REVIEW ARTICLE

Progress in study on metalloporphyrin mimicking metalloenzymes*

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Abstract The development of research on metalloporphyrins, which are used as model compounds for some metalloenzymes, is reviewed, including the survey of oxygen binding of heme, the catalytic oxidation of cytochrome P450, the molecular recognition to amino acid esters, the self-assembly and self-aggregation of metalloporphyrins.

Keywords: **metalloporphyrins, metalloenzymes, model compound.**

Among the biomacromolecule systems, different biological functions can be represented by different enzymes with similar metalloporphyrin active sites[1] such as hemoglobin, myoglobin, cytochrome P450 monooxygenase, catalase, peroxidase, cytochrome C and nitric oxide synthetase. Due to the different molecular environment surrounding the porphyrin active site, especially the different axial ligands, the enzymes show different biological functions and different mechanisms of interaction. In order to uncover the profound mystery of life, the study of the relationship among structure-function-mechanism of interaction is the subject of recent investigation in bioinorganic chemistry. In this work, the research on model metalloporphyrins which were used to mimic the metalloenzymes is discussed in details in terms of experimental methods, reaction mechanism, recent development and characteristic properties of the model compound.

1 Design and synthesis of heme model compounds based on the mechanism of interaction

1.1 Studies of the interaction mechanism of heme

The oxygen binding active site of hemoglobin and myoglobin is the cofactor of heme-iron porphyrin. The mechanism of oxygen binding to the hemoglobin active site has been studied in details from the point of structural chemistry. In the process of the binding of O_2 to Fe(Ⅱ), the axial ligand of imidazole group plays an important role. Imidazole group causes the direct interaction of Fe(Ⅱ) porphyrin agon to globulin, and the interaction affects the distribution of electron in the Fe(Ⅱ) porphyrin agon-O_2 adduct. Imidazole is a π electron donor which can donate electrons to Fe(Ⅱ). This improves the ability of electron-donor of the t_{2g} orbit of Fe(Ⅱ) and favors the formation of back donating bonding between Fe(Ⅱ) and O_2, and therefore the binding of O_2 to Fe(Ⅱ). Chemists have been trying to synthesize iron porphyrin oxygen-carrier since the 1950s. The attempt was not successful in the beginning due to the formation of Fe(Ⅱ)—O_2—Fe(Ⅱ) resulting from the binding between the synthesized iron porphyrin and O_2. The O—O bond in Fe(Ⅱ)—O_2—Fe(Ⅱ) parted easily, and then coordinates to the none oxygen-bonded Fe(Ⅱ) porphyrin to form μ oxygen dimmer which cannot transfer oxygen reversibly. Since agon of Fe(Ⅱ) porphyrin of hemoglobin and myoglobin are in the coil of polypeptide, the steric hindrance inhibits the nearness of the Fe(Ⅱ) ions of two heme and the formation of Fe(Ⅲ)—O—Fe(Ⅲ).

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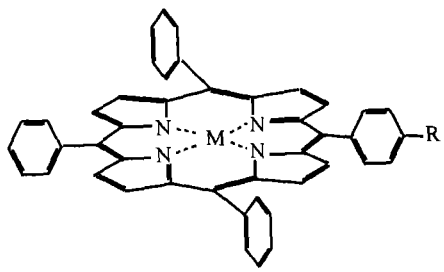
1.2 Design and synthesis of the heme enzyme model

Base on the new knowledge of the action mechanism of hemoglobin, scientists synthesized picket-fenced [Fe(TpiVpp)(1-MeIm)], capped[1], strapped, pocket and crowned Fe(Ⅱ) porphyrins which can bind oxygen reversibly and function in a similar way to heme enzyme.

UV-Vis spectroscopy was used by authors to test the ability of binding oxygen reversibly via the tailed Co(Ⅱ) 5[P-4-(m-pyridyloxy)butoxy] phenyl-10, 15, 20-triphenyl porphyrin (PyBPTPP) (Fig. 1)[2]. The model compound can bind oxygen reversibly at room temperature in benzene. The coordination between oxygen and Co(Ⅱ) in the molecule of O_2-Co(Ⅱ) porphyrin can be further demonstrated via IR.



	M	R
HPTPP	2H	-H
BrBPTPP	2H	$-(CH_2)_4Br$
PyBPTPP	2H	$-(CH_2)_4-O-$
MPyBPTPPCl	Fe or Co	$-(CH_2)_4-O-$

Fig. 1. The tailed porphyrins and the iron or cobalt porphyrin coordination compounds.

