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ORIGINAL CONTRIBUTION

Porphyrin-cyclodextrin supramolecular complexes as myoglobin model in water

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Abstract This mini-review reports supramolecular system composed of O-methylated β-cyclodextrins and metalloporphyrins that mimic the functions of myoglobin (Mb) in aqueous solution. Although many Mb functional models have been demonstrated so far, most models can bind dioxygen only in organic solvents such as toluene. Recently, we prepared the model systems composed of O-methylated β-cyclodextrin dimers having pyridine and imidazole linkers and tetrakis(4-sulfonatophenyl)porphinato iron(II) (hemoCD and Fe(II)PImCD). HemoCD binds dioxygen reversibly in aqueous solution, and the dioxygen adduct of hemoCD is very stable (a half-lifetime is 30 h at pH 7). Although the dioxygen affinity of Fe(II)PImCD is much higher than hemoCD, the stability and the reversibility of this system is lower. This review compares the functions of these model systems with those in biological systems.

Keywords Per-O-methylated β-cyclodextrin · Cyclodextrin dimer · Ferrous porphyrin · Myogloboin model · Aqueous solution

Introduction

Myoglobin (Mb) as well as hemoglobin (Hb) is a hemoprotein that binds dioxygen to its ferrous center of heme located inside a protein, globin. Globin plays very important role to protect formation of μ-oxo dimer of iron porphyrin and autoxidation of dioxygen adduct of heme. Many attempts have been carried out to mimic the functions of Mb (or Hb) in artificial systems [1, 2]. A representative example is "picket-fence porphyrin" that was prepared by Collman et al. [3, 4] in 1973. The point of this model is four bulky substituents at α-,α-,α-,α-positions of tetraphenylporphinato iron. The regularly aligned bulky substituents inhibit formation of μ-oxo-dimer of iron porphyrin and help five-coordination to ferrous porphyrin whose axial ligand is an imidazole derivative. The picket fence porphyrin forms a stable dioxygen adduct in absolute toluene. Until today, many Mb (Hb) models were prepared by applying almost the same concept as picket-fence porphyrin [5]. Although these model compounds gave us important information about dioxygen binding to ferrous porphyrin, there was a strict limitation. These model compounds act as dioxygen receptors only in absolute organic solvents such as toluene. If a trace amount of water exists in the system, the dioxygen adduct is immediately destroyed. Tsuchida et al. [6–9] studied dioxygen binding to amphiphilic porphyrins placed at very hydrophobic environments such as liposomal membranes and albumin in aqueous solution and demonstrated that dioxygen can be captured by a ferrous porphyrin in aqueous solution if the porphyrin is placed in a sufficiently hydrophobic microenvironment. Other groups also tried to mimic the function of Mb in aqueous solution [10–12]. However, there are no examples of dioxygen binding to simple model ferrous porphyrins in aqueous solution except for the Tsuchida's ones. Then we tried to prepare new Mb (Hb) functional models that work in aqueous solution. Before start this project, we found a novel behavior of per-O-methylated β-cyclodextrin to include peripheral aryl substituents of water-soluble tetraarylporphyrins in its cyclic cavity.

This review was written to dedicate to Professor Janos H. Fendler on the occasion of his 70th birthday. He gave me a chance to study biomimetic chemistry when he was a professor of Texas A&M University.

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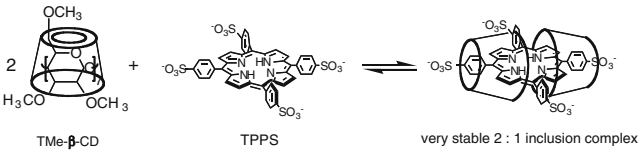
Table 1 Thermodynamic parameters for coordination of inorganic anions to Fe(III)TPPS complexed with TMe-β-CD in 0.1 M succinic acid buffer at pH 4.0 and 298.15 K

