Iron(II) Complex of Octopus-Porphyrin with a Covalently Linked Proximal Imidazole; Self-Assembly and O_2-Coordination in Aqueous Media

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The iron complex of tetrakis[2,6-di(alkanoyloxy)phenyl]porphyrin derivative with seven phosphocholine head groups and an *N*-alkylimidazole side-chain as the proximal base (octopus-porphyrin) has been synthesized. The ferrous complex (**7b**) was easily dispersed in water to provide a stable colloid. Transmission electron microscopy and scanning force microscopy of the evaporated solution showed spherical micelles with a diameter of 8 nm, which probably consists of a dimer of **7b**. These micellar aggregates could reversibly coordinate O_2 at 25 °C; and the O_2-binding parameters are also given.

Symmetric octopus-porphyrins having eight amphiphilic alkyl chains *as long legs* are self-organized in water to form beautiful molecular assemblies, e.g., monolayer fiber, planar sheet, and bilayer vesicle.[1] Transmission electron microscopy (TEM) and scanning force microscopy (SFM) have always revealed the morphologies of these aggregates in nanometer units, and excitonic interactions appearing in the Soret band absorption have also provided useful information as well. Furthermore, these porphyrin assemblies show unique functions, i.e., O_2-transport like hemoglobin (Hb), selective light-induced charge separation, and vectrial electron transfer.[1] For dioxygenation of the iron(II) complex, however, a large excess amount of an external nitrogenous base, *namely N-alkylimidazole*, as an axial ligand is needed, because the steric hindrance of the octopus-legs inhibits the coordination of the externally added base. Therefore, its O_2-binding affinity is quite low.[1a,c] To overcome this fault, we recently synthesized a new octopus-porphyrinatoiron complex with seven alkylphosphocholine groups and an intramolecularly coordinated proximal imidazole. The ferrous complex (**7b**, Chart 1) is easily dispersed in water to produce a spherical micelle, which can reversibly coordinate O_2 at 25 °C with a similar O_2-binding affinity of the red blood cells. The kinetics of the O_2-binding to the **7b** micelle is also described herein.

Experimental

Materials and Appratus. Infrared spectra were recorded with a JASCO FT/IR-410 spectrometer. ^{1}H NMR spectra were measured using a JEOL Lambda 500 spectrometer. FAB-MS spectra were obtained from a JEOL JMS-SX102A spectrometer. UV-vis absorption spectra were recorded on a JASCO V-570 spectrophotometer. 5,10,15,20-Tetrakis(2,6-dihydroxyphenyl)porphyrin and 5-(1-imidazolyl)pentanoic acid hydrochloride were prepared according to our previously reported procedures.[2] All of the solvents were purified by distillation before use. Other chemicals were of commercial high-purity grades and were not further purified. The water used was deionized using a ADVANTEC GS-200. Transmission electron microscopy (TEM) and scanning force microscopy (SFM) were observed, as previously reported.[1b]