By mimicking the active site of hemoglobin, it has been known that the physiological function of hemoglobin is to transport oxygen from lungs to tissues, where carbon dioxide produced and bound to amino group of hemoglobin to form carboxyamide hemoglobin.

2 Design and synthesis of the cytochrome P450 model compounds

2.1 Mechanism of interaction

Cytochrome P450 monooxygenase exists extensively in organisms, and can activate oxygen molecule at room temperature and oxidize hydrocarbon selectively[1], the active site of cytochrome P450 is Fe porphyrin, its fifth axial ligand is the sulfur of cysteine residue. The bond length of Fe(Ⅲ)—S is about 0.22 nm. The sixth axial ligand of the porphyrin is still unsettled. It was suggested that the sixth axial ligand may be H_2O, hydroxyl or the oxygen of amino acid[3] and that different groups will coordinate to Fe porphyrin under different conditions at this position.

With the effort of many scientists made in the past 20 years, the cycle diagram of the oxidation reaction of substrate catalyzed by cytochrome P450 can be summarized as Fig. 2[4].

The static cytochrome P450 is in the equilibrium between two kinds of Fe porphyrin complexes — six-coordinated low-spin P450 and five-coordinated high-spin P450. When the substrate near to the hydrophobic part of protein binds to the active site of

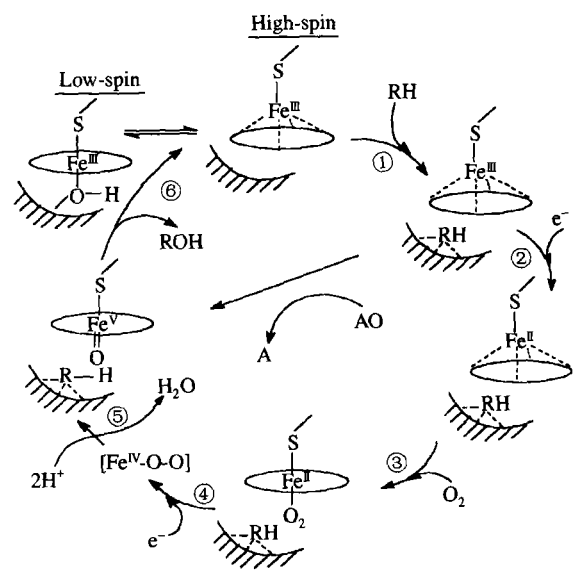


Fig. 2. Oxidation of substrate catalyzed by cytochrome P450.

Fe porphyrin, the equilibrium shifts to five-coordinated high-spin P450, and the high-spin Fe(Ⅲ) complex receives the electrons transmitted by nicotineamide adenine dinucleotide phosphate (NADPH) and is reduced to five-coordinated Fe(Ⅱ) complex, the latter then binds to oxygen to form six-coordinated complex. This complex receives an electron to activate the oxygen forming a [Fe(V)=O] active intermediate. This intermediate transmits its oxygen to substrate to accomplish the cycle of catalysis.

2.2 Composition of the cytochrome P450 model enzyme system

Recent studies on cytochrome P450 include two

aspects: (i) to further understand the mechanism of interaction of cytochrome P450 with other molecules and the cycle of catalysis, (ii) to mimic the reaction paths of P450 partially or entirely and to develop the catalysts that can oxalate hydrocarbon selectively at mild condition by synthesizing the metalloporphyrins.

In 1979, a simple model system of cytochrome P450 monooxygenase was reported by Groves. By using phIO as the oxygen source, and FeTPPCl as catalyst, the olefin oxidated into epoxy ring and the hydroxylation of alkane were achieved in this system[5].

As shown in Fig. 2, the cytochrome P450 model system is composed of the five parts.