Anion	$K_{X,app}$ (M^{-1})	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J $mol^{-1}K^{-1}$)
Cl^-	237±2	−13.5±0.1	−2.2±0.1	37.9±0.7
N_3^-	(1.28± 0.03)×10^4	−23.4±0.1	−13.9±0.1	31.9±0.7
Br^-	54±1	−9.9±0.1	−1.2±0.1	29.2±0.7
SCN^-	130±2	−12.1±0.1	−15.1±0.1	−10.1±0.7

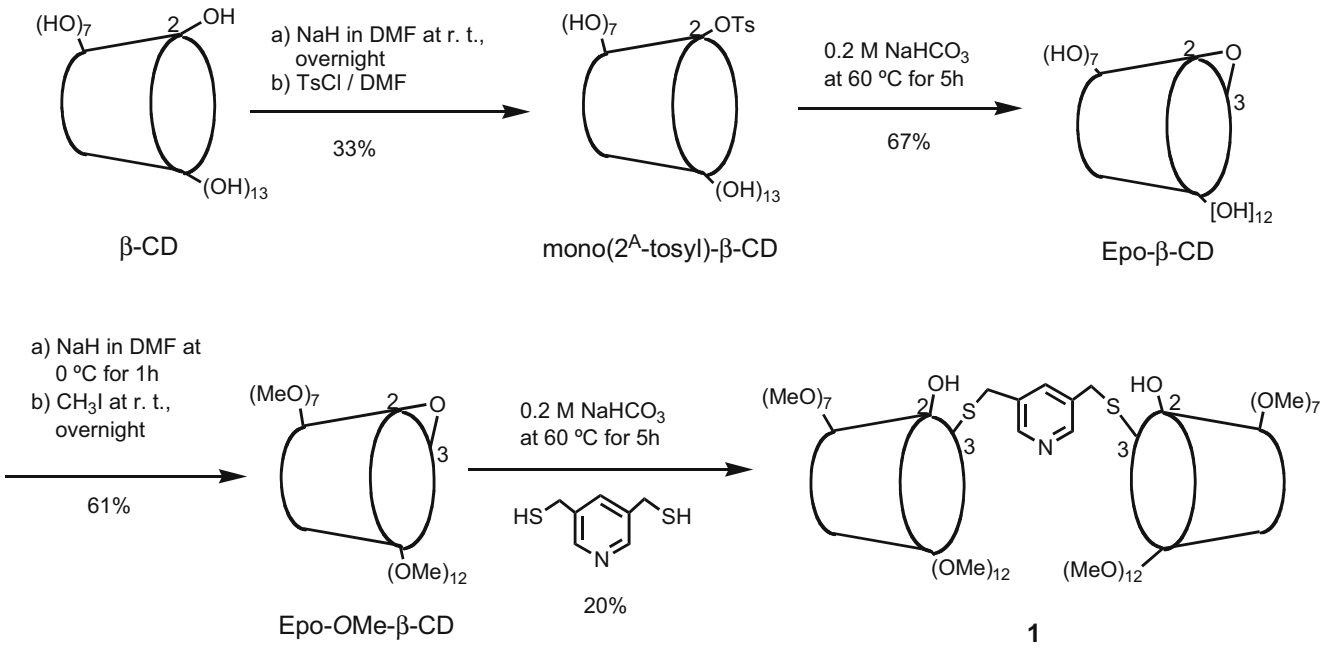
The thermodynamic parameters were determined by means of isothermal titration calorimetry [17].