5-[2-(5-Bromopentanoyloxy)-6-hydroxyphenyl]-10,15,20-tris(2,6-dihydroxyphenyl)porphyrin (1). After 5-bromopentanoic acid (1.5 g, 8.3 mmol) was dissolved in oxalyl chloride (3.6 mL, 41 mmol) under a nitrogen atmosphere, the mixture was stirred for 1.5 h at room temperature. Following the appearance of $\nu_{C=O}$ (acid cloride) at 1800 cm^{-1}, excess oxalyl chloride was removed in vacuo. Then, a small amount of this acid chloride (63 μL, 471 μmol) dissolved in dry THF (100 mL) was slowly added dropwise to 5,10,15,20-tetrakis(2,6-dihydroxyphenyl)porphyrin (0.5 g, 673 μmol) in a dry THF solution (400 mL) including 4-dimethylaminopyridine (DMAP) (58 mg, 471 μmol) at room temperature for 2 h under nitrogen. After reacting for 12 h, the solvent was brought to dryness on a rotary evaporator and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous Na_2SO_4. The organic phase was concentrated and the residue was dissolved in $CHCl_3$/MeOH, 8/1 (v/v). The unsolved part was filtered with a G4 glass-filter, the filtrate was chromatographed on a silica-gel column. The second band from the last was collected and dried in vacuo to give a purple product (**1**) (126 mg, 19%). R_f = 0.35 ($CHCl_3$/MeOH, 8/1, v/v). ^{1}H NMR (CD_3OD, 500 MHz): δ −0.1–0.2 (4H, m, –$(CH_2)_2$–), 6.7–7.6 (12H, m, phenyl), 8.8 (8H, s, pyrrole β-H). IR (KBr) 3300–3800 (OH), 3318 (NH, porphyrin), 2926, 2856 (CH_2, alkyl), 1735 (C=O, ester) cm^{-1}. UV-vis (CH_3OH) 417, 513, 548, 587, 640 nm. FAB-MS *m/z* 905.1 [M$^+$].

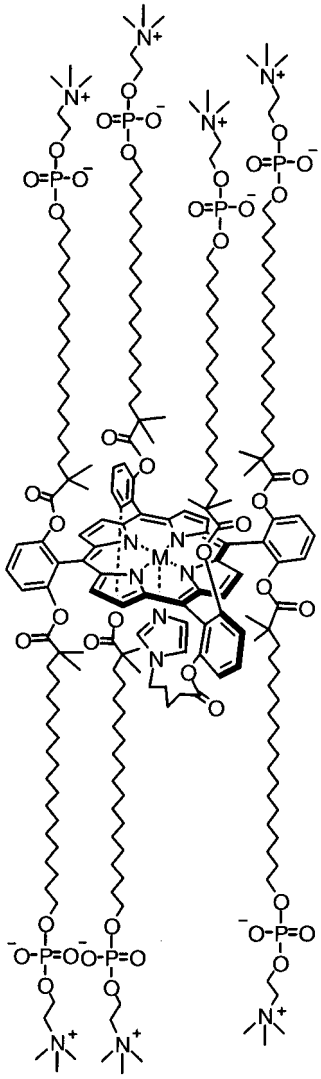
5-[2-(20-Benzyloxy-2,2-dimethyleicosanoyloxy)-6-(5-bromopentanoyloxy)phenyl]-10,15,20-tris[2,6-bis(20-benzyloxy-2,2-dimethyleicosanoyloxy)phenyl]porphyrin (2). 20-Benzyloxy-2,2-dimethyleicosaoic acid (1.73 g, 3.9 mmol) was dissolved in oxalyl chloride (1.7 mL, 19.4 mmol) and stirred for 1.5 h at

CREST investigator, JST.

room temperature. After confirming the appearance of $\nu_{C=O}$ (acid chloride) at 1800 cm^{-1}, excess oxalyl chloride was removed in vacuo. The addition of *N*-bromotrimethylsilane (2.6 mL, 19.4 mmol) in dry THF (5 mL) into this acid chloride under a nitrogen atmosphere gave the corresponding acid bromide. After stirring for 30 min at room temperature, excess *N*-bromotrimethylsilane was removed in vacuo. A dry THF solution (14 mL) of **1** (126 mg, 139 μM) and DMAP (475 mg, 3.9 mmol) was then poured into the acid bromide, and reacted for 12 h at 60 °C under nitrogen. The solution was evaporated to dryness and the residue was extracted with $CHCl_3$. The organic phase was concentrated, and the residue was washed with CH_3OH at 40 °C. The mixture was chromatgraphed on a silica-gel column using $CHCl_3/Et_2O$, 45/1 (v/v) as the eluent, to give a purple product (**2**) (383 mg, 71%). R_f = 0.54 ($CHCl_3/Et_2O$, 45/1, v/v). ^1H NMR ($CDCl_3$, 500 MHz) δ −3.0 (2H, s, inner H), −1.2–−0.5 (42H, m, dimethyl), 0.6–1.6 (244H, m, $-(CH_2)_{17}-$), 3.0 (2H, t, $-CH_2Im$), 3.5 (14H, t, $-CH_2O-$), 4.5 (14H, s, $-OCH_2Ph$), 7.3–7.8 (47H, m, phenyl), 8.8 (8H, s, pyrrole β-H). IR (NaCl) 3317 (NH, porphyrin), 2923, 2852 (CH_2, alkyl), 1758 (C=O, ester), 1225 (CO, phenol)), 1108 (CO, ether) cm^{-1}. UV-vis ($CHCl_3$) 415, 509, 538, 584, 639 nm.

