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Supramolecular Ferric Porphyrins as Cyanide Receptors in Aqueous Solution

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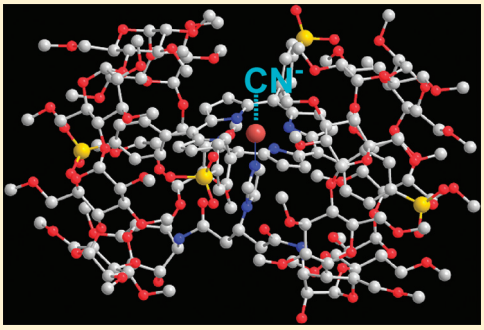
Supporting Information

ABSTRACT: All fundamental data about binding of the cyanide to a supramolecular complex composed of a per-*O*-methylated *β*-cyclodextrin dimer having an imidazole linker (Im3CD) and an anionic ferric porphyrin ($Fe^{(III)}$TPPS) indicate that the $Fe^{(III)}$TPPS/Im3CD complex is much better as an cyanide receptor in vivo than hydroxocobalamin, whose cyanide binding ability is lowered by its strong binding to serum proteins in the blood.



KEYWORDS: *Cyclodextrin dimer, ferric porphyrin, supramolecules, cyanide binding, cyanide receptor*

Cyanide anion (CN^-) is a versatile diatomic ion that exhibits a strong ability to coordinate to various metal ions and a strong nucleophilicity to react with various haloalkanes and carbonyl compounds. Meanwhile, cyanide[1] is highly toxic, because it binds strongly to ferric cytochrome c_3 to block electron transfer in the mitochondrial respiratory chain.[2] Although many methods for detecting cyanide anion have been proposed,[3] only two cyanide receptors have been practically applied as antidotes for cyanide poisoning. One of the major therapies for cyanide poisoning is nitrite/thiosulfate treatment. This method is based on the coordination of CN^- to methemoglobin (metHb) derived from the oxidation of Hb by the nitrite anion. Because the nitrite/thiosulfate therapy requires the oxidation of Hb to metHb, which is inactive for O_2 binding, this method is not adequate for a patient affected by cyanide poisoning during a fire accident, whose maximum oxygen uptake has been reduced by strong binding of carbon monoxide to Hb.[4] We determined the association constant (K_{ass}) for the binding of the cyanide to metHb to be 8.14×10^5 M^{-1} at pH 7.0 and 37 °C (vide infra). Therefore, a cyanide receptor with a value of $K_{ass} \geq 8 \times 10^5$ M^{-1} may act as an antidote for cyanide poisoning, although many other requirements should be satisfied for its practical use as an antidote. Hydroxocobalamin (OHCbl) is another practically applied antidote.[2,4] The K_{ass} of OHCbl for cyanide in vitro is 7.96×10^6 M^{-1} at pH 7.0 and 37 °C (vide infra). Although OHCbl is bound to serum proteins in vivo, thereby reducing the K_{ass},[5] this material has been regarded as a good antidote with minimal side effects.[6] Nonetheless, OHCbl has drawbacks, such as high cost, long residence time in the body, potential allergic response, and slow association rate toward the cyanide. In the present study, attempts were made to prepare completely artificial cyanide receptors that overcome the limitations of OHCbl.