Interactions of a porphyrin-free base and its metal complex with TMe-β-CD

Preparation of microscopically hydrophobic environment around a porphyrin center should be very important subject to prepare a hemoprotein mimic. It has been known that heptakis(2,6-di-O-methyl)-β-cyclodextrin (DMe-β-CD) forms a stable *trans*-type 1:2 inclusion complex with a water-soluble porphyrin [13]. We found that heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TMe-β-CD) has stronger ability to include the aryl groups of *meso*-tetraarylporphyrins in water [14, 15]. Hydrophobicity at the clefts of the 2:1 inclusion complexes of CD-porphyrin complexes was evaluated by measuring the pK_a values of the porphyrins. The pK_a value for diprotonation to two pyrrole nitrogens of a water-soluble porphyrin can be determined from pH titration for the UV-vis spectrum of the porphyrin. For example, the pK_a value of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS) in the absence of cyclodextrin is 4.8, which dramatically decreases to 0.4 upon addition of TMe-β-CD, while the effects of native β-CD (pK_a=4.2) and 2,6-DMe-β-CD (2.2) are not so remarkable. Such results clearly indicate that the environment near the porphyrin center of the TPPS-TMe-β-CD complex is very hydrophobic. The binding constants (K_1 and K_2) for complexation of TPPS with TMe-β-CD could not be determined by a usual method because of too large K values. The K_1 and K_2 values in ethylene glycol (75%)-water (25%) mixture are 2×10^4 and 5.8×10^5 M^{-1}, respectively. Further study revealed that O-methylation of secondary OH groups of β-CD is essentially important for strong inclusion [16]. Destruction of intermolecular hydrogen-bond belt at secondary OH side due to O-methylation makes it possible to alter the shape of CD to maximize van der Waals interaction between the porphyrin and the CD ("induced-fit" type complexation) [15, 16].



Fe(III)TPPS also forms a very stable 2:1 complex with TMe-β-CD [17]. The K_1 and K_2 values at pH 1.5 are 3.06×10^5 and 5.6×10^3 M^{-1}, respectively. Meanwhile, the K_1 and K_2 values at pH 5.0 are 2.85×10^5 and 6.96×10^4 M^{-1}, respectively. The K_1 value is almost independent of pH, while the K_2 value increases with increasing pH until pH 5.



Scheme 1 Preparation of the CD dimer **1**

Table 2 $P_{1/2}$ values and kinetic data for dioxygen binding to biological and biomimetic systems

System[a]	$P_{1/2}$ (Torr)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	Reference
Mb (sperm whale)	0.54	1.7×10^7	15	[22]
Mb (human)	0.69	1.9×10^7	22	[22]
Hb (human, R state)	0.22	3.3×10^7	13.1	[1]
Hb (human, T state)	26	–	–	[5]
$FePiv_3$5Cim	0.58	2.6×10^8	2.9×10^3	[23]
hemoCD	16.9	4.7×10^7	1.3×10^3	[21]

[a] Figure 1 explains the abbreviations and the structures of the oxygenated iron porphyrins.

There is an equilibrium between $(H_2O)_2$Fe(III)TPPS and (OH^-)Fe(III)TPPS and its pK_a value determined from the UV-vis spectroscopic titration is 4.3. Because the net charge at the center of Fe(III)TPPS is +1 for $(H_2O)_2$Fe(III)TPPS, complexation with the second TMe-β-CD molecule is more difficult than the case of (OH^-)Fe(III)TPPS whose net charge at the porphyrin center is zero. In aqueous solution without cyclodextrin, μ-oxo dimer of Fe(III)TPPS is formed in a higher pH range (pK_{app}=6.6). However, in the presence of TMe-β-CD, no μ-oxo-dimer is formed, at least, below pH 12.

The 2:1 complex of Fe(III)TPPS and TMe-β-CD is selectively coordinated by inorganic anions [17]. The thermodynamic parameters for anion binding to the 1:2 Fe(III)TPPS-TMe-β-CD complex at pH 4 are shown in Table 1.