5-[2-(20-Benzyloxy-2,2-dimethyleicosanoyloxy)-6-hydroxyphenyl]-10,15,20-tris[2,6-bis(20-benzyloxy-2,2-dimethyleicosanoyloxy)phenyl]porphyrin (3). Compound **2** (383 mg, 98 μmol) and imidazole (534 mg, 7.8 mmol) were dissolved in dry DMF (17 mL) and reacted for 4 h at 80 °C under a nitrogen atmosphere. After the solvent was removed in vacuo, the residue was extracted with $CHCl_3$. The organic layer was washed several times with water and dried over anhydrous Na_2SO_4. The organic phase was then concentrated and the residue was chromatographed on a silica-gel column using $CHCl_3/Et_2O$, 45/1 (v/v) as the eluent. The main band was collected and dried in vacuo to give a purple product (**3**) (283 mg, 77%). R_f = 0.50 ($CHCl_3/Et_2O$, 45/1, v/v). ^1H NMR ($CDCl_3$, 500 MHz) δ −3.0 (2H, s, inner H), −1.1–−0.4 (42H, m, dimethyl), 0.6–1.6 (244H, m, $-(CH_2)_{17}-$), 3.5 (14H, t, $-CH_2O-$), 4.5 (14H, s, $-OCH_2Ph$), 7.3–7.8 (47H, m, phenyl), 8.8 (8H, s, pyrrole β-H). IR (NaCl) 3300–3800 (OH), 3318 (NH, porphyrin), 2923, 2852 (CH_2, alkyl), 1758 (C=O, ester), 1225 (CO, phenol)), 1109 (CO, alcohol) cm^{-1}. UV-vis ($CHCl_3$) 415, 509, 538, 584, 639 nm. FAB-MS *m/z* 3743.0 [M^+].

5-{2-(20-Benzyloxy-2,2-dimethyleicosanoyloxy)-6-[5-(1-imidazolyl)pentanoyloxy]phenyl}-10,15,20-tris[2,6-bis(20-benzyloxy-2,2-dimethyleicosanoyloxy)phenyl]porphyrin (4). Oxalyl chloride (1.3 mL) was poured into a dehydrated acetnitrile solution (3 mL) of 5-(1-imidazolyl)pentanoic acid hydrochloride (202 mg, 15 mmol), and stirred for 1 h at 60 °C under an argon atmosphere. Excess solvents were removed in vacuo and dry a THF solution (7.6 mL) dissolved **5** (283 mg, 76 μmol) and DMAP (370 mg, 3.0 mmol) was added. After reacting for 12 h at 60 °C under argon, the solvent was evaporated and the residue was extracted with $CHCl_3$. The organic layer was washed with water and dried over anhydrous Na_2SO_4. A rotary evaporator then concentrated the $CHCl_3$ phase and the residue was chromatgraphed on a silica-gel column using $CHCl_3$/MeOH, 30/1 (v/v) as the eluent, to give a purple product (**4**) (173 mg, 59%). R_f = 0.68 ($CHCl_3$/MeOH, 30/1 (v/v)). ^1H NMR ($CDCl_3$, 500 MHz) δ −3.0 (2H, s, inner H), −1.5–−0.5 (42H, m, dimethyl), 0.6–1.6 (244H, m, $-(CH_2)_{17}-$), 3.5 (14H, t, $-CH_2O-$), 4.5 (14H, s, $-OCH_2Ph$), 7.3–7.8 (47H, m, phenyl), 6.6–7.1 (3H, t, Im), 8.8 (8H, m, pyrrole β-H). IR (NaCl) 3318 (N-H, porohyrin), 2923, 2856 (CH_2, alkyl), 1758 (C=O, ester), 1225 (CO, phenol), 1110 (CO, ether) cm^{-1}. UV-vis ($CHCl_3$) 415, 509, 536, 585, 640 nm.



