b]	2.97×103	1.93×10^{-3}	8.25×10^{-4}
[Zn3(L153)(NCS)2]$^{2+}$	[b]	9.28×102	1.88×10^{-3}	2.58×10^{-5}
[Zn3L86(-O2CCH3)2(CH3OH)4]	[b]	1.33×103	1.06×10^{-3}	3×10^{-3}
Na4[Zn2(L141)2](OAc)^{2}	[b]	9.13	1.78×10^{-3}	1.27×10^{-7}

- **Shreya Mahato and her team** submitted a research work in 2021 which demonstrates the synthesis, crystal structure, supramolecular architecture, 4-methylcatechol oxidation and bactericidal activity of a newly designed zinc complex containing a protonated Schiff base of zwitter ion type[Zn(HL)2Cl2], [Schiff base (HL) = 2-(2-methoxybenzylideneamino)phenol]. Crystal structure analysis of the zinc-Schiff base reveals that zinc centre exists in a distorted tetrahedral geometry. This zinc-Schiff base complex has been examined towards the bio-mimetic oxidation of 4-methylcatechol (4-MC) in methanol and portrays its good efficacy with good turnover number, 1.45 × 103 h−1. Electro-chemical study, electron paramagnetic resonance analysis and electrospray ionization mass spectrometry results for the zinc-Schiff base complex in presence of 4-MC ensures that the catalytic reaction undergoes through enzyme-substrate binding, and generation of radical in the course of catalysis drives the catalytic oxidation of 4-MC.

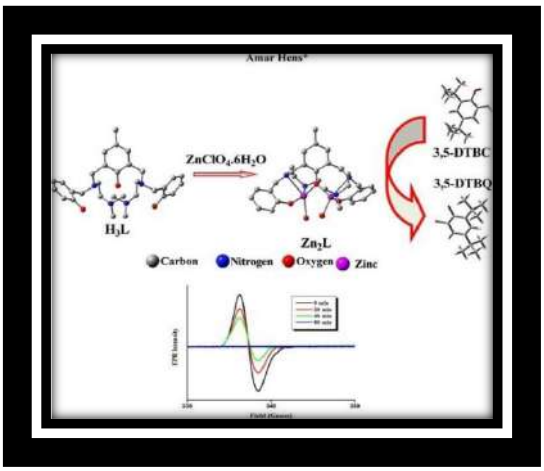


- In 2014 **Ayan Patra and his coworkers** synthesized anew dinuclear zinc(II) complex, $Na_4[Zn_2(hdpa)_2](OAc)_2$ with a new dinucleating ligand, H_3hdpa (H_3hdpa = 2-({[2-hydoxyethyl]-[2-hydroxy-3-(1-oxo-1,3-dihydro-isoindol-2-yl)-propyl]-amino}-methyl)-benzoic acid), Catechol oxidase activity of the complex has been investigated in methanol solution by the UV–Vis spectrophotometric technique using 3,5-di-tert-butylcatechol as a model substrate. DFT calculations have been performed to find the Fukui functions at the metal centers in the complex to predict the possible metal centers involved in the binding process with 3,5-DTBC during the catalytic oxidation reaction.

- In 2015, **Sunit Kumar Maal, Chandra Sekhar Purohit, Rajarshi Ghosh & Merry Mitra** worked on the synthesis and X-ray crystallographic characterization of a trimetallic zinc(II) complex $[Zn_3(L)_2(fu)_2]$ [H_2L = N,N-(salicyaldene)-1,3-diaminopropan-2-ol, fu = 2-furoate] reveal its distorted square pyramid geometry at the two terminal Zn(II) centres and distorted octahedral geometry at the central Zn(II). **It** behaves as an effective catalyst towards oxidation of 3,5-di-tert-butylcatechol in 1:1 methanol:dichloromethane mixture to its corresponding quinone derivative in aerial oxygen. The reaction follows Michaelis–Menten enzymatic reaction kinetics with turnover number (K_{cat}) 30.40×10^3 h^{-1} .