The Fe(III)TPPS-TMe-β-CD complex shows high N_3^- selectivity. The large binding constant for N_3^- is ascribed to negative and large ΔH and positive ΔS. Table 1 suggests that linear anions such as N_3^- and SCN^- show negative and large ΔH, while spherical ones such as Cl^- and Br^- show larger ΔH. ΔS value decreases in the order of hydrophobicity of the anions (Hofmeister series), suggesting that dehydration from a hydrophilic anion upon complexation with Tme-β-CD strongly participate in the anion binding to the Fe(III)TPPS-Tme-β-CD complex. The negative heat capacity changes (ΔC_p) for Br^- (−345 J $mol^{-1}K^{-1}$) and Cl^- (−320 J mol^{-1} K^{-1}) also support the participation of dehydration. The anion binding is very similar to that of metMb [18], suggesting that Fe(III)TPPS-per-O-methylated β-CD system might be used as Mb model in aqueous solution.

Myoglobin model composed of a cyclodextrin dimer having a pyridine linker and Fe(II)TPPS

To achieve construction of a Mb model, the Fe(II) center should be placed at a hydrophobic environment and coordinated by a π-electron donor such as imidazole or pyridine. At first, we designed an O-methylated β-CD dimer having a pyridine linker (**1**) as shown in Scheme 1 [19]. The CD dimer **1** forms an extremely stable 1:1 inclusion complex with Fe(III)TPPS in aqueous solution [20]. The 1:1 Fe(III) TPPS-**1** complex is easily reduced to the Fe(II)-**1** complex (hemoCD) by a minimum amount of sodium dithionite. The inorganic salts can be removed by passing the mixture

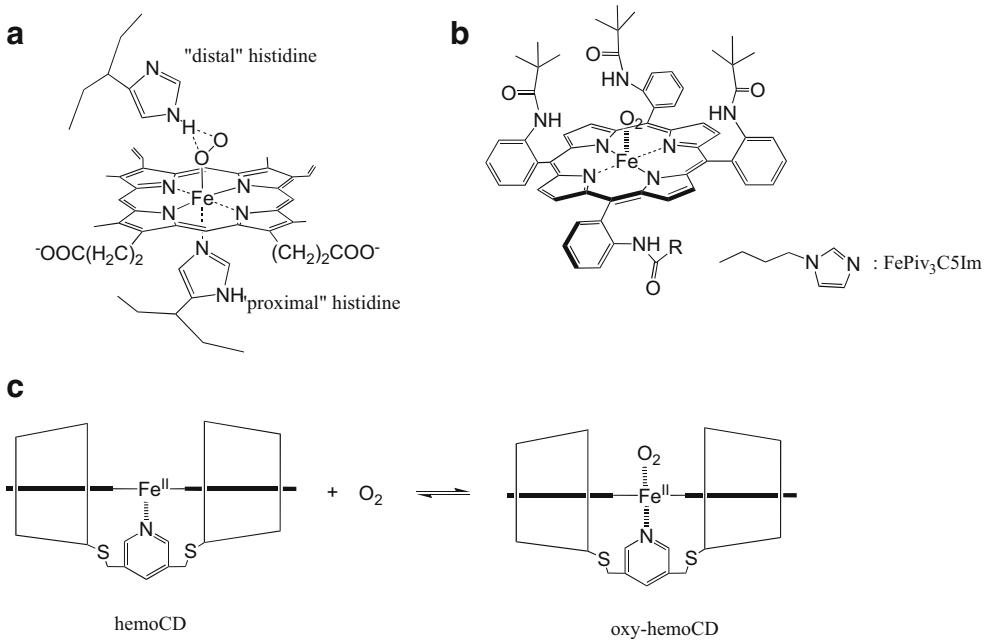


Fig. 1 Oxygenated heme in a biological system (**a**) and the dioxygen adducts of the model systems (**b**, **c**)

Table 3 Thermodynamic parameters for dioxygen association to biological and artificial systems[a)]

System	ΔH (kJ mol^{-1})	ΔS (J $mol^{-1}K^{-1}$)	K (M^{-1})	Reference
Hb (human)	−52.3	−77.2	1.4×10^5	[24]
Mb (sperm whale)	−79.9	−153.0	1.0×10^6	[25]
Mb (horse heart)	−87.8	−182.3	7.5×10^5	[25]
hemoCD[b)]	−65.2	−134	2.7×10^4	[21]