2.2.1 Catalyst The catalyst in this model system is metalloporphyrins with the ability of catalyzing oxidation and reduction. Previous studies indicated that the metal ions with different valences and the ability of producing two-electron oxidation are good active center of the model enzyme, therefore the number of d electrons, the self-spin state and the energy level of the central ion are important. The transition metals used in porphyrin complex are Fe, Co, Mn, Mo, Ru and Rh at present[5,6], and the metalloporphyrins can be classified as:

(i) Symmetrical metalloporphyrin. We synthesized many different kinds of meso-tetraphenylporphyrin derivatives as a function of substituting positions (ortho, meta or para) of the phenyl group, chemical property of the substituting group including electron donors or acceptors and their metal ions (Fe, Mn and Co) complexes[7~9]. The structures of these compounds have been confirmed by X-ray diffraction[10,11]. A cytochrome P450 model system was constructed using molecular oxygen as the oxygen source, ascorbic acid as reducing agent and thioalcohol compounds as axial ligand. Benzene was oxidized to phenol directly at room temperature by this model system with a turnover rate at about 40%[7]. Cyclohexane can be oxidized to cyclohexanol and cyclohexanone by the same model system[12].

(ii) Asymmetric metalloporphyrin. Since 1992, the authors have synthesized several kinds of asymmetric porphyrins and their complexes with different electron donor and acceptor groups in phenyl group[13], and the cytochrome P450 model system was used in the hydroxylation of cyclohexane. The results indicated a stronger catalytic ability of asymmetric porphyrin compared to symmetric porphyrin.

(iii) Tailed metalloporphyrin. Since the metalloporphyrin with the fifth axial ligand shows similar behavior to cytochrome P450 monooxygenase, we synthesized several kinds of tailed porphyrins with tailed group, such as pyridine, imidazole[13~15], benzimidazole group[16], benzothiazole group[17], para-hydroxy-phenyl, para-carboxylphenyl, para-nitrosophenyl, benzoxazole group[18,19] and thiophen group[20] linked by the alkyl chain $-(CH_2)_n$ to the ortho, meta or para position of the phenyl group in the porphyrin ring, according to the character of the structure of porphyrin. The coordination of imidazole group and benzimidazole group to the iron porphyrin was demonstrated by magnetic circular dichroism spectra. The magnetic characterization of the different tailed iron porphyrin-nitrogenous ligand adducts was investigated by electronic spin resonance (ESR). It was found that some adducts showed novel ESR signals due to the coordination of tailed group. For example, the tailed iron porphyrin with imidazole tailed group showed new coordinated mode and unique magnetic characterization[21,22]. From the investigation on catalytic oxidation of cyclohexane to cyclohexanol and cyclohexanone under mild conditions, the above tailed porphyrins were proved to be more effective in the catalytic function due to the stabilization effect of the tailed group to intermediate. Low-spin cytochrome P450 in organism showed similar electron paramagnetic resonance spectrum to the complex of low spin six-coordinated hemoglobin with a ligand of —SH due to the polarizable sulfur ligand which causes electrons to shift toward the ligand of para position via the centered iron. During the investigation of rearrangement reaction of nitromethane to aldehyde catalyzed by cytochrome P450, it was discovered that S can stabilize active intermediate and inhibit the formation of π positive ion radical of porphyrin, and the tailed metalloporphyrin with S terminal group showed the most effective catalytic activity in the studies[12,19] due to its similar active site to that of natural cytochrome P450. Other experiments demonstrated that the tailed group with the S terminal group was linked to the phenyl group in porphyrin ring via flexible or rigid chain and was coordinated to the central ion of porphyrin and functioned as the axial ligand, which resulted in electron pulling and could weaken the O—O bond and make the O—O bond split, and favoring the formation of active intermediate of Fe(v) = O.

(iv) Polymer-bonded metalloporphyrins. The studies of polymer-bonded metalloporphyrins as the model system of cytochrome P450 have drawn extensive attention due to the facts that metalloporphyrin can mimic the active center of cytochrome P450 and bonded polymer can mimic the polypeptide chain and hydrophobic surrounding of active center, and that the polymer effect can enhance the catalytic activity of metalloporphyrin[23]. The catalytic oxidation of substrates, such as thioalcohol, paradioxybenzene and hydroquinone, by polymer-bonded Cu(Ⅱ) or Co(Ⅲ) porphyrins with amine, carbonyl or ester linkage, has been reported previously[24,25]. Polystyrene-bonded Fe(Ⅲ) porphyrin in a high-spin state ($S = 5/2$) has been synthesized via ether linkage by us. Compared to the other porphyrins, the catalytic activity of polymer-bonded iron porphyrin is two- to four-fold more effective due to the hydrophobic surroundings of iron porphyrin which resembles the protein surrounding in hemoglobin and myoglobin[26]. On the other hand, manganese porphyrin system was 5.9-fold more effective. The relationship between the configuration of model enzyme and the catalytic activity has been investigated by authors systematically.