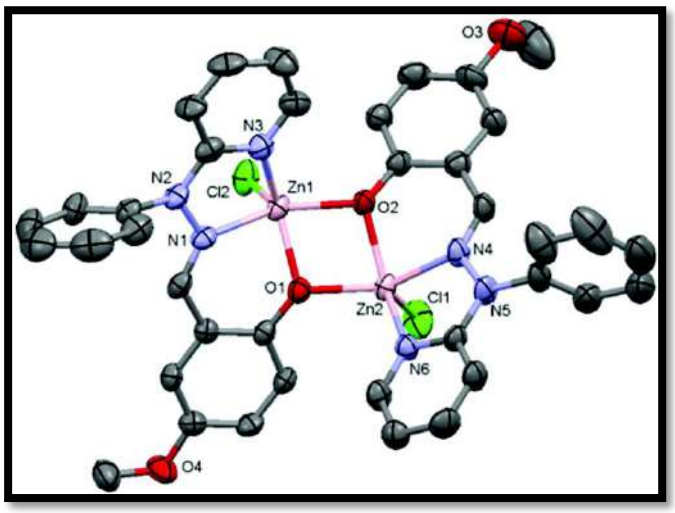


- **Amar Hens** in 2020 reported that aheptadentate N4O3 coordinating dinuclear zinc complex was synthesized and characterized by 1H NMR spectroscopy, IR spectroscopy and ESI MS spectroscopy studies. X-ray single crystal structure of the dinuclear complex revealed that both zinc atoms have pentacoordinated environment realized by the N2O2 donor set of ligand and one water molecule. The theoretical optimized structure of the dinuclear complex in solution phase was indicated a larger elongation take place in the bond distance between zinc and oxygen atom of coordinated water molecule which leads to come closer of two zinc atoms in solution phase in comparison of crystalline structure. This proximity of two zinc atoms fulfilled my aim for investigating the catalytic catecholase activity. The catecholase activity of the complex has been investigated under completely aerobic conditions in MeOH water medium at pH 8.0 against the model substrate 3,5-di-tert-butylcatechol (3,5-DTBC). Saturation kinetic studies have shown the order of conversion of substrate to product quinone. The mechanistic path of the oxidation process has been confirmed by UV–vis, CV and EPR spectral studies are made-up to be responsible for the oxidation of 3,5-DTBC. EPR experiment



suggested generation of radical in the presence of 3,5-DTBC and that finding has been strengthened by cyclic voltammetric study. Thus, it proposed that the radical pathway is responsible for conversion of 3,5-DTBC to 3,5-DTBQ promoted by complex of redox-innocent ZnII ion. The ligand-centered radical generation has been further verified by density functional theory calculation.

- In 2016**, Ghosh et al.** reported some diphenoxo bridged dinuclear zinc complexes and characterized spectroscopically and X-ray crystallographically. DFT and TD-DFT calculations were performed to optimize the molecular geometry, interpret the spectroscopic results and investigate the contribution of the ligands to the redox properties of the complexes. Phenoxyl radical complexes were generated in solution via chemical oxidation using ceric ammonium nitrate (CAN) and the redox properties were examined through cyclic voltammetric measurements. All the dinuclear zinc complexes were found to be highly active in catalyzing the oxidation of the primary alcohols 3,5-di-tert-butylcatechol and o-aminophenol. An EPR experiment clearly hinted at the generation of a radical during the oxidation of catechol and o-aminophenol. Reaction models for these processes were proposed and theoretical studies were performed to support the proposed mechanism. ESI-MS spectra clearly indicate the formation of a catalyst–substrate adduct by removal of one Cl− ion.



Conclusion

We have presented the biomimetic Zn based model complexes of Zn, discussed about their activity, collated the known mechanistic pathways involved during the catalysis and disseminated them based on the reports. It appears that almost all model complexes act by binding to the substrate and then oxidizing it while the catalyst gets reduced; this step is

followed by oxidation of the catalyst by molecular oxygen. The first step in the substrate oxidation, leads to the formation of radicals and the next step completes the product formation while the catalyst goes back to its oxidised state. Except for a few cases, most model systems show that the oxidation of catechol involves reactive oxygen species and $H_2O_2$is formed as a by-product. In contrast, the enzymatic pathway produces water instead of hydrogen peroxide, involving a four electron reduction of oxygen. Hence, most model complexes are capable of only performing a two electron reduction of dioxygen while oxidizing catechol to o-quinone. The knowledge of the oxidation mechanism of catechol learned through studies on the enzyme and design of biomimetic Zn model complexes led to the hypotheses that at times dinuclearZn^{II}complexes have a higher reaction rate then their mononuclear analogues. The literature data strongly suggests that many Zinc based complexes show quite higher activity. Many metal complexes have been designed to activate molecular oxygen with the objectives to develop bioinspired catalysts for oxidation reactions but in most of the cases the turn over numbers is low compared to enzyme. It should be borne in mind that nature has multiple constraints while designing a metalloenzyme viz. the designed enzyme has to function in cellular environment, it should have a metal that is available for uptake, it should not participate in undesirable reactions, the geometry of the protein active site hosting the metal should be such that the desired redox chemistry is feasible. In contrast in the laboratory while designing a catalyst we preferably are performing the reaction in a pot which does not have many of the above constraints faced by nature and hence we have more liberty to choose the metal and ligand with the basis, that the designed catalysts should function efficiently. In addition, probing the catalytic promiscuity based on the designed complex and the available literature knowledge also provides insight to the various possible mechanistic pathways that may be adapted by a model complex. Hence, designing the metal complex similar to the active site of the metalloenzyme may not always be needed if one's objective is only to carry out efficient transformation rather than following a pathway same as that of, or similar to the metalloenzyme for the desired reaction. Thus, to design efficient functional model systems of catechol oxidase (CO), ZnII/III has proven through various studies and reviews a good choice for laboratory or industrial purposes based on the activity of ZnII/III catalysts in literature.

