PREFACE

Inorganic biochemistry is an emerging subject and interdisciplinary field between inorganic chemistry and biochemistry. The task of inorganic biochemistry is to research the interaction between the metal complex (or metal ion) with biological ligand at the molecular level. There exists wide research about inorganic biochemistry such as biological macromolecular, oxygen carrier, hemoglobin, mimic enzyme, DNA-binding and cytotoxicity, biological mineralization, etc. Encompassing the varied and disparate roles of metal ions in biology, the field of bioinorganic chemistry has reached a certain level of maturity. This book provides leading research from around the globe in this field.

Expert Commentary - Since the introduction of solid-phase synthesis, a variety of alternative methodologies have been proposed and demonstrated to be viable for the quick and efficient removal of catalysts and/or reagents from the reaction mixture, avoiding the use of chromatographic techniques for the purification of the desired products. These alternative methodologies often overcome the disadvantages of crosslinked, insoluble polymer beads, which, as a consequence of their insolubility and their being necessarily heterogeneous in the reaction mixture, do have operational drawbacks. Enzymatic methods have opened up advantageous alternatives to classical chemical techniques since enzyme-catalyzed transformations often proceed under very mild conditions and very high selectivities. Stability limitations of the biocatalyst in unnatural conditions (i.e. organic solvents, high temperatures) have been avoided by the use of immobilization-stabilization techniques. The authors comment here, with recent examples, on the use of crosslinked beads or linear macromolecules, in combination with biocatalyst, either immobilized or in their free form, on their advantages and drawbacks.

Chapter 1 - Artificial nucleases, exerting new types of function and specificity and/or higher activity than their native counterparts are expected to be important future tools of modern genetics. Several strategies have been applied to develop such new proteins. Among these, the most well-known ones are the mutation of the wild-type enzymes, the directed evolution method and the construction of chimeric enzymes. Since metal ions often take part in hydrolytic catalysis in nature, bioinorganic research has also addressed efforts toward this field. Large number of model coordination compounds has been tested against activated and natural macromolecular substrates, and were found to be efficient catalysts. From these studies the authors learned about the basic role of, and the requirements against, metal ions. In spite of the similarity between proteins and peptides, only in rare occasions latter compounds were applied in these model studies. The reason for this is that the peptides with smaller

numbers of amino acids either coordinated metal ions through their deprotonated amide groups [platinum (II), palladium (II), copper (II)], or the hydrolysis of the metal ions occurred at physiological pH [zinc (II)]. In addition, such peptides can't form stable backbone conformation to allow preformed binding sites for metal ions. All these problems can, however, be overcome by designing suitably embellished oligopeptides of 20-40 amino acids, the metal ion complexes of which may function either as a catalytic centre or as a recognition domain of an artificial enzyme. Peptide fragments also possess the advantage that they can easily be inserted in the amino acid sequence of a protein by means of recombinant DNA technology, resulting in a new family of chimeric enzymes. Recently, the metal ion binding, function and the possibility of application in biology of such oligopeptides were extensively studied. The present review collecting and summarizing the results of these trials is intended to provide help researchers to navigate within the available chemical and biological information and to further develop this exciting field of bioinorganic chemistry.