M: Fe^{3+} **7a**

Fe^{2+} **7b**

Chart 1.

5-{2-[5-(1-Imidazolyl)pentanoyloxy]-6-(20-hydroxy-2,2-dimethyleicosanoyloxy)phenyl}-10,15,20-tris[2,6-bis(20-hydroxy-2,2-dimethyleicosanoyloxy)phenyl]porphyrin (5). The $Et_2O{\cdot}BF_3$ complex (0.78 mL, 6.2 mmol) and EtSH (4.6 mL, 62 mmol) were added into a dry CH_2Cl_2 solution (3 mL) of **4** (173 mg, 45 μmol) under an argon atmosphere. After stirring for 4 h at room temperature, the resulting solution was cautiously dropped into ice-water. $CHCl_3$ extracted the suspension, and the organic layer was washed with water and dried over anhydrous Na_2SO_4. The organic phase was concentrated and the residue was chromatographed on a silica-gel column using $CHCl_3$/MeOH, 10/1 (v/v) as the eluent, to afford a purple product (**5**) (104 mg, 72%). R_f = 0.50 ($CHCl_3$/MeOH, 10/1 (v/v)). ^1H NMR ($CDCl_3$, 500 MHz) δ −3.0 (2H, s, inner H), −1.5–−0.5 (42H, m, dimethyl), 0.6–1.6 (244H, m, $-(CH_2)_{17}-$), 3.5 (14H, t, $-CH_2OH$), 7.3–7.8 (12H, m, phenyl), 6.6–7.1 (3H, t, Im), 8.8 (8H, m, pyrrole β-H). IR (NaCl) 3300–3800 (OH), 3318 (NH, porphyrin), 2920, 2851 (CH_2, alkyl),

1756 (C=O, ester), 1111 (CO, alcohol) cm^{-1}. UV-vis ($CHCl_3$) 415, 507, 536, 583, 638 nm. FAB-MS *m/z* 3262.2 [M^+].

Iron(Ⅲ) Chloride Complex of 5 (6). Iodine (16 mg, 63 μmol) was added to a dry toluene solution (9 mL) of **5** (104 mg, 32 μmol) under an argon atmosphere, and $Fe(CO)_5$ (334 μL, 2.5 mmol) was injected into the solution via a microsyringe. The solution was stirred for 18 h at 100 °C. After confirming that the reaction had completed by the disappearance of the fluorescence of the free-base porphyrin (ca. 18 h), the solution was cooled to room temperature and aqueous NaCl was added. $CHCl_3$ extracted the suspension, and the organic layer was dried over anhydrous Na_2SO_4. The organic phase was concentrated and the residue was chlomatgraphed on a silica-gel column using $CHCl_3$/MeOH, 10/1 (v/v) as the eluent, giving a purple product (**6**) (94 mg, 88%). R_f = 0.50 ($CHCl_3$/MeOH, 10/1 (v/v)). IR (NaCl) 3300–3800 (OH), 2920, 2850 (CH_2, alkyl), 1754 (C=O, ester), 1225 (CO, phenol), 1107 (CO, alcohol) cm^{-1}. UV-vis ($CHCl_3$) 348, 415, 504, 582, 682 nm. FAB-MS *m/z* 3351.8 [M^+-Cl].