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REVIEW ARTICLE

TOPIC: COBALT BASED PHENOXAZINONE OXIDASE: BIOMIMETIC FUNCTIONAL MODEL AND MECHANISTIC STUDIES

UNDER THE GUIDANCE OF: DR. ANANGAMOHAN PANJA

SUBMITTED BY: RAUSHNI KHATOON

CU ROLL NO.: 193013 – 11- 0103

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CERTIFICATE

This is to certify that RAUSHNI KHATOON (CU Roll No.: 193013-11-0103) has completed the review on the topic entitled **"COBALT BASED PHENOXAZINONE SYNTHASE: BIOMIMETIC FUNCTIONAL MODEL AND MECHANISTIC STUDIES".** This is record of an original and independent study carried out by her under supervision and guidance of **Dr. Anangamohan Panja**, Gokhale Memorial Girls' College, for the partial fulfillment of the B. Sc. Hons course in Calcutta University.This review nor any part of it has been submitted for either any degree/diploma or any academic award anywhere before.

All help received by his from various sources have been duly acknowledged.

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DISSERTATION: REVIEW REPORT

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Department Of Chemistry

UNDER THE GUIDANCE OF:

Dr. ANANGAMOHAN PANJA

TOPIC

PHENOXAZINONE SYNTHASE: BIOMIMETIC FUNCTIONAL MODELS ON IRON COMPLEXES.

SUBMITTED BY: AMREEN SOHAIL

CU ROLL NO.: 193013-11-0003

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GOKHALE MEMORIAL GIRLS' COLLEGE

DEPARTMENT OF CHEMISTRY

TO WHOM IT MAY CONCERN:

THIS IS TO CERTIFY THAT THIS DISSERTATION REVIEW ENTITLED FERROUS BASED CATECHOL OXIDASE: BIOMIMETIC FUNCTIONAL MODEL AND MECHANISTIC PATHWAY' SUBMITTED BY AMREEN SOHAIL (CU REG. NO.:013-1211-0239-19), DEPARTMENT OF CHEMISTRY OF GOKHALE MEMORIAL GIRLS' COLLEGE IS RECORD OF AN ORIGINAL AND INDEPENDENT STUDY CARRIED OUT BY HER UNDER THE SUPERVISION AND GUIDANCE OF **DR. ANANGAMOHAN PANJA**, GOKHALE MEMORIAL GIRLS' COLLEGE, AND THAT NEITHER THIS REVIEW NOR ANY PART OF IT HAS BEEN SUBMITTED FOR EITHER ANY DEGREE/DIPLOMA OR ANY ACADEMIC AWARD ANYWHERE BEFORE.

ALL HELP RECEIVED BY HER FROM VARIOUS SOURCES HAVE BEEN DULY ACKNOWLEDGED.

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PHENOXAZIONON SYNTHASE : BIOMIMETIC FUNCTIONALMODELS ON IRON COMPLEXES

CONTENT

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PHENOXAZIONON SYNTHASE : BIOMIMETIC FUNCTIONALMODELS ON IRON COMPLEXES

CONTENT

ABSTRACT:

The impetus to modelling of enzyme active sites comes from their potential to provide insight to the mechanistic pathways of the native enzymes, establishing the role of that particular metal in the active site and to design better catalysts inspired by nature. Most of the metalloenzyme are capable of activating molecular oxygen due to the presence of the metal ions. Among the various metalloenzymes, phenoxazinone synthase (PHS) is an enzyme that is of interest for its oxidizing ability to generate phenoxazinone. In this review we discuss the progress made so far in the area of Fe-based models on phenoxazionone synthase enzyme. The studies on phenoxazinone synthase are quite detailed and the mechanistic pathways reasonably well disseminated as discussed here.

Introduction :

1.1 Activation of molecular oxygen

Oxidation reactions are fundamentally important component of organic synthesis and play an important role in rendering the desired functionality to the intermediates of valuable compounds such as pharmaceuticals, agrochemicals, and other fine chemicals.[1-3] For economic and environmental reasons, the oxidation processes of bulk chemical industries predominantly involve the use of molecular oxygen as the primary oxidant[4-8] .The application of oxidation reactions in scaled-up synthesis is limited due to the use of heavy metals, thermal hazards, and moderate chemo selectivity for highly functionalized compounds in most oxidation reactions[9,10]. Classical oxidation methods with stoichiometric quantities of inorganic oxidants are toxic and enrich the environmental pollution. That is why oxidations using catalytic amount of activator which can activate molecular oxygen with minimum chemical waste is inspiring. The challenges faced to activate molecular oxygen for its use in oxidation reactions is due to its kinetically inert nature. However if the organic substrate gets converted to a radical then its reaction with oxygen is a spin-allowed process. Among the other possibilities, the orbital overlap of oxygen with a suitable metal ion may help its activation through electron transfer from the metal. Such organic co-factors have been reviewed elsewhere and are beyond the scope of this review [11,12]. A major problem while using dioxygen in chemical transformation is that its reactivity is not easily controlled and often may lead to low selectivity and over-oxidation[13]. Nature has evolved an elegant solution to overcome the kinetic barrier of dioxygen activation by using transition metal incorporated in proteins, the so called ‘metalloenzymes’[14-19].Inorganic chemists have largely exploited the concept of nature by designing oxygen activation catalysts which act as small molecule mimics of the metalloenzymes and help to understand the mechanistic pathways. Using the knowledge of co- ordination chemistry, redox potential and electronic factors,[14,20-25]the enzymes

