Coordination Chemistry of Life Processes: Bioinorganic Chemistry

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Many enzymes and proteins have metal ions at their active sites that play key roles in catalysis. An important goal of an interdisciplinary field like bioinorganic chemistry is the development of small inorganic coordination complexes that not only reproduce structural and spectroscopic features, but also function in a manner similar to their natural counterparts. To highlight this burgeoning field, some selected results on synthetic modelling of (i) the electronic structure of manganese cluster of photosystem II and (ii) functional modelling of the dinuclear copper enzyme tyrosinase are described in this article.

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Preamble

A Chakravorty has portrayed the life of Alfred Werner, the inventor of coordination theory, in this issue. Needless to say that today's inorganic chemistry research centres around Werner's coordination theory. Therefore it would be most appropriate to highlight how the discovery of structure and bonding of coordination compounds finds its expression in enriching our understanding of the inorganic chemistry of life processes.

It is now well known that many inorganic elements and their compounds are essential or beneficial for life on earth. Organic compounds are of course essential, because they provide organisms with such essential compounds as proteins, nucleotides, carbohydrates, vitamins and so forth. Inorganic compounds, particularly metallic ions and complexes, are essential cofactors in a variety of enzymes and proteins. They provide essential services that cannot be or can only poorly be rendered by organic compounds. *The roles played by essential inorganic elements and compounds are (i) structural, (ii) carrying and transporting electrons and oxygen,*

Suggested Reading

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(iii) catalytic roles in oxidation-reduction (including oxygenation) reactions and (iv) catalytic roles in acid-base and other reactions. It has also been known for a long time that excesses of these *elements* can be very dangerous. In fact, a narrow concentration window exists for most of the so-called trace elements.

Bioinorganic chemistry is a leading discipline at the interface of chemistry and biology. The field is undergoing a phase of explosive growth, partly because of exposure and insights obtained by increasing numbers of metalloenzyme X-ray structures. There are three major avenues of investigation in bioinorganic chemistry. The first involves direct study of the structure and function of 'metallobiomolecules', an area which is traditionally that of a biochemist. The second major avenue involves an indirect approach, commonly the domain of the inorganic or organic chemists. The third involves the addition of metal ions or complexes as probes to biochemical structure and function.

Similarity with Werner-type Coordination Complexes

Intuitively, one can anticipate that the behaviour of metal ions in proteins cannot be vastly different from that governed by the fundamental chemistry of the particular metal. *The synthetic analogue approach*, the primary focus of this article, is based on the premise that the chemistry of the metal-binding site ('active site') is dependent, for the most part, on the immediate coordination environment of the metal ion. For most metalloproteins, the immediate coordination environment consists of donors from the side-chains of amino acids. Sometimes, a prosthetic group (e.g., a porphyrin ring) completes the coordination sphere of the metal ion. Thus it could be generalised that the 'metallo-biomolecules' are highly elaborated coordination complexes whose metal-containing sites (coordination units), comprising one or more metal atoms and their ligands, are usually the loci of electron transfer, binding of exogenous molecules and catalysis. Two major factors control the properties of metal ions in biological systems: (i) the stereochemistry of the metal site and the nature of the ligands

attached to the metal and (ii) the protein environment, which plays a crucial role in controlling the reactivity of the metal site. In some cases the protein can force metal ions into unusual geometries; the protein environment may be the determining factor controlling the activity of the increasing number of functionally distinct metalloproteins that have essentially identical metal centres. From the aforesaid discussion, it is understandable that inorganic chemists can contribute considerably to the understanding of the structural, electronic and mechanistic aspects of metal ions in metalloproteins, by synthesizing small coordination compounds, which mimic the specific properties of those metal sites. These synthetic models are usually intended to serve as stereochemical and electronic analogues of these sites and have the substantial advantage of being amenable to characterization at a very high level of detail. In fact, these synthetic models could be used to investigate the effects of systematic variations in coordination geometry, ligand, local environment and other factors. Simultaneous attainment of biological structure and function in a synthetic system has proven more difficult. The problem becomes more demanding when catalysis is involved. The purpose of models is not necessarily to duplicate natural properties but to sharpen or focus certain questions. A synergistic approach (*Figure* 1) to the study of metalloproteins can and has provided insights that cannot be easily attained from protein studies.