Octopus-porphyrinatoiron(Ⅲ) Chloride (7a). 2-Chloro-2-oxo-1,3,2-trioxaphospholane (58 μL, 587 mmol) was added to a dry CH_2Cl_2 solution (17 mL) of **6** (94 mg, 28 μmol) and triethylamine (82 μL, 587 mmol) at 0 °C under an argon atmosphere. After stirring the mixture for 1 h and an additional 1 h at room temperature, the solvent was removed in vacuo. The resultant phosphate triester was then dissolved into dry DMF (17 mL) containing trimethylamine (1 mL). The mixture was sealed in a pressure bottle and allowed to react for 1 h at room temperature. After reacting for an additonal 16 h at 60 °C, the solvent was removed and the residue was reprecipitated into a cold acetone/water solution (4/1). The obtained brown precipitate was purified by gel column chromatography on a Sephadex LH-60 using benzene/MeOH (3/1) as the eluent, to give a red-brown product (**7a**). IR (KBr) 2923, 2852 (CH_2, alkyl), 1756 (C=O, ester), 1225 (CO, phenol), 1107 (CO, alcohol), 1252 (P=O), 1088 (POC) cm^{-1}. UV-vis ($CHCl_3$) 376, 414, 507, 581, 642 nm. Found: C, 60.43; H, 8.79%. Calcd for $C_{241}H_{416}N_{13}O_{44}P_7FeCl\ 3CHCl_3$: C, 60.22; H, 8.68%.

Preparation of Aqueous Octopus-porphyrinatoiron(Ⅱ) Solution. A small excess molar of aqueous ascorbic acid was added to a **7a** methanol solution (50 μL, 1.2 mM) under a CO atmosphere. The injection of this methanolic carbonyl-**7b** into water (3 mL, 70 °C) gave a red-colored solution, which was incubated for 4 h at room temperature. Excess ascorbic acid was removed by dialysis with a cellulose tube in deionized water for 4 h at 4 °C.

O_2-Binding Equilibrium and Kinetics. O_2-Binding to the octopus-porphyrinatoiron(Ⅱ) was expressed by

$$\mathrm{Fe(II)P} + \mathrm{O_2} \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} \mathrm{Fe(II)P\text{-}O_2}. \quad (K = k_{on}/k_{off}) \qquad (1)$$

The O_2-binding affinity (gaseous pressure at half O_2-binding for Fe(Ⅱ)P, $P_{1/2} = 1/K$) was determined by UV-vis absorption spectral changes during O_2 titration.[2,3] The porphyrin concentrations of 10–20 μM were normally used for the absorption spectroscopy. The spectra were recorded within the range of 350–700 nm. The O_2-association and -dissociation rate constants (k_{on}, k_{off}) were determined using a Unisoku TSP-600 laser-flash photolysis apparatus.[2–5]