donor sites are modelled with small molecule called ligands , which are then incorporated with metals to form complexes that are probed as structural and functional models. In general, the catalytic activity of most of the metal complexes is highly encouraging. Many of the complexes may seem not efficient but they undoubtedly are very useful in providing us with useful insight about the important mechanistic aspects of the metalloenzymes. Development of catalytic reactions with the perspective of understanding and mimicking the enzymatic functions of metalloenzymes has been the focus of bio-inorganic chemists during the past few decades. Other than understanding the possible mechanistic pathway of the enzyme through such mimics, a long term ideas to develop complexes which would be very useful as catalysts.

1.1 Nature's choice and role of biomimetics :

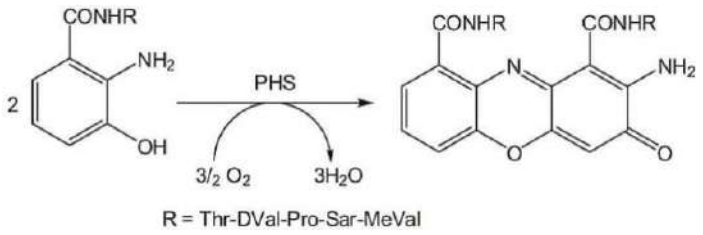
Nature uses several metalloenzymes to catalyze the controlled and selective oxidation of organic compounds. The geometry and structural feature of enzyme active sites and the choice of incorporated metals are very diverse and fully optimized to the function of the proteins or enzymes. In addition, it also takes into account the availability of the metal ion in environment. Establishing the correlation of the geometric and electronic structure with function is one of the main objectives of the bioinorganic chemists. The activation of dioxygen on metal sites requires the availability of different accessible redox states. Metalloenzymes capable of dioxygen activation consist mainly of enzymes with copper, iron or manganese active sites. A wide variety of different mono- or multinuclear iron and copper enzymes has been discovered and catalyzes major biological transformations [26,27]. The primary goal in designing mimics of metalloenzymes is to understand the structures of active sites and reactive intermediates and the mechanistic details of dioxygen activation and oxygenation reactions occurring at the active sites. Metalloenzymes use diverse active sites such as heme iron sites, mono and dinuclear non heme iron sites, mono and dinuclear

copper sites, a heteronuclear heme iron–copper site, and other metal sites to activate dioxygen[25,28,29]. Progress made so far in terms of understanding the mechanistic pathway of oxidative enzymes has been very encouraging.[30,31] The huge library of complexes available to us as model complexes of various enzymes shows that, in spite of sincere efforts, we have not obtained turnovers close to native enzymes but the dissemination of mechanistic pathways and introduction to alternative pathways has been possible. The works in this area that have appeared in the literature over the past few decades shows the impetus to understand the chemistry of these metalloenzymes. We have tried to take into account all the reports but it is not possible always to provide an exhaustive account of all related previous works.

Phenoxazinone synthase (PHS):

One of the well-known metalloenzymes is Phenoxazinone synthase (PHS). Active site structure and function of PHS is a pentanuclear copper containing enzyme that catalyzes the formation of the phenoxazinone chromophore during synthesis of Actinomycin D (Scheme 1). Actinomycin D is an aromatic heterocyclic natural product in which the 2-aminophenoxazinone chromophore is linked to two cyclic pentapeptides [32]. These classes of compounds are potent antineoplastic agents. Their clinical use, however, are limited to the treatment of choriocarcinoma,Wilms tumors, rhabdomyosarcoma,and Kaposi's sarcoma due to their high toxicity[33]. It has been shown that actinomycin binds to DNA by