[a] ΔS and the binding constants (K) for the biological system are not the original values. The detail is explained in [21].
[b] The thermodynamic parameters were determined by means of isothermal titration calorimetry.

through a Sephadex G-25 column. During this procedure, hemoCD is coordinated by dioxygen to form oxy-hemoCD. The formation of oxy-hemoCD has been proved by means of UV-vis, resonance Raman, and ^{1}H NMR spectroscopy. Oxy-hemoCD is very stable even in aqueous solution, the half-lifetime ($t_{1/2}$) of oxy-hemoCD being 30 h in phosphate buffer at pH 7.0 and 25 °C.

Dioxygen affinities of Mb, Hb, and their model compounds have been evaluated by $P_{1/2}$ that is the partial dioxygen pressure at which half of the O_2-receptor molecules are dioxygenated. The $P_{1/2}$ values of hemoCD and other dioxygen receptors are summarized in Table 2 together with the kinetic data obtained from laser-flash photolysis [21].

Because Mb must receive dioxygen from oxyHb, the $P_{1/2}$ value of Mb is considerably low. Meanwhile, oxyHb in a T state must transfer its dioxygen to deoxyMb. Therefore, the $P_{1/2}$ value of Hb in the T state is much higher than that in the R state. Dioxygen affinity of hemoCD is intermediate between those of Hb in the R and T states. Pyridine as an axial ligand of hemoCD seems to lower the dioxygen affinity of hemoCD (vide infra). The rate of dioxygen association of hemoCD is comparable to that of biological systems, while the rate of dioxygen dissociation from oxy-hemoCD is much faster than those from oxyMb and oxyHb. In the biological system, a distal histidine stabilizes the dioxygen adduct due to hydrogen bonding. Absence of distal histidine may cause destabilization of the dioxygen adducts in the model systems (see Fig. 1).

The thermodynamic parameters for dioxygen binding to biological and artificial systems are shown in Table 3. In both biological and artificial systems, the dioxygen association to the ferrous centers is enthalpically favorable and entropically unfavorable process. In the case of inorganic anion binding to the ferric porphyrin, the favorable entropy change due to dehydration from a ligand (anion) participates in ligation (Table 1). Meanwhile, ligation of more hydrophobic dioxygen to the ferrous center is the enthalpy-driven process where the bond energy of Fe(II)-O_2 is essential to dominate the association of dioxygen. One of