Chapter 2 - Inorganic biochemistry is an emerging subject and interdisciplinary field between inorganic chemistry and biochemistry. It began as an independent subject with the establishment of international issue, *Journal of Inorganic Biochemistry*, and developed gradually over the past forty years. Inorganic biochemistry has fascinated scientists' attention and is a very active field among nature science. The task of inorganic biochemistry is to research the interaction between the metal complex (or metal ion) with biological ligand at the molecular level. There exists wide research about inorganic biochemistry such as biological macromolecular, oxygen carrier, hemoglobin, mimic enzyme, DNA-binding and cytotoxicity, biological mineralization, etc. Encompassing the varied and disparate roles of metal ions in biology, the field of bioinorganic chemistry has reached a certain level of maturity. In this chapter, the role of metal ion in inorganic biochemistry is discussed in detail and it covers the interaction of metal complex with biological molecular, DNA-binding mode and biological activity, the binding to the membrane molecules (human erythrocyte membrane) and the functions of metal complex in biological hydrolysis process. Researching fields are as follows: 1) the interaction of metal complex with biological molecular (DNA and α-amino acid): The metal complex maybe interact with calf thymus DNA by intercalative and coordination binding mode. Additionally, the contribution from the electrostatic interaction between DNA and aliphatic side chain moiety in the metal complex cannot be ignored. The longer the aliphatic side chain is, the stronger the electrostatic interaction is. However, the Pt/Pd complexes may interact with DNA by non-intercalation. It has been found that linear free energy relationships, linear enthalpy relationships and linear entropy relationships exist in the system containing metal ion and biological small molecules for example α-amino acid; 2) biological activity: Some Pt^{2+}/Pd^{2+} complexes exhibit significant cytotoxicity against cancer cell lines and the pyridine ring moiety coordinated with Pt^{2+}/Pd^{2+} play an important role in its antitumor activities. Additionally, Ln^{3+} complexes containing amino acid moiety with nonpolar aliphatic side chains show interesting antitumor activity *in vitro* against different cell lines. Moreover, the cytotoxic properties of Ln^{3+} complexes seem to increase gradually as the lipophilicity of nonpolar aliphatic side chains increase; 3) the effect of metal ions to hemoglobin (Hb): Rare earth metal ions binding to the membrane molecules (human erythrocyte membrane) induce the phase transition of lipid bilayer, conformational changes and aggregation of membrane proteins; 4) metalloenzyme catalysis: metal complexes are more efficient catalyst hydrolysis of ATP, NPP, HPNP, NA, CA, and etc biological small molecules than protonated ligand at a certain pH range. The water molecule (or –OH)

participates in the catalytic hydrolysis of biological small molecule in different steps with different functions.

Chapter 3 - In living cells, different biological functions such as molecular oxygen transport, hydroxylation of organic compounds, peroxide homolytic and heterolytic cleavages and electron transport are performed by one exclusive moiety: iron protoporphyrin IX. This multiplicity of functions is possible due to the different microenvironments and heme iron ligands provided by different apoproteins. In this study, is described a mimicking of Nature strategy, in which, different iron or manganese porphyrins had catalytic properties modulated by the hydrophobic microenvironment of micelles, liposomes, the mesoporous silica MCM-41 and apocytochrome *c*. The association to micelles, liposomes and protein and the activity of these catalysts were probed for hydrogen peroxide, *tert*-butylhydroperoxide (*t*-BuOOH) and diphenylacetaldehyde (DPAA) and analyzed by electronic absorption spectra, EPR measurements and atomic force microscopy (AFM).

The Fe(III) protoporphyrin IX, in homogeneous medium, exhibited catalase-like activity on hydrogen peroxide and peroxidase activity on *t*-BuOOH and exclusively peroxidase activity when associated to cationic micelles of CTAB and DODAB liposomes. In SDS liposomes, protoporphyrin IX became more buried in the micelle core than in CTAB micelles and exhibited a more intense bleaching during the reaction with peroxides. Besides peroxides, DPAA was also used as the reducing agent for these systems and accelerated the completion of the peroxidase catalytic cycle. Peroxidase and oxidase activities were also exhibited by the non-covalent complex of protoporphyrin IX with apocytochrome *c*. The peroxidase activity of heme *c* in microperoxidases and native cytochrome *c*, was modulated in micelles and liposomes by the partition of the substrates in the lipid aggregates. For cytochrome *c*, the limiting catalytic step was the access of the peroxide to the heme pocket. The capacity of histidine to act as an acid-base catalyst in the peroxidase cycle of microperoxidases was favored in conditions in which the coordination with the metal was disfavored, i.e., in DODAB liposomes and replacing iron by manganese. The catalytic activity of the meso-tetrakis(2,6-dichloro-3-sulfonatophenyl) porphyrin (Fe(III)TDC($SO_3^-Na^+$)PP) was also investigated. This porphyrin exhibited peroxidase but not catalase activity in homogeneous and heterogeneous media. In DODAB liposomes the addition of Fe(III)TDC($SO_3^-Na^+$)PP led the vesicles to assembly in a wire-like manner. The Fe(III)TDC($SO_3^-Na^+$)PP and its $Zn2^+$-substituted form were extremely efficient to induce folding of apocytochrome *c* that did not exhibit peroxidase activity. The peroxidase activity of Mn(III)TDC($SO_3^-Na^+$)PP was also studied in CTAB micelles and DODAB liposomes in the absence and in the presence of histidine that significantly favored the catalytic activity.