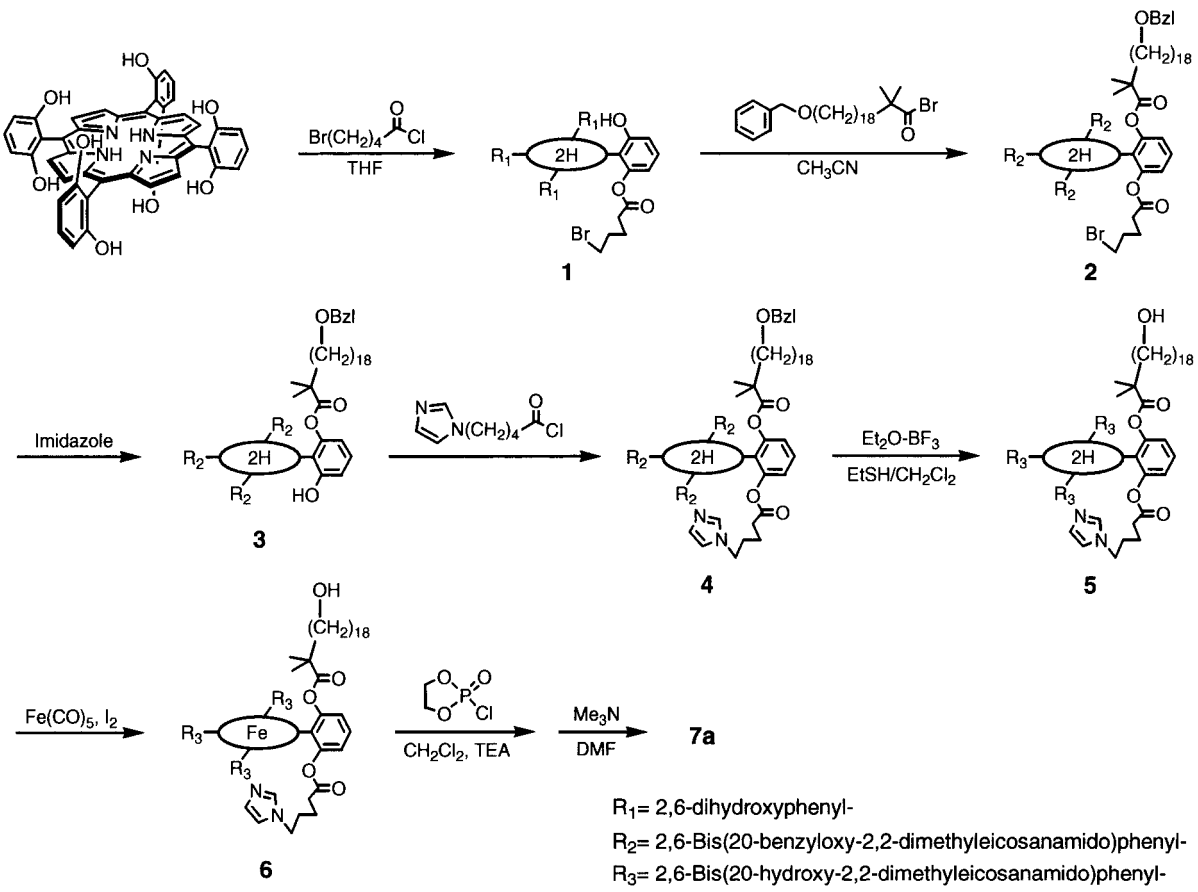
Results and Discussions

Our first attempt to introduce seven C_{20}-alkyl chains to the eight hydroxy groups of the tetrakis(2,6-dihydroxyphenyl)porphyrin (TDPP) unfortunately failed. We obtained only six- and eight-substituted products, which were clarified by ^1H NMR spectroscopy. The 5-bromopentanoyl chloride was then reacted with TDPP to yield the mono 5-bromopentanoylated TDPP (**1**) (19%) (Scheme 1). After introducing of the C_{20}-alkyl chains into the remaining seven hydroxy groups, the first pentanoyloxy residue was selectively saponified to give **3** (77%). The 5-(1-imidazolyl)pentanoyl side-chain was then attached to this position, and $Et_2O{\cdot}BF_3$ with EtSH allowed cleavage of the benzyl end groups, thus yielding **5** (72%). The insertion of the central iron was performed by $Fe(CO)_5$, however, it took an unusually long time (18 h) for the fluorescence based on the free-base porphyrin to disappear, which is caused by the octopus-legs' encumbrance on the porphyrin ring plane.