The carriers of metalloporphyrin are ion exchange resin, high molecular polymer, diatomite, alumina, molecular sieve, crystalline silica, aluminate and the material of inorganic clay. Carbon black[27], black lead[28], inorganic mineral soil[29], the material of organic biomembrane and some inorganic and organic polymer composites can also be used as the carriers of metalloporphyrins[30,31]. Despite of some special characterizations, carrier-bonded porphyrins are well studied and are not to be discussed in this article.

(v) Metalloporphyrin dimmer. The active site of cytochrome P450-metalloporphyrin is in the surrounding of protein chain and the binding of substrate undergoes near to a hydrophobic protein environment in hemoglobin, therefore hydrophobic surroundings are essential to the improvement of catalytic activity. Furthermore, from the catalytic cycle of cytochrome P450 monooxygenase, the reduction of Fe(Ⅲ) ion of iron porphyrin at the early stage is the key process. Despite of the discovery of the biologic effect of natural porphyrin dimmer and polymer in photosynthesis, mimic studies of porphyrin dimmer are seldom reported. Deisenhofer[32] determined the X-ray three-dimensional structure of the photosynthesis active center of the bacteria RP Viridis and won the Nobel prize of chemistry in 1988.

Besides the photosynthesis system in nature, there are many kinds of metal proteins and proteinases with porphyrin dimmer or polymer as the active center. These dimmers or polymers play important biologic roles in the transfer and activation of oxygen, photocatalysis and photoconductivity, therefore, the mimic study of the biological active porphyrin dimmers is an important aspect in bioinorganic chemistry. A series of porphyrin dimmers with different electron donors or acceptors as substituting groups in the phenyl, such as P-Fe(Ⅲ)TPPCl/P-H_2TPPCl, P-Fe(Ⅲ)TPPCl/P-Zn(Ⅱ)TPP and P-Fe(Ⅲ)TPPCl/P-Fe(Ⅲ)TPPCl, have been designed and synthesized by authors as the model system of cytochrome P450. These porphyrin dimmers were linked by flexible alkoxyl chain. The effect of the above mentioned substituting groups on the intramolecular energy transfer and the catalytic reaction to hydrocarbon was studied by us too. Hydrocarbon reacts with O_2 in the cavity of "face-face" hydrophobic surroundings bridged by alkoxyl in these model systems. Compared to that of unmodified porphyrin monomer, the catalytic activity of these model systems can be 6-fold in some systems[33]. ^{1}H NMR and ^{1}H-^{1}H COSY studies indicated an equilibrium between the closed and open conformations in the solution of porphyrin dimmer. This equilibrium can be gradually moved from the open to the closed conformation with the increase in the length of the alkoxyl chain, for example, the alkoxyl chain with 4 carbon atoms favored the formation of closed conformation mostly[34]. The closed conformation of porphyrin dimmer enhanced the π-π interaction and the catalytic activity[35], and the bidentate ligand DABCO (1, 4-diazobicyclo[2, 2, 2] octane) further increased the catalytic activity due to the formation of the special structure of the ternary DABCO-dimeric iron(III) porphyrin adduct[36](Fig. 3).

2.2.2 The source of oxygen (including mono- and di-oxygen donor) Oxygen source of the cytochrome P450 model system can be mono- and di-oxygen donor. The mono-oxygen can oxidize metalloporphyrin to high valence intermediate directly to accomplish the short cycle of catalysis (Fig. 2) without using oxygen molecule or reducing agent. The yield of this kind of reaction is high because of avoiding the reaction inertia of oxygen molecule. Using mono-oxygen donors including PhIO, NaOCl, H_2O_2, ROOH, CN(Ph)NO$(CH_3)_2$ and $KHSO_5$, hydrocarbon can be selectively oxidized with high effeciency. However, PhIO is expensive, ROOH is an explosive, the