Chapter 4 - This chapter deals with development of novel metal based drugs. Due to the large list of metallo drugs, this is limited only to platinum, Ruthenium and Rhodium complexes with greater emphasis on their bioactivities and their mode of action. Metal based anticancer chemotherapies are making significant advances in clinical trials. Until recently, the focus has been on coordination complexes, and mechanisms such as "activation by reduction", "ligand exchange kinetics" and "transferrin-targeted delivery" have been proposed to account for the excellent cytotoxicity and low general toxicity of these complexes. More recently organometallic compounds, which to some extent appear not to follow the established rules, are now being investigated. Despite such differences, similar activities between certain coordination and organometallic compounds suggest similar modes of action are present. DNA, the classic target, is believed to be the dominant mechanism for

cytotoxicity with certain metal based drugs, while with others, non-classical targets are thought to be more important.

Chapter 5 - Flavonoids are a large group of phytochemicals ubiquitously found in many food products of vegetable origin. They have been reported to have a broad spectrum of pharmacological activity. In addition, due to the presence of hydroxyl groups in their molecular structure, flavonoids exhibit strong antioxidant properties. Previous works in this field show that their antioxidant activity is modified in the presence of metal ions by the formation of soluble complex species which change the ability to scavenge free radicals. This chapter includes: (i) a critical review of the published reports concerning the electrochemistry of flavonoids and their metallic complexes in different media, (ii) a comparative study on the electrochemical behavior (cyclic voltammetry) of the flavonoids quercetin, rutin, rhamnetin and isorhamnetin on gold electrodes, and (iii) the influence of the relevant metal ions Cu(II) and Fe(III) on the electrochemical oxidation of the four flavonoids. The results show, as a general trend, that complexation with these metal ions produces a change in the capability of flavonoids to be oxidized. This is quite remarkable in the case of Cu(II). The evaluation of the species distribution diagrams of the systems together with the oxidation potentials allows the changes in the radical scavenging ability of the flavonoids upon complexation to be discussed.

Chapter 6 - Cytochrome *c* oxidase (C*c*O), the fourth metalloenzyme in the respiration system of mitochondria membrane, catalyzes the four-electron reduction from O_2 molecule to two H_2O molecules. The authors proposed in their previous work that the H_2O molecule coordinated to the Cu atom of the Cu_B site in the active site of C*c*O provides a proton to Fe=O of the heme a_3 site at the stage of formation of the second H_2O molecule. In this paper, they show that the H_2O molecule on the Cu atom does not provide a proton to produce FeOOH from FeOO in heme a_3 at the early stage of the O_2 reduction, but it supports the electron transfer from the Cu_B site to FeOOH. The proton to yield the FeOOH comes from the H_2O molecule that connects to both His290 and Tyr244 through the hydrogen bonds. Tyr244 also connects to the farnesylethyl group of heme a_3 and K-channel through the hydrogen-bond network. The reduced Cu atom of Cu_B site simultaneously induced the electron transfer to FeOOH with the proton transfer, leading the mechanism of proton-coupled electron transfer.