Upon rapid injection of the methanolic carbonyl complex of **7b** into deionized water, a red-colored homogeneous colloid was obtained ([MeOH] $<$ 2 vol%). This solution did not change for more than six months at room temperature, and no precipitation was observed. The TEM of the negatively stained colloid showed spherical micelles with a diameter of 8 nm, which corresponds to the molecular length of **7b** (7.6 nm) (Fig. 1a). Other procedures used to disperse in water, e.g. vortex mixing or sonication, also consistently gave the same micelles. This micellar morphology was not sensitive to the presence of electrlyte, e.g. the addition of NaCl (0.1 M) caused no precipitation, in the range of pH 6–10. An SFM measurement of an evaporated solution of the **7b** micelle exhibited a flattened round particle with a height of 1.96 nm on the graphite surface (Fig. 1b). This value is in good agreement with the double length of the rigid tetrakis[2,6-di(alkanoyloxy)phenyl]porphyrin center (2.2 nm). These observations are quite different from the former results of our symmetric octopus-porphyrin with *eight* alkylphosphocholine groups, which produces a long fiber with a stacked porphyrin axis and μm length.[1b] In this fiber, the hydrophobic and symmetrical porphyrin center involved eight 2,2-dimethyl groups strongly aligned like a string of pearls. We made a hypothesis that the interaction of these hydrophobic porphyrin centers is mainly responsible for the formation of the fibers, as well as the hydrophobic interaction of the eight C_{20}-alkyl chains.[1b,d] However, the introduction of the (1-imidazolyl)alkyl arm to the porphyrin periphery produced a decrease in the symmetry of the porphyrin center; therefore, it cannot grow into a long fiber, and the aggregation is probably stopped by the dimer (Fig. 2).

In the UV-vis absorption spectrum of the carbonyl-**7b** micelle, the Soret band was significantly shifted to the red-region relative to that of the monomeric methanol solution (λ_{max}: 418 → 426 nm). The red-shift of the Soret band is presumably due to an exciton interaction, which can be attributed to a laterally slipped stacking arrangement of the two porphyrin chromospheres (Fig. 2).[1b] We can conclude that the bimolecular dimer of **7b** is the basis for each spherical micelle.

Light irradiation of this colloid under N_2 allowed CO dissociation, giving the five-*N*-coordinated iron(Ⅱ) complex (λ_{max}: 441, 538, 562 nm). Upon exposure of these micelles to O_2, the UV-vis absorption spectrum changed to that of the O_2-adduct complex (λ_{max}: 422, 550 nm). The dioxygenation was kinetically stable and reversible at 25 °C, depending on the partial



Scheme 1.

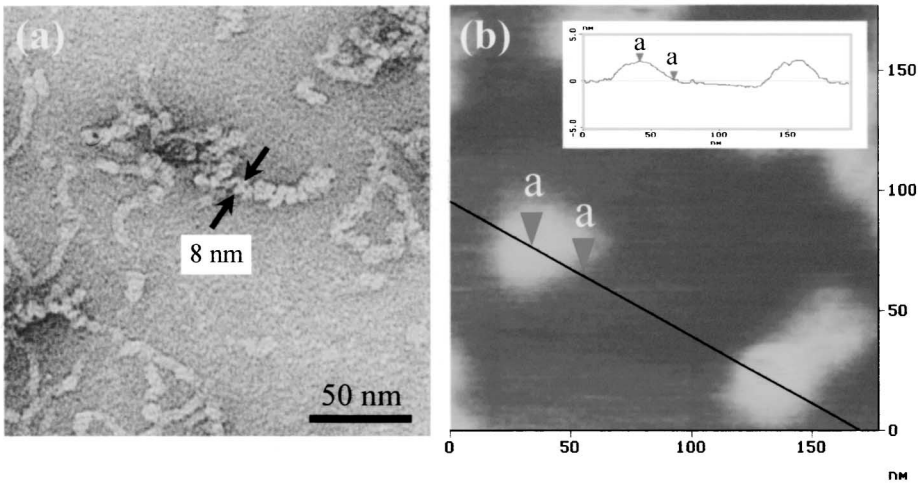


Fig. 1. (a) TEM of the evaporated **7b** micelle stained by uranyl acetate, and (b) SFM image (tapping mode) of the evaporated sample of the **7b** micelle on HOPG surface. Image size is 180 × 180 nm^2 and vertical a-a distance is 1.96 nm.