intercalation of the phenoxazinone chromophore and that the cyclic pentapeptide lactone confers sequence specificity to adjacent GC base pairs. This interaction results in highly specific inhibition of DNA-dependent RNA synthesis [32,34]. In 1962, Katz and Weissback first isolated the enzyme, phenoxazinone synthase from Streptomyces antibiotus[35]. Later it was cloned and overproduced in Streptomyces liuidans and isolated in 100 mg quantities. The subunit molecular weight is 88000 daltons. The enzyme PHS, had also been isolated from S. antibioticus[35]. However, structure was unknown until in 2006, James P. Allen and Wilson A. Francisco reported the crystal structure of PHS from Streptomyces antibioticus. The structural data showed that each subunit of the hexamer contains five copper atoms and it confirms the presence of all three-type copper-binding motifs, as usually known for multicopper oxidases: one type 1 (blue), two type 2 (normal), and one binuclear type 3 centres. The fifth copper centre which is a type 2 copper, is located at a distance of 25A˚ from the blue copper and the other normal type 2 copper, and the requirement of five copper atoms for maximum activity suggest that the fifth copper atom is not merely advantageously bound but has a structural role as well. The hexameric form has been reported as the most active form of PHS [36] (Fig. 1). The high activity of the hexameric form is likely due to a number of factors viz. stabilization of hexameric form of the protein relative to the dimer, accessibility of the active site, geometry of the copper centres, and the availability of proper solvent channel.



The above factors play important role in the regulation of PHS activity. Four of the metal atoms are located primarily in domains 1 and 3. These copper atoms form one mononuclear type 1, one mononuclear type 2 and one binuclear type 3 copper centres, which is similar to that observed for other multicopper oxidases. The fifth metal atom is in the loop connecting domains 2 and 3 and represents a new cofactor not previously described in any other multicopper oxidases. On the basis of the coordination of the fifth copper, it was expected to be a type 2 centre. The type 1 copper centre, identified as Cu1, has four ligands (His524, His608, Cys603, and Met613) that are entirely from domain 3. The geometry around the Cu1 centre is distorted bipyramidal with a missing axial ligand which is common to type 1 copper. The Cu-ligand bond distances in PHS are similar to that observed in other multicopper oxidases. The type 1 copper centre is ca. 12.5A˚ away from Cu2 of the type 3 centre and is connected to the type 3 centre through a central bridging ligand that facilitates the transfer of electrons from the type 1 to the type 3 centres (Scheme 2). The bridging ligand however though modeled as OH, other ligands such as water or various anions would also be compatible with the electron density. Hence the type of bridging ligand is not unambiguous. The 3.88A˚ distance between the two Type-3 copper atoms, which are identified as Cu2 and Cu3, and the Cu2-X-Cu3 bond angle of 153.14◦ are comparable with other multicopper oxidases. The copper atom of the type 2 centre, identified as Cu4, is 3.63 and 3.86A˚ away from the Type-3 Cu2 and Cu3 copper atoms respectively. The two histidines (His161 and His527) coordinated to the Type-2 Cu is contributed by domain 1 and 3, respectively. The third ligand to the Cu4 is a water molecule.

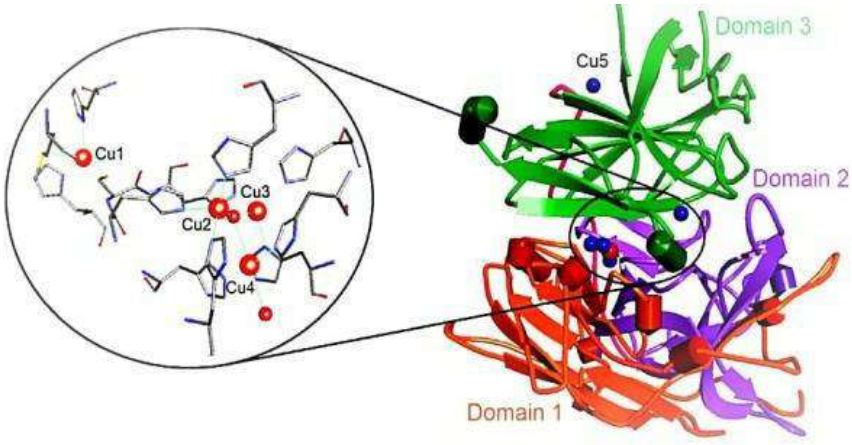


Fig. 1. Crystal structure of phenoxazinone synthase (right). Inset showing active site structure of PHS surrounding ligands.

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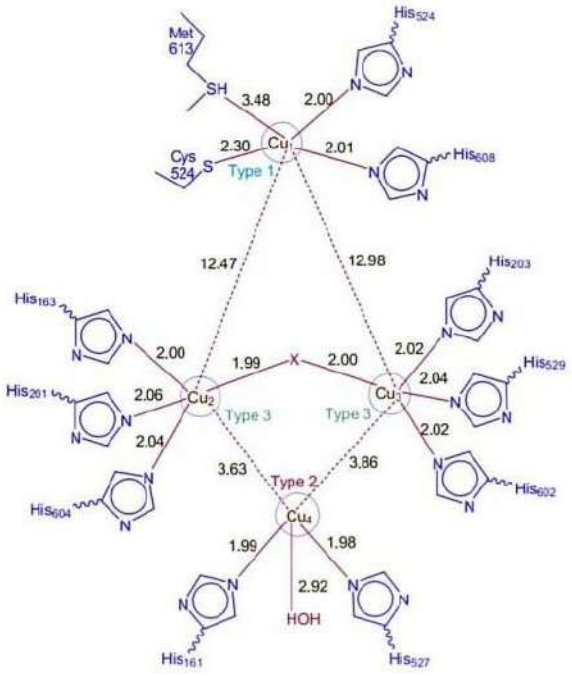