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In this article, the present day views on the functioning of two metalloenzymes, *photosystem II* and *tyrosinase*, are discussed. The important concepts of coordination chemistry have been pointed out. Many unfulfilled goals and current attempts to chemically duplicate such properties are also highlighted.

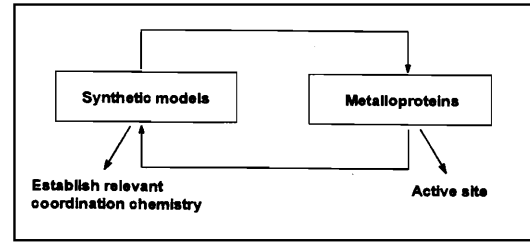

Figure 1. The synergistic relationship between studies involving metalloprotein biochemistry and inorganic modelling.

Photosynthesis

Human beings require reduced organic compounds – carbohydrates, fats and protein – as a food source. Stored in these molecules is a great amount of useful energy. Humans can tap the energy from these food sources and can use it to

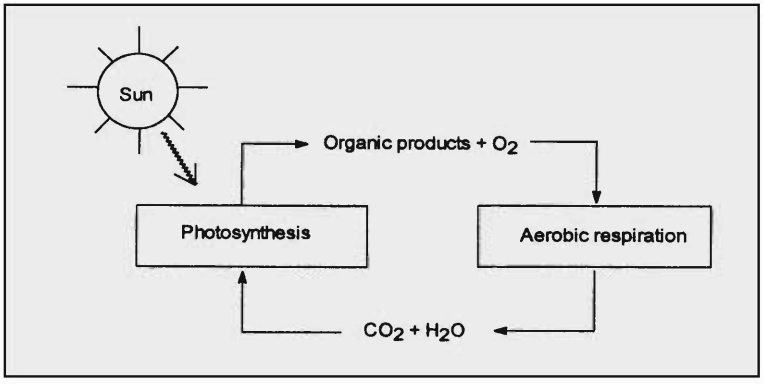

Figure 2. A cycle of dioxygen metabolism that is critical to both plant and animal life on earth.

build up their own complex molecules, to move about, to sense the environment and to keep warm. Without this input of energy, they would soon die. Fortunately, this external source of energy does grow on trees and elsewhere. However, plants do not ingest large fuel molecules themselves, but are capable of synthesizing glucose and other such molecules from CO_2 and H_2O, which are waste products of animal metabolism. However, a large increase in free energy (a positive ΔG) is required for the production of glucose and oxygen. This endergonic process is accomplished through the use of an external energy source – sunlight. Plants release O_2 as a by-product of the splitting of water (*Figure* 2).

Photosynthesis is the system of reactions by which green plants, blue-green algae and some cyanobacteria capture solar energy, convert it to chemical energy and use this energy in the reduction of CO_2 to carbohydrates. Most of the oxygen in the atmosphere, which supports aerobic life on earth, is generated by the photo-induced oxidation of water to dioxygen:

$$2H_2O \rightarrow O_2 + 4e^- + 4H^+. \quad (1)$$

Through photosynthesis, energy-poor compounds such as CO_2 and H_2O are converted to energy-rich compounds, carbohydrates and O_2. The overall process in photosynthesis is expressed as

$$6CO_2 + 6H_2O + \text{light} \rightarrow C_6H_{12}O_6\,(\text{glucose}) + 6O_2. \quad (2)$$

Photosynthesis can be divided into three steps:

1) Light collection via chlorophyll and other pigments and con-

veying the energy to a *reaction centre.*

2) The oxidation of H_2O to O_2 leads to a series of reactions that generate biological energy in the form of ATP and the reduction of $NADP^+$ to NADPH. The overall equation is

$$NADP^+ + H_2O + \text{light} \rightarrow H^+ + O_2 + ATP + NADPH\ [\text{reductant}]$$