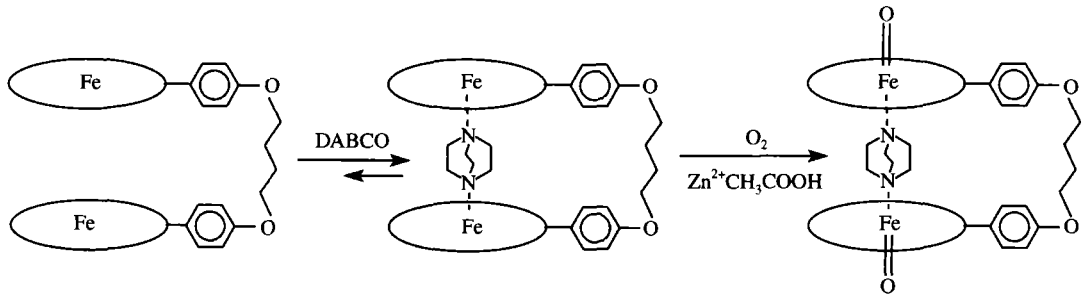


Fig. 3. The ternary DABCO-dimeric iron(Ⅲ) porphyrin complexation in the presence of 1 equiv. DABCO, dioxygen and reducing agent.

synthesis of $KHSO_5$ is very complicated under rigorous condition, and the phase transfer catalyze is need in the reaction involving NaOCl.

2.2.3 Reducing agent (to the source of O_2) The properties of reducer are very important in the catalysis cycle (Fig. 2) by regenerating the catalysts in the model system of using O_2 as oxygen resource. The reducers include chemical reducers, such as $NaBH_4$, sodium ascorbate, H_2/colloid Pt and zinc/acetic acid, and other reducers, such as electrochemical reducers and photochemical reducers. In the epoxy reaction using *cis*-1, 2-diphenyl ethylene as substrate in the system of MnTPPCl/O_2/ sodium ascorbate, the ratio of *cis*- and *trans*-epoxide is 37:67. This ratio is the same as that using PhIO or NaOCl as oxygen resource. It may be due to the similar intermediate structures formed by metalloporphyrin via three different oxygen sources.

2.2.4 Axial ligand The influence of an axial ligand on metalloporphyrin can be presented by electron factor and steric hindrance. We studied three kinds of axial ligands[37]. The first kind is electron donor with —SH group, such as thiosalicylic acid, thiophenol, mercaptan, thioglycolic acid, thiohydracrylic acid and tetrahydro thiophene. The catalytic activity with such electron donor ligand is high. They are not only axial ligands, but also co-catalysts. They can transfer charge to the central ion via σ coordinated bond and activate O_2. The efficiency of sulfhydryl benzoic acid is the highest. The second kind is the nitrogenous ligand, such as pyridine, imidazole and their derivatives. The nitrogen atom of these ligands coordinate to the central ion via σ or π bond, and compete with O_2 for the central ion, and the catalytic efficiency is relatively low. In the last catalytic system, sulfhydryl compound and nitrogenous ligand were added at the same time, and the catalytic efficiency increased significantly. The electron spectra of this system were similar to those of cytochrome P450, suggesting that the state of electron is similar to that of cytochrome P450 monooxygenase.

2.2.5 Other factors The model system can be affected by other factors, such as the pH value of the catalytic system, the concentrations of the compositions (catalyst, reducer and axial ligand), the time and temperature of reaction and solvent. The catalytic efficiency is relatively better when the system is weakly acidic. The catalytic activity can be reduced in either strong acidic or strong basic environment. Catalytic activity can be affected by solvent and reaction time, however, it was seldom reported.

3 Mimic studies of selective catalysis of the active site to substrate—the assembly of metalloporphyrin

3.1 Molecular recognition of metalloporphyrin

The driving force of the binding of heme to apomyoglobin is the interaction between porphyrin and phenyl in the side chain of amino acid[38]. When heme is removed from myoglobin, the stability of protein decreases significantly with the diminished interaction between heme and the side chain of amino acid[39]. The molecular recognition can be defined by the selective interaction between guest and host with special structure and functions. Therefore, the synthesis of porphyrin with good recognition groups is the key in constructing a good recognition system of porphyrin to amino acid derivatives.

3.1.1 Recognition of 5-(2-carboxylphenyl)-10, 15, 20-triphenylporphyrinatozinc(Ⅱ) ($ZnTPPCO_2H$) to amino acid esters Authors synthesized $ZnTPPCO_2H$ and studied the molecular recognition of this porphyrin to amino acid esters and discovered another binding mode of porphyrinatozinc(Ⅱ) to amino acid

esters through hydrogen bonding[40].