Several years ago, we found that 1:1 inclusion complexes of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphinatoiron(II) ($Fe^{(II)}$TPPS) with per-*O*-methylated *β*-cyclodextrin dimers having nitrogenous axial ligands at their linkers, such as Py3CD, Py2CD, and Im3CD (Figure 1), show biomimetic functions quite similar to those of the oxygen-storing hemoprotein, myoglobin (Mb).[7−10] These supramolecular ferrous complexes reversibly bind molecular oxygen as well as carbon monoxide in aqueous solution. The previous results suggest that the $Fe^{(III)}$TPPS complexes of the cyclodextrin dimers bind the cyanide in a similar manner to metHb and metMb. Before initiating this project, we knew that ferric hemoCD1 (met-hemoCD1) injected into the femoral vein of a rat is reduced in the blood to afford ferrous hemoCD1, which captures O_2 and endogenous CO in the blood and is rapidly excreted in the urine.[11] This nature of ferric hemoCD1 renders it inappropriate for use as an antidote for cyanide poisoning because the cyanide scarcely binds to a ferrous porphyrin. In this study, it was found that the $Fe^{(III)}$TPPS/Im3CD complex (Fe(III)PIm3CD, Figure 1) was the most suitable choice for a cyanide receptor for in vivo use.

It is well established that two CN^- anions coordinate to porphinatoiron(III) in organic solvents.[12−17] Quite recently, a spectroscopic and kinetic study of the cyanide binding to a water-soluble ferric porphyrin (5,10,15,20-tetrakis(2,4,6-trimethyl-3-sulfonatophenyl)porphinatoiron(III)) revealed the formation of mono- and dicyano-adducts with K_{ass} values of

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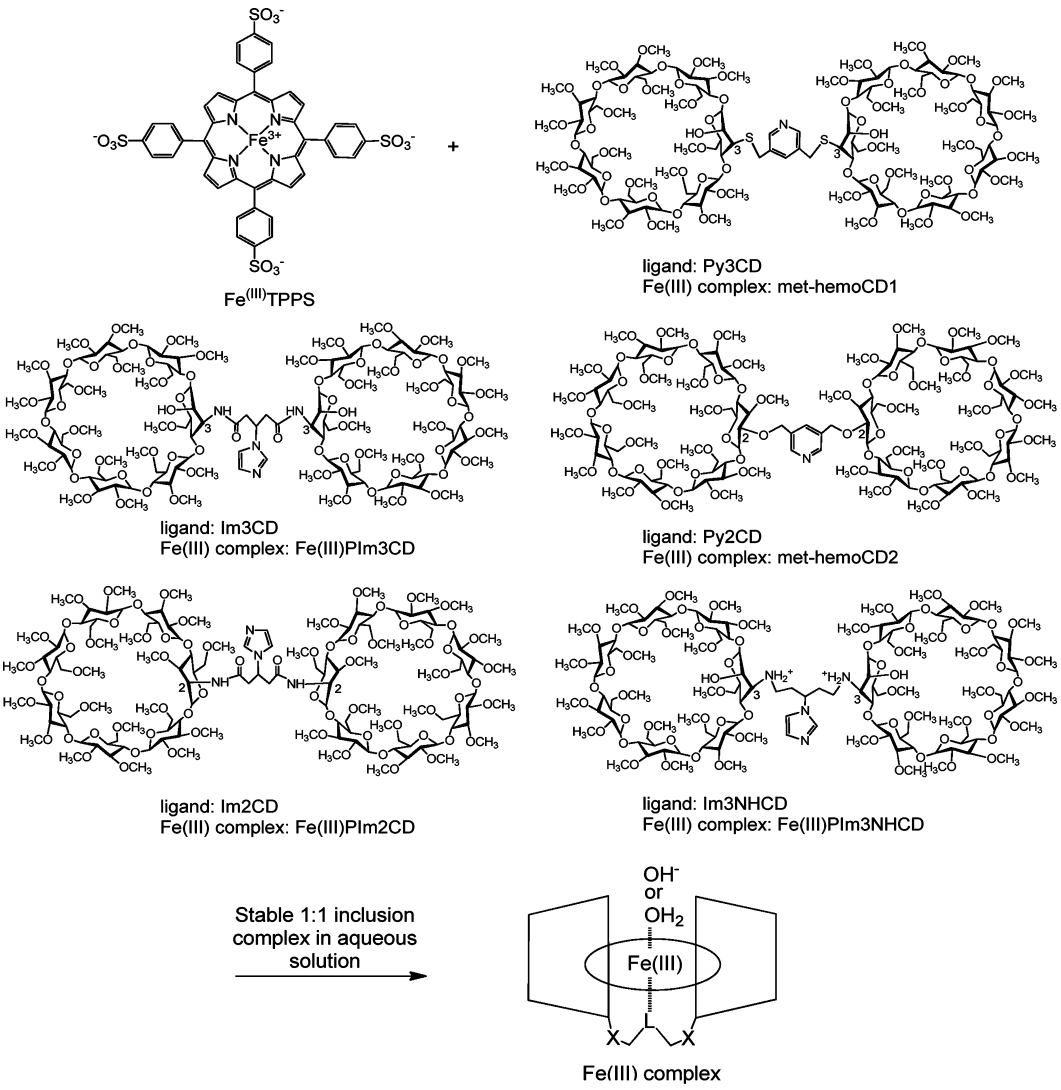