Chapter 7 - Heme enzymes are a versatile group of biocatalysts involved in a large variety of (bio)chemical reactions. Thus, peroxidases convert molecular oxygen to hydrogen peroxide while the cytochromes P450 (P450) are involved in the monoxygenation of substrates. Albeit the large differences in biochemical functions the active sites of these two enzyme groups are very similar: namely a protoporphyrin IX with a ligated iron atom. The active species of P450 enzymes that catalyzes the monoxygenation reactions is the oxo-iron species, but its lifetime is very short and therefore it has never been detected experimentally. Thus, in order to obtain information on reactivity patterns and catalytic properties of these short-lived species, theoretical modelling is useful as it can give predictions of reaction mechanisms and the stability of the various proposed oxidants. In this Chapter the authors will discuss the fundamental differences of heme enzymes, such as peroxidases and the P450s, and related synthetic catalysts and describe how subtle changes to the catalytic center influence the reactivity patterns of the oxidants. The key property that establishes the reactivity differences between peroxidases and the cytochromes P450 is the nature of the axial ligand trans to the oxo group, which is a histidine group in peroxidases whereas it is a cysteinate group in the P450s. Thus, anionic ligands, such as cysteinate push electrons toward

the iron center while neutral ligands like histidine pull electrons away from the center. In this chapter the authors will review studies on the axial ligand effect in heme enzymes and compare the electronic properties of the oxo-iron species of various heme and non-heme enzymes and biomimetics. In particular, a series of systematic studies on the reactivity patterns of models of cytochrome P450, horseradish peroxidase, catalase and cytochrome *c* peroxidase with respect to a common substrate are discussed and show how the axial ligand determines the properties of the catalytic center as a monoxygenase or peroxidase. These studies have quantified the nature of the push/pull-effect of the axial ligand and make predictions on how to improve synthetic oxidants to generate systems that can regio-, stereo- or chemoselectively react with substrates.

Chapter 8 - The di-heme peroxidase enzymes provide a protective defense for a number of pathogenic bacteria, against host peroxide challenge, following infection. The overall mechanism of peroxide reduction by these enzymes is complex. Following initial formation of an active mixed valence form of the enzyme, the enzymic cycle involves a number of very rapid reactions. No reaction intermediates have been identified. Using Brownian dynamic simulation the authors have studied the activation process of the enzyme and have identified the formation of an ensemble of weak intermolecular complexes between the peroxidase enzyme and its natural protein electron donors. Predicted reaction rates for the formation of the active mixed valence form of the enzyme closely match those determined experimentally. Recent crystallographic studies of the mixed valence form allow us to model peroxide binding and cleavage at the active site of the mixed valence form of the enzyme using density functional theory. These calculations identify a crucial role for Glu114 in peroxide binding, hydrogen bonding and subsequent peroxide cleavage. The authors' modeling of the activation process of the enzyme and the reduction of peroxide to water provides a rationale for the known actions of this important group of enzymes.

Chapter 9 - Mn catalases catalyze disproportionation of H_2O_2 into H_2O and O_2 by using a $Mn_2(\mu\text{-}O_2CR)(\mu\text{-}O/OH/H_2O)_2$ structural unit that cycles between the $Mn^{II}{}_2$ and $Mn^{III}{}_2$ oxidation states. Because of the fast kinetics of this enzymatic reaction each independent step of the catalytic cycle has not yet been characterized. In this context, biomimetic compounds provide a unique way for testing mechanisms in these enzymes. The fine-tuning of Mn redox states is a critical feature when using artificial compounds to model the enzymatic activity. The authors have evidenced some of the key structural factors that control the oxidation states of Mn during H_2O_2 decomposition by comparing the catalase activity of diMn complexes of 1,3-bis[(2-hydroxybenzyl)(2-pyridylmethyl)amino]propan-2-ol (hbpmpnOH) with that of diMn compounds including other dinucleating ligands. New complexes, $[Mn_2(hbpmpnO)(\mu\text{-}O_2C_2H_3)(\mu\text{-}OCH_3)]BPh_4$ and $[Mn_2(X\text{-}hbpmpnO)(\mu\text{-}O_2C_2H_3)_2]BPh_4$ (X = Cl, OMe), were synthesized and characterized, and their catalase-like activity was evaluated. These compounds possess a triply bridged $diMn^{III}$ core, including either bis(μ-alkoxo)(μ-carboxylato) or (μ-alkoxo)bis(μ-carboxylato) bridges, with the remaining coordination sites of the two Mn's occupied by the six donor atoms of the polydentate ligand. These complexes show catalytic activity toward disproportionation of H_2O_2, without a lag phase and first-order kinetic on [catalyst]. Spectroscopic monitoring of the H_2O_2 disproportionation reaction showed that these diMn compounds dismutate H_2O_2 by a mechanism involving redox cycling between $Mn_2{}^{II}/Mn_2{}^{III}$ levels with retention of dinuclearity during catalysis. A correlation between the electronic properties of the different ring substituents, the redox potentials of the dinuclear