Upon the addition of amino acid to ZnTPP-CO_2H, the UV-Vis spectra changed significantly. The red shift of the Soret band and Q bands indicated the coordination between the central zinc of ZnTPP-CO_2H and amino acid, and the binding constants suggested an existing hydrogen bond between ZnTPP-CO_2H and amino acid esters. The ^1H NMR titration experiment of $ZnTPPCO_2H$ with amino acid esters demonstrated that the amino group of amino acid esters formed hydrogen bond with the carboxyl of $ZnTPPCO_2H$ and the carbonyl group coordinated with $ZnTPPCO_2H$. London's dispersion force was also observed between the guest and the recognition cavity of $ZnTPPCO_2H$[41].

3.1.2 Molecular recognition of chiral amino acid modified porphyrinatozinc(Ⅱ) to chiral amino acid esters During the synthesis of protein, aminoacyl-transfer RNA synthases can recognize a specific amino acid, and the synthases only interact with *L*-amino acid rather than *D*-amino acid. The chiral molecular recognition to amino acids and their derivates has been a challenging subject in biomimetic chemistry. The synthesized chiral amino acid modified zinc(Ⅱ) porphyrins (AAN-C_2-(TPP)Zn) exhibits chiral recognition to both *L*- and *D*-amino acid esters[42]. Upon the addition of amino acid to AAN-C_2-(TPP)Zn, the red-shifted of the Soret band and Q band of the UV-Vis absorption spectra indicated the coordination between amino acid esters and zinc(Ⅱ) porphyrins.

Our experiments indicated that the achiral amino acid (glycin) modified porphyrinatozinc(Ⅱ) exhibited no chiral recognition. The selectivity of the *L*-amino acid modified porphyrinatozinc (Ⅱ) to *L*- and *D*-amino acid esters is about 2∶1 and that of the *D*-amino acid modified porphyrinatozinc(Ⅱ) to *D*- and *L*-amino acid esters is about 2∶1.

The decrease in the binding constant between the host and the guest in higher concentration of alcohol in $CHCl_3$ suggested the formation of hydrogen bonding between the porphyrin and amino acid esters. When alcohol was added into the solution of host-guest, the hydrogen bonding between alcohol and the host would weaken the hydrogen bonding between the guest and the host, leading to a decreased binding constant.

The repulsion force plays a key role in the chiral recognition between host and guest. When a host binds to amino acid esters, the repulsion force between *L*-amino acid-modified porphyrin and *L*-amino acid esters or between *D*-amino acid modified porphyrin and *D*-amino acid-esters is small, and the constant is larger than that between *L*-amino acid-modified porphyrin and *D*-amino acid-esters or between *D*-amino acid-modified porphyrin and *L*-amino acid-esters.

In order to admire chiral recognition, there should be at least three points of interaction between host and guest and these three points of interaction must form chiral pocket. Although multiple interactions, such as coordination interaction, hydrogen bonding, hydrophobic interaction and charge-transfer interaction, can be employed to recognize amino acid, it is still difficult to predict and control these interactions to a specific position and in a certain direction, therefore, a precise molecular design for a host is needed in chiral molecular recognition.

3.2 Self-assembly and self-aggregation of metalloporphyrin

In nature, metalloporphyrins cannot function until the formation of the self-assembly of porphyrin with protein or cell membrane. Some amphiphilic porphyrins can self-aggregate in water. This self-aggregation can mimic the biological process of heme[43,44]. The mimic study of the self-assembly and self-aggregation of metalloporphyrin to metalloenzyme is an important subject.

3.2.1 Chiral linear assembly of amino acid bridged dimeric porphyrin (C_2-AA-C_2-$(TPP)_2Zn_2$ In $CHCl_3$, split-induced dichroism (ICD) of the Soret absorption band was observed for the amino acid-bridged dimeric porphyrinatozinc(Ⅱ), which showed that the configuration of these compounds was relatively fixed due to the π-π stacking of the zinc porphyrins in the dimmer. Namely the observed ICD was caused by chiral exciton coupling interactions. $^1H\{^1H\}$-COSY, UV-Vis absorption spectra and ICD titration methods demonstrated the origin of the ICD of C_2-AA-C_2-$(TPP)_2Zn_2$[45].