O_2 pressure. The half-life of the dioxygenated complex was determined to be ca. 11 h at 25 °C, which is 2.5-fold longer compared to that of the similar amphiphilic terakis-(2-alkanamidophenyl)porphyrinatoiron(II) fiber reported previously.[3] The O_2-binding affinity [$P_{1/2}$ (= $1/K$); 0.98 kPa] was almost the same as the red blood cell's ($P_{1/2}$ = 1.2 kPa),[6] but the O_2-binding profile of the **7b** micelle did not show a cooperativity like that seen in Hb; the Hill coefficient was 1.0 (Fig. 3).

The O_2-association and -dissociation rate constants (k_{on}, k_{off}) were determined to be 6.7 × 10^6 M^{-1} s^{-1} and 81 s^{-1}, respectively; k_{on} was relatively slower than those of the other both-faces encumbered porphyrinatoiron(II) (10^7–10^8 M^{-1} s^{-1}).[2,7] This might be caused by a steric hindrance of the flexible long alkyl chains.

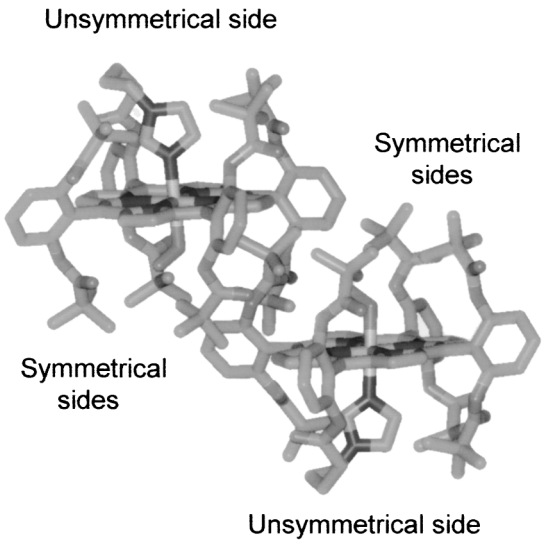


Fig. 2. Proposed structure of the **7b** micelle center which consists of the dimeric porphyrin moieties. The laterally slipped stacking geometry of the symmetrical side (Fe–Fe distance: 1.3 nm) is based on the results in Ref. 1b.

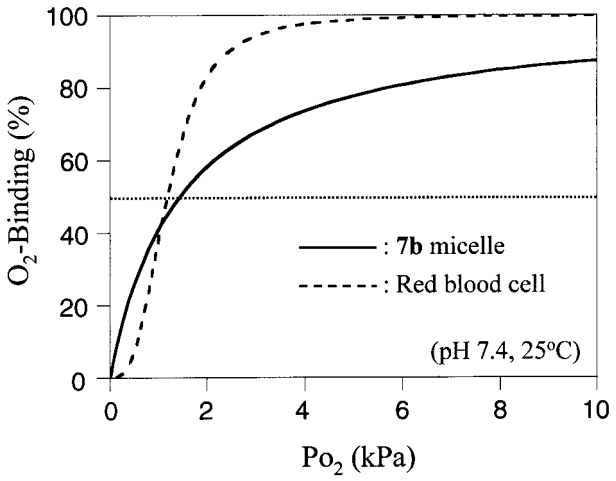


Fig. 3. The O_2-binding equilibrium curves of the **7b** micelle and red blood cells.

In conclusion, the iron(II) complex of the octopus-porphyrin (**7b**) is self-organized in water to give a minimum-size molecular assembly (ϕ = 8 nm), which can reversibly bind and release O_2 in aqueous media. The O_2-binding affinity is high enough and almost identical to those of the red blood cells.

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