Figure 1. Various supramolecular cyanide receptors in aqueous solutions.

5.5×10^5 and 3.8×10^5 M^{-1}, respectively, at pH 8.0 and 20 °C.[18] $Fe^{(III)}$TPPS (7 μM) also formed $(CN^-)_2Fe^{(III)}$TPPS in aqueous solution, with the K_{ass} values for the 1:1 and 2:1 complex formation being 832 ± 97 and 2593 ± 474 M^{-1}, respectively, at pH 7.0 (0.1 M phosphate buffer) and 37 °C (Supporting Information, Figure S1). In the presence of 28 μM heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMe-β-CD), both the K_{ass1} and K_{ass2} values were reduced to 354 ± 86 and 152 ± 26 M^{-1}, respectively (Supporting Information, Figure S2). Because the two TMe-β-CD molecules encapsulate $Fe^{(III)}$TPPS to form a *trans*-type 2:1 inclusion complex,[19] a TMe-β-CD capsule may prevent the approach of the cyanide to the ferric center of the porphyrin. The K_{ass} values for $Fe^{(III)}$TPPS by itself and the 2:1 TMe-β-CD-$Fe^{(III)}$TPPS complex are too small for practical application as antidotes for cyanide poisoning.

It was expected that Fe(III)PIm3CD would be a promising antidote because of the following reasons. Although a ferrous form of this supramolecule exhibits functions similar to those of Mb, the autoxidation of the dioxygen adduct of Fe(III)-PIm3CD (O_2-Fe(III)PIm3CD) proceeds at a much faster rate than that of O_2-hemoCD1.[9] The fast autoxidation of O_2-Fe(II)PIm3CD is ascribed to a wider interspace between the two cyclodextrin units because of the relatively rigid amide bonds at the linker positions. The wider interspace causes easy penetration of H_2O into the capsule, leading to H_2O-promoted autoxidation of O_2-Fe(II)PIm3CD to Fe(III)PIm3CD and a superoxide ion. In addition, the carbon monoxide affinity of Fe(II)PIm3CD ($P_{1/2}^{CO}$ = 1.6 × 10^{-3} Torr at pH 7.0 and 25 °C) is much lower than that of hemoCD1 (1.5 × 10^{-5} Torr). These previous results suggest that Fe(III)PIm3CD is stable in the blood. Indeed, we confirmed that Fe(III)PIm3CD injected into the femoral vein of a rat was rapidly excreted in the urine without reduction to its ferrous form (Supporting Information, Figure S3).