Scheme 2. Schematic diagram of four conserved copper atoms and their surrounding ligands together with the bonding and nonbonding distances in Å

2. Mechanism of the oxidation of OAP by Phenoxazinone Synthase (PHS):

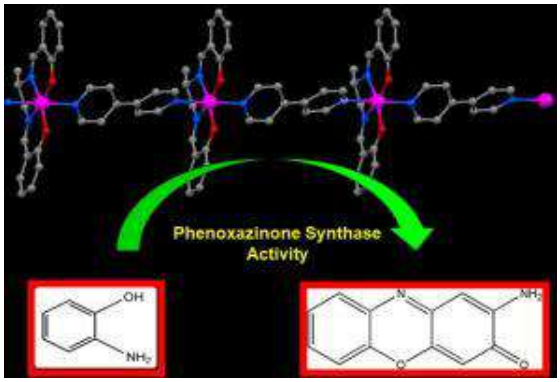
After the discovery of PHS in 1962 although the structure of the enzyme was unknown the

enzymatic catalysis and mechanistic studies of the phenoxazinone synthase (PHS) was initiated by Tadhg P. Begley and co-workers [37, 38] where they concluded that among four possible mechanistic pathways (Scheme 13) phenoxazinone synthesis proceeds via a quinine imine intermediate [I] that undergoes a conjugate addition, at the active site, with a second molecule of OAP. The resulting intermediate [II] then under-goes a 2- electron oxidation to the p-quinoneimine [III] (Scheme 13).This reaction occurs, in part, at the active site. A second conjugate addition followed by a final 2-electron oxidation gives the phenoxazinone chromophore.They proposed that both of these steps occur outside the active site, and the phenoxazinone synthesis occurs via a cascade of three consecutive 2-electron aminophenol oxidations in which the2- aminophenol (OAP) functionality is regenerated, after each conjugate addition, by a facile tautomerization reaction. In 1993, Villafranca et al. showed that the enzyme requires 4-5 copper atoms/monomer for full catalytic activity and additional copper inhibits the enzyme activity [39]. Their spectroscopic studies indicate the presence of three functional copper atoms that can accept electrons from substrate and two additional copper atoms with unidentified functional behavior. It is strange to find that although the enzyme was isolated by more than one research group, no detailed kinetic study to determine the turn over number of the enzyme have been performed.

Fe- based model systems of phenoxazinone synthase (PHS)

Sourav Chatterjee and his team reported a new 4,4′-bipyridine (4,4′-byp) mediated 1D- polymeric Fe^{III} complex (complex **1**) of Schiff base ligand H_2L, a 1:2 condensation product of 1,2-diaminopropane and salicylaldehyde, has been synthesized. Complex **1** is structurally characterized by single crystal X-ray diffraction. A phenoxo bridged dinuclear Fe^{III} complex (complex **2**) of analogous ligand has been synthesized also. Dioxygen activation in terms of Phenoxazinone synthase activity using *o*-aminophenol (OAPH) as a model substrate catalyzed by both the complexes are thoroughly investigated here. ESI-MS spectral study reveals that polynuclear complex **1** dissociates into mononuclear units while dissolve in methanol during catalytic study. The kinetic study illustrates that both the complexes have well competence towards *o*-aminophenol oxidation where dinuclear Fe^{III} species demonstrate higher activity than mononuclear intermediate species. Important finding from the mass spectral and electrochemical study provide significant information of the mechanistic pathway of the functioning phenoxazinone synthase like