The studies of ICD and ^1H NMR indicated that chiral linear zinc porphyrin arrays can be built with C_2-AA-C_2-$(TPP)_2Zn_2$ and the bidentate ligand ethylenediamine[46]. Molecular orientation in por-

phyrin-based aggregates can be conveniently monitored by CD spectroscopy. The ICD spectra of C_2-AA-C_2-$(TPP)_2Zn_2$ are dramatically changed reduced upon the addition of ethylenediamine, as shown in Fig. 4.

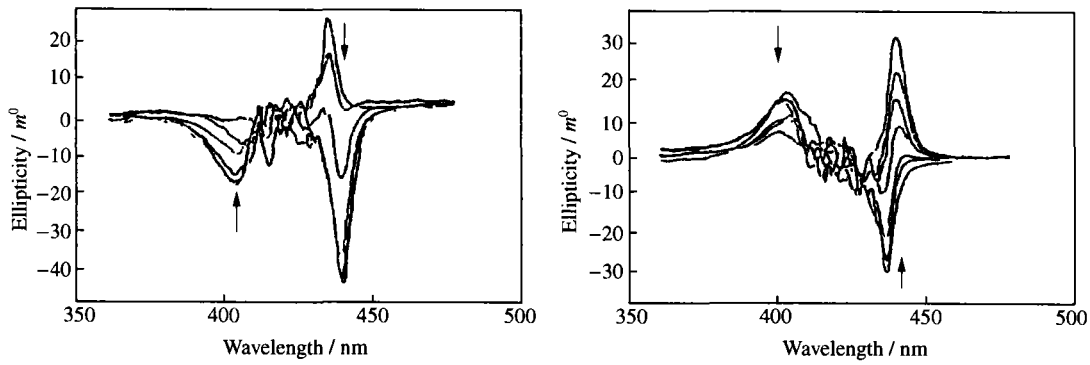


Fig. 4. Changes in the CD spectra upon addition of ethylenediamine to a solution of C_2-AA-C_2-$(TPP)_2Zn_2$ (AA = threonine, in $CHCl_3$ at room temp, [ethylenediamine]:[Zine porphyrin]≤1:1; (a) C_2(*L*-Thr)-C_2-$(TPP)_2Zn_2$, (b) C_2(*D*-Thr)-C_2-$(TPP)_2Zn_2$.

At first, the CD signal at about 436 nm was gradually reversed with increasing ethylenediamine. With further addition of ethylenediamine, the ellipticities gradually decreased, and finally disappeared when a large excess of ethylenediamine was added.

The reverse of the ICD upon the addition of ethylenediamine is due to the formation of chiral linear porphyrin arrays which reached the highest concentration when [ethylenediamine]/[porphyrin] ratio was 1 : 1. At higher ethylenediamine content, this bidentate ligand would act as monodentate ligand and result in the dissociation of the arrays. Borovkov further interpreted the mechanism of the ICD reverse of the porphyrin assembly using the dynamic methods of CD and ^1H NMR[47].

^1H NMR studies further demonstrated the formation of the assembly of C_2-AA-C_2-$(TPP)_2Zn_2$-ethylenediamine. In the porphyrin dimmer, the chemical shifts of the proton of bridge were in the high field (δ: $-5 \sim 0$) due to the current effect of porphyrin ring. The signals of the bridged protons in high field disappeared in the adduct of C_2-AA-C_2-$(TPP)_2Zn_2$-ethylenediamine ([ethylenediamine] : [porphyrin] was 1 : 1). The protons of ethylenediamine could be observed in high field (δ: -5.66, NH_2; -4.71, CH_2). The significant shift of the proton of ethylenediamine to high field indicated the two amino groups of ethylenediamine coordinated to zinc atom in porphyrin. The disappearance of the bridged proton signals suggested that the coordination of ethylenediamine to porphyrin dimmer resulted in weakening the current effect of porphyrin ring to the bridged proton. If the two amino groups of ethylenediamine coordinated to the zinc atom of two molecules of C_2-AA-C_2-$(TPP)_2Zn_2$ respectively, the bridged proton and the protons of ethylenediamine should be observed in the high field at the same time.

Theoretically, countless porphyrin unit can be linked by this "module-linker", and owing to the exciton coupled action between colouring group of porphyrin, this kind of assembly can achieve long distance electron transfer.

3.2.2 Self-aggregation of chiral amino acid modified porphyrin Besides the construction of the chiral porphyrin assembly using module-linker strategy in organic solvent, we synthesized another kind of amphiphilic chiral amino acid modified porphyrin[48,49] which can be self-aggregated in aqueous medium[50].