Figure 2 shows the UV−vis spectral changes of Fe(III)-PIm3CD ([$Fe^{(III)}$TPPS] = 5.0 × 10^{-6} M, [Im3CD] = 6.5 × 10^{-6} M) upon addition of NaCN in 0.05 M phosphate buffer at pH 7.0 and 37 °C. The titration curves were fitted to an equation for the 1:1 complexation to give a K_{ass} value of (2.61 ± 0.34) × 10^6 M^{-1}. A continuous variation method also supported the 1:1 complex formation. Therefore, it can be concluded that no ligand exchange of the nitrogenous axial ligand with the cyanide anion occurs. Because the pK_a value of HCN is 9.0,[20] the K_{ass} at pH 7.0 is apparently represented by K_{ass} = [(CN^-)Fe(III)PIm3CD][H^+][L]/[(L)Fe(III)PIm3CD]-[HCN], where L = H_2O or OH^-. Similar titration experiments

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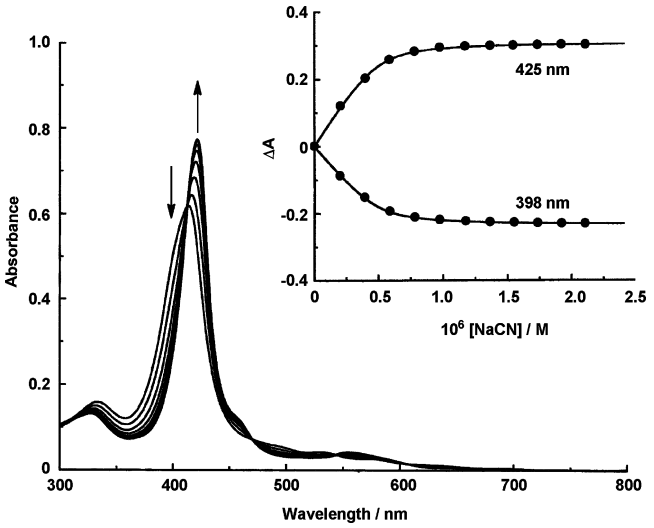

Figure 2. UV−vis spectral changes of Fe(III)PIm3CD ([$Fe^{(III)}$TPPS] = 5.0×10^{-6} M; [Im3CD] = 6.5×10^{-6} M) upon addition of various amounts of NaCN in phosphate buffer (pH 7.0, 5.0×10^{-2} M) at 37 °C. Inset: Plots of absorbance changes at 398 and 425 nm. The black solid lines represent the best fit of data to the theoretical equation for 1:1 complexation, which determined the K_{ass}.

provided the K_{ass} values for the other supramolecular receptors shown in Figure 1 and for the natural receptors such as OHCbl, metHb, and metMb (Table 1). The K_{ass} values for the

Table 1. Rate Constants (k_{on} and k_{off}), Association Constant (K_{ass}), and pK_a (H_2O/OH^-) Values of Various CN^- Receptors in Phosphate Buffer (pH 7.0, 5.0×10^{-2} M) at 37 °C[a]

receptor	$10^{-6}K_{ass}$ (M^{-1})	k_{on} (M^{-1} s^{-1})	$10^6 k_{off}$ (s^{-1})	pK_a (H_2O/OH^-)
met-hemoCD1	2.11 ± 0.63	14.9	7.06	5.5[8]
met-hemoCD2	2.20 ± 0.23	39.3	17.9	6.9[10]
Fe(III)PIm3CD	2.61 ± 0.34	193	73.9	7.7
Fe(III)PIm3CD/serum proteins[b]	1.34 ± 0.43	104	77.6	N. D.[c]
Fe(III)PIm2CD	1.71 ± 0.06	101	59.1	7.4
Fe(III)PIm3NHCD	6.91 ± 2.59	24.1	3.49	6.8
OHCbl	7.96 ± 0.73	81.5	10.2	8.1[22]
OHCbl/serum proteins[b]	0.013 ± 0.008	6.5	500	N. D.[c]
metHb (human)	0.814 ± 0.033	320	393	8.33[23]
metMb (horse)	0.193 ± 0.040	305	1580	8.93[24]

[a]The values of k_{off} were calculated from the following equation: $k_{off} = k_{on}/K_{ass}$. [b]Experiments carried out in fetal bovine serum at pH 7.0 and 37 °C. See ref 21. [c]N. D. = not determined.