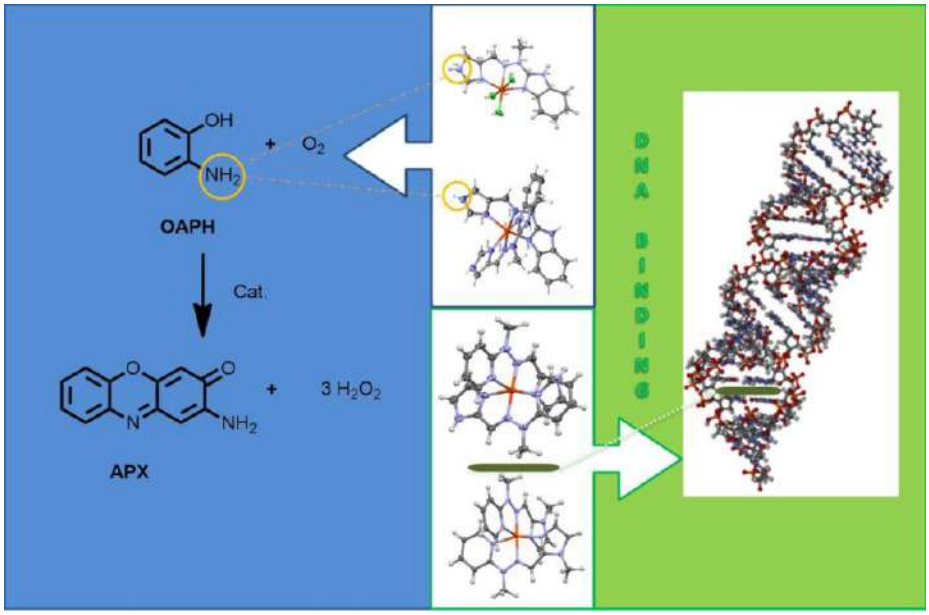
activity of synthesized iron complexes.



Sekar Indira and his team reported a new class of aminophenol ligands, bis(5-(*tert*-butyl)-2-hydroxybenzyl)glycine (H_3L^1) and bis(2-hydroxy-5-methylbenzyl)glycine (H_3L^2) were synthesized by the Mannich reaction. The mixed ligand mononuclear iron(III) and cobalt(III) complexes have been synthesized by using these ligands and bipy/phen. All the complexes were characterized by IR, UV, EPR and mass spectral analysis. In the electronic spectra, the higher electron donating nature of the p-substituent $-C(CH_3)_3$ (H_3L^1) compared with the $-CH_3$ (H_3L^2) of the phenolic ring causes a bathochromic shift in the LMCT-charge transfer band. EPR studies revealed the paramagnetic nature of the complexes. Cyclic voltammograms showed three characteristic reduction peaks for iron(III) complexes and two reduction peaks for cobalt(III) complexes in the cathodic direction. There is an anodic shift in the reduction of the metal centre when the electron donating nature of the p-substituent of the phenolic ring decreases or the flexibility of α-diimine ligands increases.

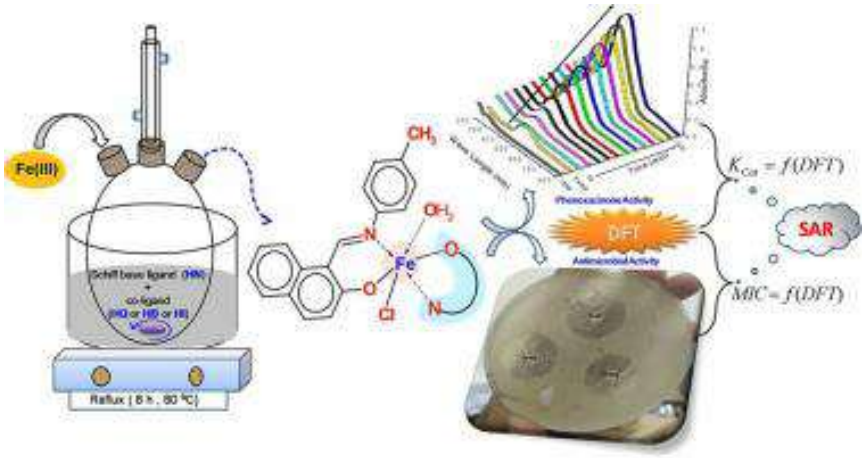
The synthesized complexes were effectively used as models for mimicking the activities of catechol oxidase and phenoxazinone synthase. The catalytic activity of the complexes are compared and discussed on the basis of the p-substituent of the phenolic ring and the flexibility of the exogenous ligands. The structural properties of the complexes are also discussed by using molecular modeling studies.

Elucidation of the structure and function of biomolecules provides us knowledge that can be transferred into the generation of new materials and eventually applications in e.g., catalysis or bioassays. The main problems, however, concern the complexity of the natural systems and their limited availability, which necessitates utilization of simple biomimetic analogues that are, to a certain degree, similar in terms of structure and thus behaviour. We have, therefore, devised a small library of six tridentate *N*-heterocyclic coordinating agents (L^1–L^6), which, upon complexation, form two groups of artificial, monometallic non-heme iron species. Utilization of iron(III) chloride leads to the formation of the 1:1 ($Fe:L_n$) 'open' complexes, whereas iron(II) trifluoromethanosulfonate allows for the synthesis of 1:2 ($M:L_n$) 'closed' systems. The structural differences between the individual complexes are a result of the information encoded within the metallic centre and the chosen counterion, whereas the organic scaffold influences the observed properties. Indeed, the number and nature of the external hydrogen bond donors coming from the presence of (benz)imidazole moieties in the ligand framework are responsible for the observed biological behaviour in terms of mimicking *phenoxazinone synthase* activity and interaction with DNA