complexes and their catalase activity was evidenced. Comparison of the structure, kinetic parameters and redox potentials of the present diMn compounds with those of other diMn complexes also including polydentate ligands with a central bridging alkoxo revealed that the oxidation states of Mn during the catalytic cycle are not critically dependent on the redox potentials of the catalyst but strongly depend on the peroxide binding mode to the metal centre(s).

Chapter 10 - Superoxide dismutase (SOD) enzymes form an important defense line in living organisms. Through a dismutation reaction they transform the highly reactive superoxide radical ion to oxygen and hydrogen peroxide. The latter compound is further transformed by catalase or peroxidase enzymes to water and oxygen. The overall structure of the enzymes and those of the active sites are largely known, thus, it has been revealed that in eukaryotes Cu(II) and Zn(II) ions act as cofactors and they are connected with an imidazolate bridge and this structural unit is coordinated with amino acids. In prokaryotes the SOD enzymes contain Mn(II) or Fe(II) or Ni(II) in their active centres. In order to learn about the working mechanism of SOD enzymes at the molecular level various structural mimics were prepared and their structural transformations during the dismutation reaction was followed. Gathering adequate amount of information allowed the preparation of functional mimics that are not necessarily copies of the active sites of the enzymes, nevertheless, display considerable SOD activity. Both functional and structural mimics are comprehensively dealt with in this review. Although enzymes may seem to be attractive catalysts for promoting real-life reactions effectively with high selectivity, they can seldom if ever be used under industrial conditions, i.e. at high temperatures and pressures. The SOD enzymes for promoting oxygen transfer reactions are not durable enough under these conditions either. The complexes mimicking SOD activities perform better in this respect, however, their reusability is limited, because of separation problems. A solution can be the immobilization of these SOD mimicking complexes on solid or semi-solid supports. Even if the activity is not better then the support-free complexes, the catalyst can be filtered at the end of the reaction and can easily be recycled. Attempts for immobilization are also comprehensively reviewed and immobilized complexes with surprisingly high SOD activities are reported as well. Full characterization of these materials is given and rationalization of their exceptionally high activities is offered.

Chapter 11 - This article reviews recent studies of platinum(II) and platinum(IV) antitumor compounds with bis(carboxyalkylamino)ethane and -propane ligands and their derivatives. Depending on the substituents on the ligands, coordination to the platinum atom can be in a bidentate (*via* two nitrogen atoms) or tetradentate (*via* two nitrogen and two oxygen atoms) fashion. The review covers various aspects, viz, synthesis, spectroscopic and structural features and biological activity of these complexes. In the case of tetradentate coordination, various isomers can be formed which, interestingly, display varying cytotoxic activity. Some general conclusions regarding the relationship between the structure of the complexes with bis(carboxyalkylamino)ethane and -propane derivatives and antitumor activity are drawn. Finally, a brief outlook on the future development of such compounds and their potential as alternative cancerostatics is discussed.