$Fe^{(III)}$TPPS complexes of the cyclodextrin dimers were significantly larger than those for metHb and metMb. The K_{ass} of OHCbl ($(7.96 \pm 0.73) \times 10^6$ M^{-1}) was the largest of all the receptors examined in this study, whereas association of OHCbl to serum proteins decreased this value so that it became the smallest ($K_{ass} = 1.3 \times 10^4$ M^{-1}).[21] Although the K_{ass} value for Fe(III)PIm3CD also decreased in the aqueous serum protein solution, the diminution rate was much smaller than the case of OHCbl.

Because cyanide poisoning is fulminant, association of the cyanide to a receptor must occur rapidly. The second-order rate constant for the formation of each cyanide adduct (k_{on}) was determined from the linear relationship between the pseudo-first-order rate constant and the receptor concentration (Supporting Information, Figure S4). No attempts were made to distinguish between the reactions with HCN and CN^-. The k_{off} values were calculated from k_{on} and K_{ass}. The kinetic results are also summarized in Table 1. Association of the cyanide to metHb and metMb proceeds at extraordinarily fast rates, although the dissociation of the cyanide adducts of these receptors also occurs rapidly. The k_{on} for Fe(III)PIm3CD is characteristically larger than the corresponding values of the other supramolecular receptors as well as those of the OHCbl and the OHCbl/serum protein complex.[21] The k_{on} values for Fe(III)PIm3CD and the Fe(III)PIm3CD/serum protein system are ca. 30- and 16-fold, respectively, larger than that for the OHCbl/serum protein complex. The kinetic data show the primacy of Fe(III)PIm3CD over OHCbl as a cyanide receptor in vivo.

In the absence of cyanide, H_2O or OH^- functions as the sixth axial ligand of the ferric ion of each $Fe^{(III)}$TPPS/cyclodextrin dimer complex. It is assumed that the ligand exchange between H_2O and CN^- occurs more readily than the exchange between OH^- and CN^-. Therefore, there may be a correlation between the pK_a of acid-dissociation of H_2O bound to Fe(III) and k_{on}. The previously unreported pK_a(H_2O/OH^-) values were determined from the UV−vis spectral changes as a function of pH (Supporting Information, Figures S5 and S6); the pK_a(H_2O/OH^-) values of the cyanide receptors are listed in Table 1. Figure 3 shows the plot of k_{on} vs pK_a(H_2O/OH^-),

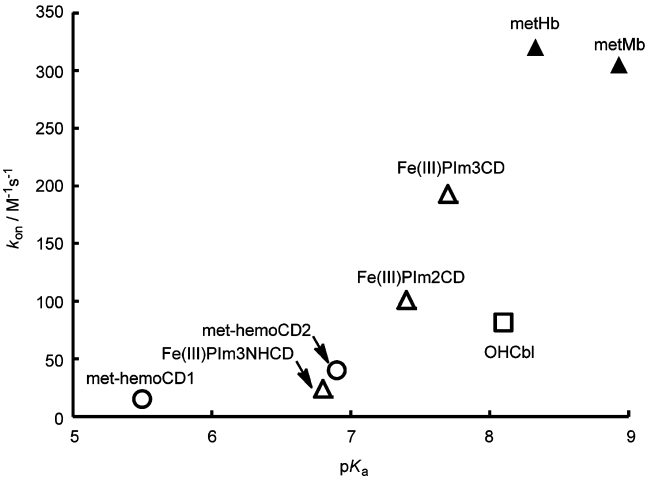

Figure 3. Plots of k_{on} as a function of pK_a (H_2O/OH^-).