3) The absorption of CO_2, oxidation of NADPH and formation of carbohydrate:

$$CO_2 + NADPH + ATP \rightarrow (CH_2O)\ [\text{'proto-carbohydrate'}]$$

The absorption of light energy by photosynthetic pigments, the transfer of energy among pigment molecules and the stabilization of the energy by charge separation are often referred to as the 'light reactions' of photosynthesis. The products of the light reactions, stored as reducing power and as chemical energy in the form of the compounds NADPH and ATP, respectively, are used in the reduction or fixation of the carbon source, carbon dioxide. The biochemical processes involved in carbon dioxide fixation (Calvin Cycle) are not directly light dependent and are called 'dark reactions'. However, this stage uses a series of enzymes and these enzymes form a cycle in which they take up CO_2, make carbon compounds for use elsewhere in the plant and supply the materials needed for the cycle to restart.

The absorption of light energy by photosynthetic pigments, the transfer of energy among pigment molecules and the stabilization of the energy by charge separation are often referred to as the 'light reactions' of photosynthesis.

A reaction centre is a protein complex that contains a precisely positioned collection of redox centres that function to convert the photon energy into redox free energy. Two reaction centres operate in series to oxidise water and reduce $NADP^+$. PS I receives electrons from PS II, which are subsequently used to reduce $NADP^+$. The function of PS II is to oxidise water.

The OEC, present in PS II of the photosynthetic apparatus, has a manganese-containing active site, which can oxidise coordinated water molecules to dioxygen.[1] In fact, the OEC is made up of a redox-active tyrosine and a tetranuclear manganese cluster that binds substrate water and accumulates oxidising equivalents. The

[1]PS I: photosystem I; PS II: photosystem II; OEC: oxygen-evolving complex; EXAFS: extended X-ray absorption fine structure; EPR: electron paramagnetic resonance; XANES: X-ray absorption near edge structure; HSAB: hard and soft acid-base.

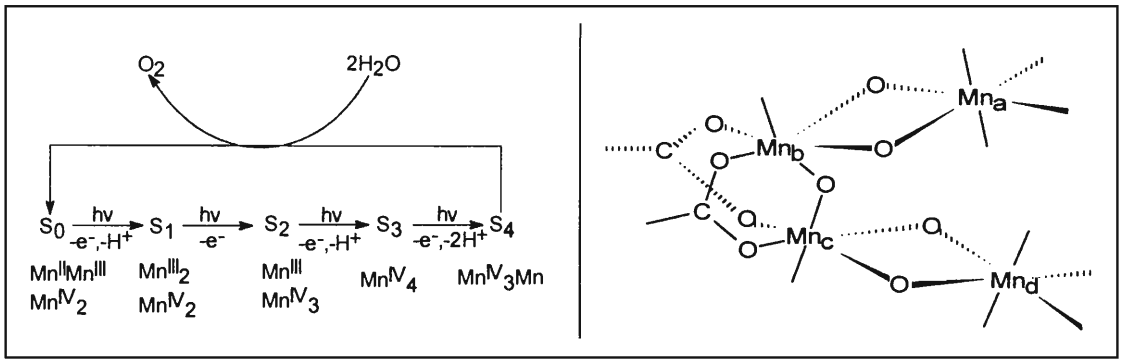

Figure 3(left). The S-State scheme for the oxidation of water to dioxygen. The Mn oxidation states (based on X-ray absorption studies) in the tetranuclear manganese cluster of PSII are indicated.

Figure 4(right). Klein's model for the OEC (the distances between Mn_a and Mn_b or Mn_c and Mn_d are ~ 2.7 A and the distance between Mn_b and Mn_c is ~ 3.2 A).