Scheme 2 Preparation of ImCD

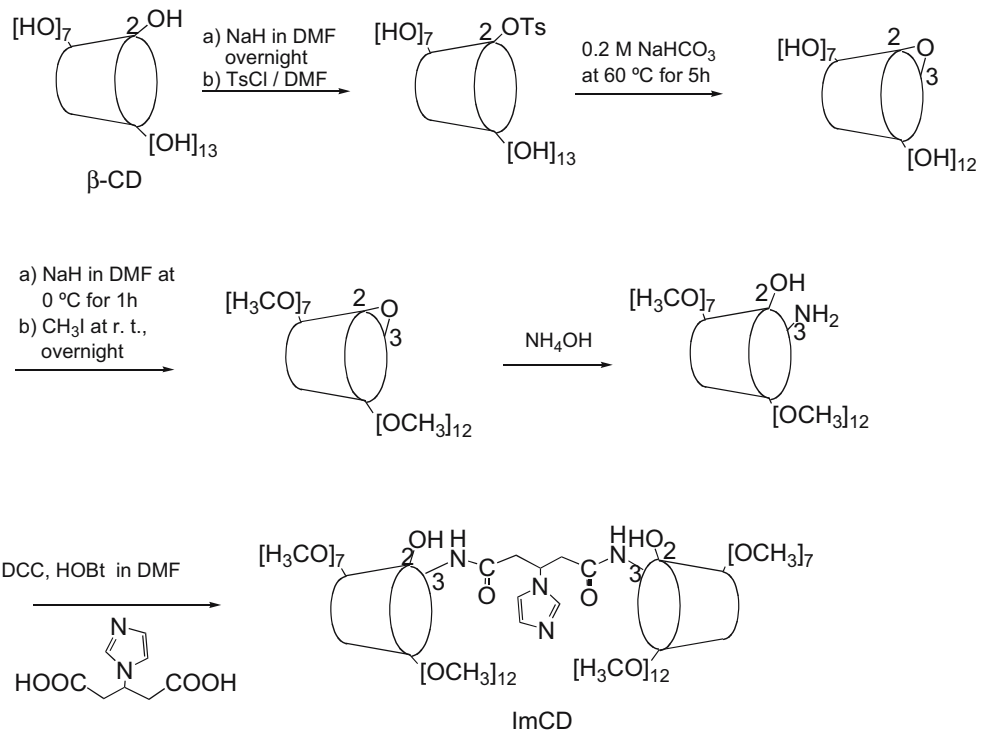


Table 4 Affinities for dioxygen and carbon monoxide

System	$P_{1/2}^{O2}$ (Torr)	$P_{1/2}^{CO}$ (Torr)	k_{on}^{CO} ($M^{-1}s^{-1}$)	k_{off}^{CO} (s^{-1})	M^a	Reference
Mb (sperm whale)	0.54	0.029	5.1×10^5	0.019	18.6	[22]
Mb (human)	0.69	0.023	7.6×10^5	0.022	30	[22]
Hb (human, R state)	0.22	1.3×10^{-3}	4.6×10^6	9×10^{-3}	150	[1]
FePiv3C5Im	0.58	2.2×10^{-5}	3.6×10^7	7.8×10^{-3}	26600	[23]
hemoCD	16.9	1.5×10^{-5}	1.3×10^7	2.5×10^{-4}	1100000	[21]
Fe(II)PImCD	1.7	1.6×10^{-3}	–	–	1040	[26]

[a] $M = P_{1/2}^{O2} / P_{1/2}^{CO}$

the effective methods to strengthen the Fe(II)-O_2 bond in the artificial system is alternation of the axial ligand.

Effects of proximal base in artificial system

Proximal base that coordinates to the ferrous center should be very important for formation of a dioxygen adduct. Then we tried to introduce an imidazole moiety in a linker between two cyclodextrin units [26]. Among several attempts, ImCD could be synthesized (Scheme 2). ImCD also forms a stable 1:1 inclusion complex with FeTPPS (FePImCD). Although hemoCD is stable in aerobic aqueous solution, Fe(II)PimCD is autoxidized with relative ease. The dioxygen ($P_{1/2}^{O2}$) and carbon monoxide affinities ($P_{1/2}^{O2}$) of Fe(II)PimCD are shown in Table 4. The data for other systems are also listed for comparison.

As expected, the dioxygen affinity is improved by changing the axial ligand from pyridine to imidazole. In contrast, the carbon monoxide affinity of Fe(II)PImCD is considerably lower than those for other artificial systems, meaning that the functions of Fe(II)PImCD are closely resemble to those of Mb and Hb. However, there is a problem of Fe(II)PImCD. That is stability of oxy-Fe(II) PImCD as well as reversibility of dioxygen binding. The half-lifetime of oxy-Fe(II)PImCD is 3 h, that is 10-times shorter than that of oxy-hemoCD.