Ankur Maji, Anshu Singh, Udai P. Singh, Kaushik Ghosh reported that the tridentate ligands ^{H}PhimpH, ^{OCH3}PhimpH, ^{CH3}PhimpH, ^{tBu}PhimpH, and ^{NO2}PhimpH have been synthesized and characterized. These tridentate ligands having non-innocent phenolato function, N_{py} and N_{im} donors upon deprotonation bind to iron(III) center resulting in a series of novel iron complexes, Complexes were characterized by elemental analysis, IR, and UV−visible, and electrospray ionization mass spectral (ESI−MS) studies. Molecular structure of complex **2** was determined by single-crystal X-ray diffraction study. Electrochemical studies depicted Fe(III)/Fe(II) couple in the range of −0.50 to −0.65 V versus Ag/AgCl. Theoretical calculation using density functional theory (DFT) was also performed to optimize the geometrical and structural parameters.

Mohamed Ismael and his team reported that new Fe(III) complexes with mixed ligands based on 1-{(E)-[(4-methylphenyl)imino]methyl}-2-naphthol (HN) as primary ligand and secondary co-ligand of O-hydroxy quinolone (HQ), 2-(1H-benzimidazol-2-yl)phenol (HB) and 2-(4,5-diphenyl-1H-imidazol-2-yl)phenol (HI) had been isolated and characterized. The isolated complexes had 1:1:1 ratio for Fe(III):ligand:co-ligand with one chloride and one water molecule coordinated to the Fe-centre, suggesting octahedral structure around the Fe-center with the formula [Fe(Ligand)(co-ligand)(Cl)(H_2O)]. Theoretical calculations using density functional theory by B3LYP with LANL2DZ basis set had been done for two possible orientations of the ligand moieties around the Fe-center, to find out the most reliable coordination modes. Calculations include geometry optimization, molecular orbital description, and energy evaluation of trans- and cis-coordination modes of the chloride and water around the Fe-center.



Abd El-Motaleb M. Ramadan a new series of Mn^{II} and Fe^{III} chelates containing pyridine and phenolate-based ligands was synthesized by treating the metal salt in ethanol with the Schiff-bases, which were formed in situ. Analytical (C, H, N, and M), thermal analysis (thermogravimetric analysis & differential thermogravimetric), and spectroscopic techniques (IR, UV–Vis, ESR) in addition to molar conductance and magnetic measurements were employed to characterize the prepared metal chelates. Mono-nuclear metal complexes, with N_5 donor containing pentadentate Schiff-base ligands were obtained. In addition, two dibridged homobinuclear iron(III) complexes with N_4O donors containing phenolate-based ligand were successfully isolated. Six-coordinate complex species in an octahedral environment is proposed for both Mn^{II} and Fe^{III} metal complexes. The density function theory study was used to optimize the geometrical shapes of three of the existing metal complexes and to identify some global reactivity descriptors.

5.CONCLUSION:

We have presented the biomimetic model complexes of IRON (phs) discussed about their activity, collated the known mechanistic pathways involved during the catalysis and disseminated thembased on the reports.It appears that almost all model complexes actby

binding to the substrate and then oxidizing it while the catalystgets reduced; this step is followed by oxidation of the catalyst bimolecular oxygenVarious metal ions would exhibit different redox properties with same ligand due to differences in their electronic properties.Hence the change in metal ion requires tuning of ligand if the redoxpotential of the metal centre is to be kept in range for a certain catalytic

process. This might be a possible explanation as to why thereplacement of the metals in the same protein may not give efficient catalytic activity. Many metal complexes have been designedto activate molecular oxygen with the objectives to develop bio inspired catalysts for oxidation reactions but in most of the casesthe turn over numbers are low compared to enzyme. It should beborne in mind that nature has multiple constraints while designinga metalloenzyme viz. the designed enzyme has to function in cellular environment, it should have a metal that is available for uptake it should not participate in undesirable reactions, the geometry ofthe protein active site hosting the metal should be such that thedesired redox chemistry is feasible.

In contrast in the laboratorywhile designing a catalyst we preferably are performing the reaction in a pot which does not have many of the above constraintsfaced by nature and hence we have more liberty to choose the metal and ligand with the basis, that the designed catalysts should func-tion efficiently. In addition probing the catalytic promiscuity basedon the designed complex and the available literature knowledgealso provides insight to the various possible

mechanistic pathwaysthat may be adapted by a model complex. Hence, designing themetal complex similar to the active site of the metalloenzyme maynot always be needed if ones objective is only to carry out efficienttransformation rather than following a pathway same as that of,or similar to the metalloenzyme for the desired reaction. Thus todesign efficient functional model systems of phenoxazinonesynthase(PHS), Mn(II/III)might be the metal ofchoice for laboratory or industrial purposes based on the activity ofMn(II/III)catalysts in literature, including our own work.

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