OEC has been shown to cycle through the 'so-called' S-states (*Figure* 3). The S-state index refers to the number of oxidising equivalents stored. Each S-state advance is associated with light-induced charge separation at the chlorophyll-containing pigment P680 (primary electron donor) to form the strong oxidant $P680^+$. Upon photoexcitation of P680, a series of electron transfer reactions between cofactors within the protein takes place. It should be noted that O_2 is released on the transition from S_3 to S_4 to S_0. The tyrosyl radical, formed upon reduction of $P680^+$ oxidises the manganese cluster which, in turn, is reduced by electrons stripped from water. PS II turns over rapidly (up to 50 molecules of O_2 released per second) in spite of having to protect itself from photochemical oxidative damage.

Structural information about PS II is still limited by the lack of suitable crystals, but biochemical, spectroscopic and kinetic studies have provided considerable insight into the catalytic centre and its mechanism. According to EXAFS experiments, Klein and others proposed a tetranuclear structural model for the OEC in PS II, involving two bis(μ-oxo)dimanganese units linked by μ-oxo bis(μ-carboxylato) bridges (*Figure* 4). Most interestingly, the S_2-state is characterized by a multi-line EPR signal which arises from an $S = ½$ ground state of the manganese tetramer. A combination of EPR and XANES data have led to the assignment of S_2 as $Mn^{III}Mn^{IV}{}_3$ although $Mn^{III}{}_3Mn^{IV}$ cannot be ruled out. This background information has set the stage ready for innovative synthetic modelling of such a di/tetranuclear oxomanganese cluster.

Synthetic Modelling of Manganese Cluster of PS II

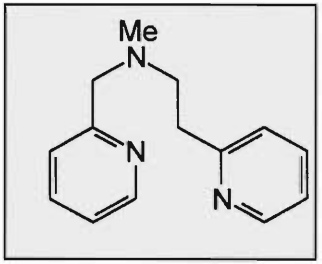

Figure 5. Structure of the tridentate ligand.

Using a common facially capping tridentate ligand, with chelate ring asymmetry (*Figure* 5), dimanganese complexes with three oxidation levels $Mn^{III}Mn^{III}$, $Mn^{III}Mn^{IV}$ and $Mn^{IV}Mn^{IV}$ have been prepared for the first time and all the forms structurally characterized. These simple coordination compounds represent important models for the active site of OEC in PSII. While the $Mn^{III}Mn^{III}$ compound has a μ-oxo-bis-μ-carboxylate bridge, the $Mn^{III}Mn^{IV}$ and $Mn^{IV}Mn^{IV}$ compounds have a bis(μ-oxo) μ-carboxylate bridge (*Figure* 6). However, the number of bridging oxo/acetate groups is one of the determining factors in achieving a desired oxidation state of manganese. Increasing the number of oxo groups from one to two and concomitantly decreasing the number of acetate groups from two to one stabilises the manganese oxidation levels from (III,III) to (III,IV) or (IV,IV). Based on the HSAB principle, a fine-tuning of the number of oxo/acetato-bridging groups as a function of metal oxidation state is understandable. The $\{Mn^{III}_2(\mu\text{-O})(\mu\text{-OAc})_2\}^{2+}$ core structurally mimics the Mn...Mn separation of 3.2 Å and the $\{Mn^{III}Mn^{IV}(\mu\text{-O})_2(\mu\text{-OAc})\}^{2+}$ and $\{Mn^{IV}Mn^{IV}(\mu\text{-O})_2(\mu\text{-OAc})\}^{3+}$ cores mimic the Mn...Mn separation of 2.7 Å of the Klein model. Additionally, these three complexes model the successive electron-transfer reactions from Mn^{III}_2 to $Mn^{III}Mn^{IV}$ to Mn^{IV}_2 oxidation levels, implicated to be operative during change in the *S*-states (*Figure* 4). Facile core interconversion among these three structures is of great importance to the understanding of the functioning of PS II. The mixed-valence compound exhibits an EPR spectrum reminiscent of the S_2-state of OEC.