UV-Vis absorption spectra indicated threonine modified porphyrin and the zinc (Ⅱ) complexes aggregated in water/alcohol and water/alcohol/NaCl[51]. Upon the addition of sodium dodecyl sulfonate (SDS) to the solution of porphyrin in water/alcohol and water/alcohol/NaCl, the UV-Vis absorption spectra changed significantly. The changes of UV-Vis absorption spectra indicated that the self-aggregation in the two medium is different. In water-alcohol-NaCl system, the aggregations may be orientated.

Fluorescence spectra also provided the evidence for the formation of threonine-linked porphyrins and their zinc (Ⅱ) complexes aggregate and the difference in structures of aggregated porphyrin in water-

alcohol system and water-alcohol-NaCl system. Threonine-linked porphyrins and their zinc (Ⅱ) complexes showed strong fluorescence in alcohol. In water-alcohol system, the fluorescence intensity decreased due to the formation of unorganized aggregate. When SDS was added, the increased fluorescence intensity indicated the dissociation of the aggregates. Upon the addition of NaCl, the more intense fluorescence was observed due to the formation of the organized and orientated aggregates of threonine-linked porphyrins and their zinc (Ⅱ) complexes.

The structure difference of the aggregated porphyrins in water-alcohol system and water-alcohol-NaCl system and the molecular orientation of these aggregates in water-alcohol-NaCl system can be monitored by CD spectroscopy. All the chiral threonine-linked porphyrins and their zinc (Ⅱ) complexes showed characterized CD spectra in alcohol. In water-alcohol system, the CD intensity decreased, but the phase disposition of CD remained constant for the formation of unorganized aggregate of porphyrin. The phase disposition of the CD of all these threonine-linked porphyrins and their zinc (Ⅱ) complexes reversed in water-alcohol/NaCl medium. The CD spectra red-shifted significantly (about 20 nm) with increased intensity (Fig. 5), which indicates that the self-aggregation of these porphyrins in water-alcohol-NaCl system is highly organized and orientated, which is different from that formed in water-alcohol system. The orientated self-aggregations may have chiral helical structure.

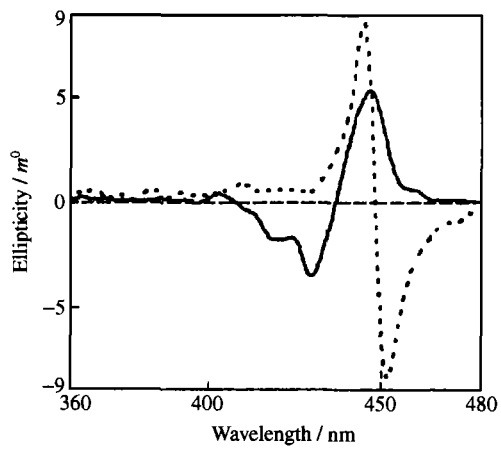


Fig. 5. CD spectra of *L*-threonine-linked porphyrinatozinc (Ⅱ) in water-alcohol system (solid line) and in water-alcohol-NaCl system(dashed line) [NaCl]: 0.05 mol/L. The concentration of porphyrin is 16 μmol/L.

4 Prospect

By comparing metalloenzyme model compounds with the natural enzymes, we are able to demonstrate that the axial ligand with —SH can activate the natural catalase. We expanded the investigation to cell and constituted a resting cell culture system and demonstrated that the axial ligands with —SH group and ions promoted the biosynthesis of catalase by aspergillus niger at different fermentation time[52,53]. Similar structure of metalloporphyrin has been discovered in different natural enzymes with different biological functions. According to the structures of these natural enzymes have produced more and more significant models, new experimental approaches have been developed to study the catalytic molecular recognition, the biological function and the interaction mechanism between chiral metalloporphyrin and substrate, which reveals the myth of life[54] and promotes the development of bioinorganic chemistry.

In order to guide and control pathways metabolism of microorganism. Above mentioned research fields are needed to be carried out to reach better understanding and full potential of metalloporphyrin model compounds.

We can predict a further renaissance of metalloporphyrin mimicking metalloenzymes, which extends to medicinal inorganic chemistry, environmental biochemistry, biogeochemical cycles, biomimetic coordination chemistry and photochemical and photobiological sciences.

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