which demonstrates that the k_{on} value increases regularly with increasing pK_a(H_2O/OH^-). Among the supramolecular cyanide receptors, the k_{on} of Fe(III)PIm3CD is characteristically large. This is ascribed to the higher pK_a(H_2O/OH^-) value (7.7) of this receptor. More than 50% of the Fe(III)PIm3CD molecules are present as an aqua complex at pH 7.0, resulting in fast ligand exchange with cyanide. In contrast, the hemoCD1 molecules having a lower pK_a(H_2O/OH^-) (5.5) exist predominantly in the hydroxo form at pH 7.0, leading to a slow exchange rate. The remarkably large k_{on} values for metHb and metMb can be interpreted in terms of the extremely high pK_a(H_2O/OH^-) values. In spite of a high pK_a(H_2O/OH^-) value, the k_{on} for OHCbl is smaller than that for Fe(III)-PIm3CD. Because OHCbl is a Co(II) complex of corrole

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ligand, the regular relationship between k_{on} and $pK_a(H_2O/OH^-)$ for the ferric porphyrin complexes is not applicable.

The efficiency of Fe(III)PIm3CD as a cyanide receptor in vivo was examined. Fe(III)PIm3CD ([Im3CD]/[$Fe^{(III)}$TPPS] = 1.2, 0.10 mmol/kg, 1.5 mL) in PBS was constantly infused into the left femoral vein of a rat (Wistar male, 270−330 g) for 5 min. Immediately after the Fe(III)PIm3CD infusion, NaCN (0.05 mmol/kg, 1.0 mL) in PBS was infused into the left femoral vein for 10 min. In control experiments without the infusion of Fe(III)PIm3CD, all three rats died within 7−16 min after the infusion of NaCN. In contrast, all four rats pretreated with 2 equiv of Fe(III)PIm3CD survived for 4 h until they were euthanized prior to awaking from anesthesia. UV−vis spectroscopic analysis indicated that approximately 69% of the infused Fe(III)PIm3CD was excreted in the urine (Supporting Information, Figure S8). About 50% of the receptor molecules in the excreted urine collected 1 h after infusion existed in the cyanide adduct form.

Fe(III)PIm3CD exhibits superior characteristics as a cyanide receptor, such as strong and fast binding with cyanide and rapid excretion of the cyanide adduct in urine. Fe(III)PIm3CD interacts minimally with serum proteins and may retain its inherent characteristics in vivo. Fe(III)PIm3CD is a completely artificial material that can be synthesized in a high yield (vide infra). For practical use of Fe(III)PIm3CD as an antidote for cyanide poisoning, further experiments in vivo are now in progress.

EXPERIMENTAL PROCEDURES

In order to provide Fe(III)PIm3CD for practical use, the synthesis of Im3CD should be optimized. The starting material, 2^A-(*p*-tosyl)-*β*-cyclodextrin, was commercially available and was converted to 2^A-hydroxy-3^A-amino-3^A-deoxy-per-*O*-methyl-*β*-cyclodextrin via a 3-step reaction (72% total yield). The linker material was 3-(1*H*-imidazol-1-yl)pentanedioic acid, which was synthesized from 1,5-diethyl-2-pentenedioate via a two-step reaction (85% total yield). Finally, the amino-*β*-cyclodextrin was reacted with the linker material in DMF in the presence of the condensation reagent to yield Im3CD (76% yield). The details of the synthesis of Im3CD are provided in the Supporting Information.

The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Doshisha University.

ASSOCIATED CONTENT

Supporting Information

Materials and apparatuses, additional details for animal experiments and the synthesis of Im3CD, raw data of the UV−vis titrations and the stopped-flow measurement, and profiles of the excretion of Fe(III)PIm3CD. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

metMb, methemoglobin; OHCbl, hydroxocobalamin; $Fe^{(II)}$TPPS, 5,10,15,20-tetrakis(4-sulfonatophenyl)-porphinatoiron(II); TMe-*β*-CD, heptakis(2,3,6-tri-*O*-methyl)-*β*-cyclodextrin; Mb, myoglobin

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