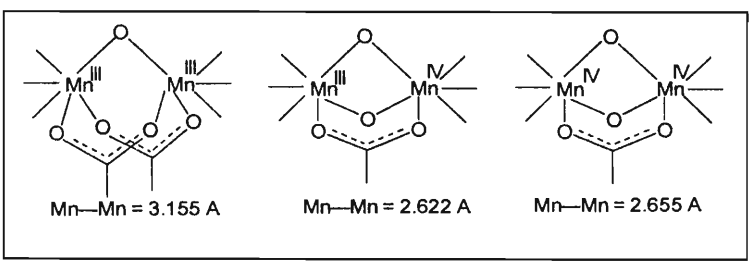

Figure 6. Structures of the bridged $Mn^{III}Mn^{III}$, $Mn^{III}Mn^{IV}$ and $Mn^{IV}Mn^{IV}$ compounds with relevant Mn...Mn distances (Lal and others).

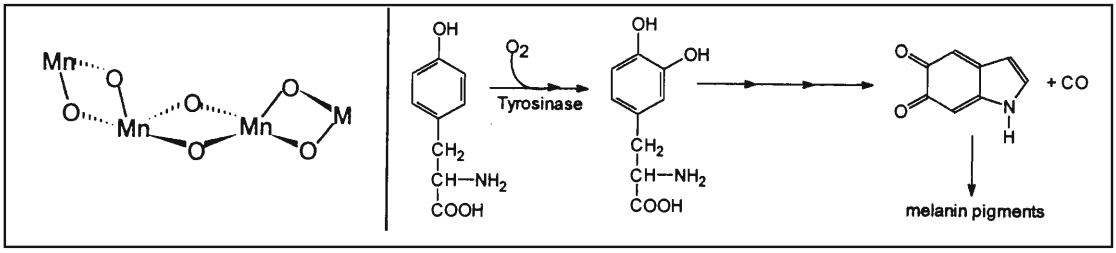

Figure 7(left). Structural core of the new tetrameric Mn compound (Blondin and others).

Figure 8(right). The role of tyrosinase in tyrosine metabolism in mammalian cells.

A major breakthrough has occurred in the understanding of the EPR spectral feature of the S_2-state of OEC by the successful generation of a mixed-valence tetrameric Mn compound (*Figure* 7). In fact, the EPR signal of $[Mn_4O_6(bipy)_6]^{3+}$ (bipy = 2, 2'-bipyridine) is the closest match from a model complex to the S_2-state multiline EPR signal of the OEC seen thus far.

It should be mentioned here that even though there has been an explosion of reports of syntheses of a large number of di-/tetrameric Mn clusters providing oxo-bridged structures, we are still far from any reasonable functional model capable of addressing the basic question of how water gets oxidised to molecular oxygen during photosynthesis!

Tyrosinase

All of us are aware of the fact that when potatoes, apples, bananas, sweet potatoes or mushrooms are injured they turn brown. This is due to the conversion of tyrosine to the pigment melanin, by the sequence of reactions shown in *Figure* 8. The same process causes skin tanning, following exposure to ultraviolet radiation. The enzymatic reactions are catalysed by tyrosinase, a *copper*-containing enzyme. The enzyme is present in the interior of the plant material and since the reaction requires molecular oxygen, the pigmentation does not occur until the interior is exposed. Tyrosinase catalyses (i) the *o*-hydroxylation of monophenols to *o*-diphenols and the further oxidation of these to *o*-quinones (*Figure* 8). These quinones undergo further enzymatic and non-enzymatic reactions that lead to polymeric pigmented material. In animals, these reactions give skin, eyes and hair their distinctive pigmentation. In order to deduce the structures and mecha-

nism of action of the protein-active sites, a major focus of research has utilised the biomimetic approach. In this section, studies done in modelling the dicopper centre in tyrosinase have been highlighted.

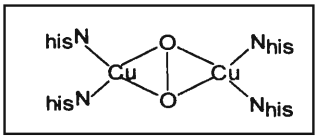

Figure 9. The Cu_2O_2 coordination (an additional histidine ligation at each terminal is not shown for clarity) unit in oxygenated arthropod hemocyanin (based on the structure of the model complex of Kitajima and others).