The difference in stabilities of oxy-hemoCD and oxy-Fe (II)PImCD is interpreted in terms of the difference in encapsulation of FeTPPS by two cyclodextrin cavities. Figure 2 shows the energy-minimized structures of hemoCD and Fe (II)PImCD. The iron center of hemoCD is completely covered by two cyclodextrin cavities while that of Fe(II) PImCD is exposed to bulk phase. Such insufficient packing of Fe(II)PImCD is ascribed to the rigid and planar amide groups at both ends of the linker, which inhibits the spatial contiguity of the two cyclodextrin cavities. The extremely large M value, which is a measure of dioxygen-carbon monoxide selectivity, for hemoCD may also be explained by the "cage effect." Resonance Raman spectrum of CO-coordinate hemoCD suggests that CO-Fe bond of CO-hemoCD is significantly weaker than that of the biological system [21]. Contrary to this result, hemoCD shows an extremely high CO-selectivity (M=1.1×10^6) as revealed in Table 4. The low $P_{1/2}^{CO}$ value for hemoCD is ascribed to the slow dissociation of CO-hemoCD (Table 4) [20]. In the artificial, homogeneous system ($FePiv_3C5Im$), the CO association rate (k_{on}^{CO}) is much faster than those of biological system. In the case of hemoCD, the k_{on}^{CO} is smaller than that for $FePiv_3C5Im$ but much faster than that for Mb and Hb. The CD capsule is not a strict barrier for CO penetration. Contrary to this, the rate of CO-dissociation for CO-hemoCD is much slower than those for biological systems, although the CO-Fe(II) bond of CO-hemoCD is weaker than

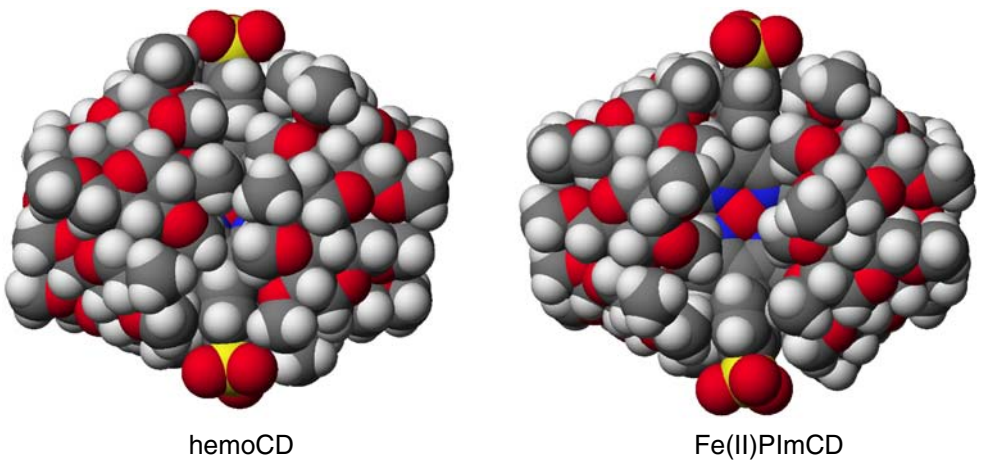


Fig. 2 Energy minimized structures of hemoCD and Fe(II) PImCD derived by MM2 calculations

those for CO-Mb and CO-Hb. Polarity around CO-Fe(II) has been assumed as a factor to control the CO selectivity [27, 28]. Although the CO-Fe(II) bond is surrounded by the polar -OCH_3 groups of the CD dimer **1**, the dissociation of CO-hemoCD hardly occurs. The cage effect is the most reasonable mechanism for slow k_{off}^{CO} of CO-hemoCD [20, 21]. A CO molecule is so hydrophobic that the CO molecule released from CO-hemoCD hardly goes out from the capsule of hemoCD to a polar aqueous phase resulting in remaining inside the capsule. Such a released CO recombines with hemoCD in a very narrow space. The biological system seems to be similar to CO-hemoCD. However, the CO molecule released from CO-Mb or CO-Hb can move to the bulk phase through hydrophobic routes in globin [29].

Conclusion

The functions of hemoCD and its related systems resemble to those of hemoproteins. For example, the redox potential of MnTPPS can be regulated by complexing with cyclodextrins [30]. It can be concluded that supramolecular systems composed of porphyrins and cyclodextrins will make it possible to study a variety of hemoprotein-mimetic chemistry.

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