The interplay between model and protein biophysical studies has provided considerable insight into Cu-O_2 chemistry occurring in O_2 carrier protein hemocyanin and tyrosinase activity. Based on the reactivity of molecular oxygen with dicopper(I) complexes of designed ligands, considerable progress has been made in the chemical modelling of tyrosinase. Such efforts led to a triumph in biomimetic chemistry because the true O_2 binding mode occurring in oxy-hemocyanin had not been considered as a possibility until a synthetic analogue revealed the actual Cu_2-O_2 coordination (*Figure 9*). Comparisons of chemical and spectroscopic properties of tyrosinase and its derivatives with those of hemocyanin, whose crystal structures in both deoxy and oxy forms have been determined, establish a close similarity of the active sites structures in these two proteins. The active site of tyrosinase apparently has greater accessibility to exogenous ligands, including substrate molecules compared to that of hemocyanin. The similarity of the oxy-states of hemocyanin and tyrosinase point to the probable close relationship between the binding of dioxygen and the ability to activate it for incorporation into organic substrates.

Synthetic Modelling of Tyrosinase

Owing to the differing stereochemical preferences of five-coordinate Cu^{II} (square pyramidal, trigonal bipyramidal or intermediate between these two geometries) relative to Cu^{I} (tetrahedral or pyramidal) ready interconversions of these two oxidation states is expected to be facilitated by use of flexible ligands which can adjust their coordination geometry to the differing demands of the two oxidation states. Keeping this basic coordination geometry of copper in mind, good model systems for tyrosinase-like mono-oxygenase activity (C-H activation incorporating one O atom from O_2 into a substrate) have been developed (*Figure* 10). As in an enzyme active site, the peroxo group (a highly reactive

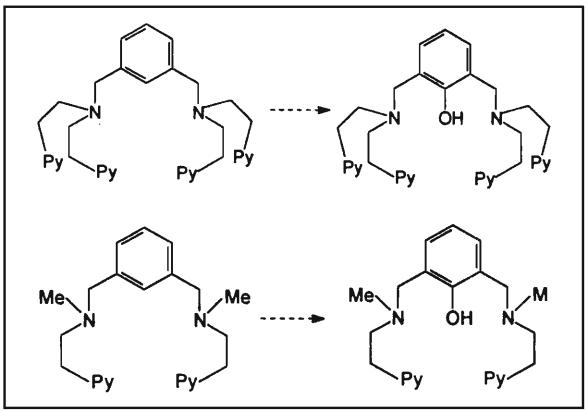

Figure 10. Dicopper-mediated hydroxylation of m-xylyl rings: (upper transformation) Karlin and others; (lower transformation) Ghosh and others.

intermediate formed due to reaction between dicopper(I) complex of the chosen ligand and O_2; *Figure* 9) is located in a highly favourable proximity to the xylyl ligand substrate and facile hydroxylation occurs by electrophilic attack on the arene substrate πsystem. These xylyl hydroxylation model systems serve as a functional mimic for tyrosinase, revealing how a Cu_2 centre can activate O_2 for hydrocarbon oxidation under mild conditions.

Concluding Remarks

Much effort has been devoted over the last 10 years to designing di- and multinuclear μ-oxo-bridged manganese complexes in order to mimic the structure, magnetic and spectral properties of this natural active centre (OEC). In contrast, few studies concerning the *reactivities* of such molecular models toward water oxidation have been published. To answer these questions, new model complexes with terminal $Mn^V=O$ moiety will certainly help and are valuable synthetic targets. Despite much effort, faithful examples of catalytically functional models are rare, especially when O_2 is used as the oxidant. It should be appreciated that recent efforts have emphasized *functional models* dealing with the mimicry of enzyme-related chemical processes or transformations. These are simple examples on the discovery and characterisation of synthetically derived coordination complexes that can bind and activate molecular oxygen. As far as the chemical modelling of tyrosinase is concerned a few good functional models are now available. However, these systems function in a stoichiometric manner. New synthetic models are sought for to demonstrate aromatic hydroxylation of externally added phenols. Such studies are still in their infancy.

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In research, you must remember not to fool yourself, for you are the easiest person to fool.

Richard Phillips Feynman