Micellar Amphiphilic Heme as Synthetic Oxygen Carrier under Semiphysiological Conditions

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As model compounds for respiratory hemoproteins, many synthetic heme complexes have been synthesized and can bind oxygen under nonphysiological conditions [1–4]. The oxidation reaction in relation to H_3O^+ must be restricted to prepare a stable oxygen complex against oxidation even in aqueous media. Recently, it has been reported that *meso*-tetra(α,α,α,α-*o*-pivalamidophenyl)porphinato iron(II) **2** can bind oxygen under semiphysiological conditions (in neutral water at 37 °C) when **2** is incorporated into the hydrophobic region of phospholipid liposome [5, 6]. This could be explained by the effect of the hydrophobic microenvironment constructed by the lipid bilayer of low polarity preventing the oxygen complex from oxidation caused by H_3O^+. But complex **2** in micelles gave no stable oxygen adduct. We then designed and synthesized a novel amphiphilic heme having alkoxyphosphocholine groups, which was expected to be more compatible with micelle or liposome because of its amphiphilicity [7].

In this communication, the incorporation of the novel amphiphilic heme **1**, *meso*-tetra{α,α,α,α-*o*-[20-(2′-trimethylammonioethoxy)phosphinatoxy-2,2-dimethyleicosanamido]phenyl}porphinato iron, illustrated in Fig. 1, in micelle and the oxygenation reaction of the complex under semi-physiological conditions will be reported.

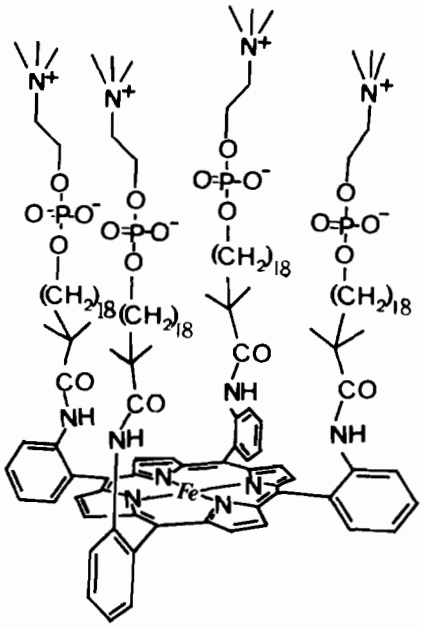

Fig. 1. Schematic representation of the amphiphilic heme **1**.

Experimental

The amphiphilic heme **1** and imidazole derivatives were synthesized according to the previous paper [6, 7]. 0.1 M Phosphate buffer (pH 7.0) was used as solvent throughout this communication.

Visible absorption spectra and fluorescence spectra were recorded on a Shimazu UV-240 spectrophotometer and a FP-500 spectrofluorometer (Nippon Bunko Ltd.), respectively.

1 (1.7 mg, 0.6 μmol), 1-lauryl-2-methylimidazole (7.6 mg, 30.0 μmol), and the surfactant (Triton X-100, Rohm & Haas Co.) (150 mg, 200 μmol) were dissolved in methanol (10 ml) and to this was added 10% Pd-on-carbon (5 mg) under nitrogen. H_2 gas was then bubbled through the solution for 40 min which was then filtered (Millipore Ltd., Millex SR) and the filtrate was transferred to a vacuum uv cell (path length: 10 mm) under N_2. The solvent was removed under reduced pressure and to the residue was added oxygen-free phosphate buffer (15 ml) to give the homogeneous solution of the corresponding deoxy heme complex. This solution was used for studying oxygenation reactions. The final concentration of the heme **1** was 40 μM. For the determination of oxygen binding equilibrium curves, the apparatus for measuring the oxygen concentration and visible absorption spectra was used at the same time [6, 8].

Results and Discussion

The amphiphilic heme **1** was soluble in alcohols, but not in water. However, **1** could be dissolved in water in the presence of synthetic surfactants like Triton X-100 (poly(oxyethylene) n-octylphenyl ether) [9] as described in the experimental section. The incorporation of **1** in micelle was studied by gel permeation chromatography and fluorescence

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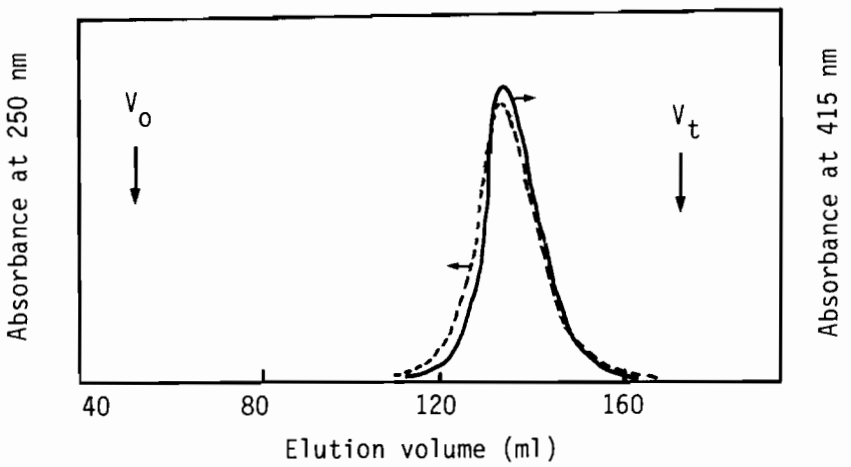

Fig. 2. The elution patterns of the **1**-surfactant mixture determined by gel permeation chromatography on Sepharose 4B (2 × 54 cm). Solvent: 0.1 M phosphate buffer (pH 7 0); Elution rate· 1.5 ml/min, Vo: void volume determined by Blue Dextrane 2000; Vt total bed volume.

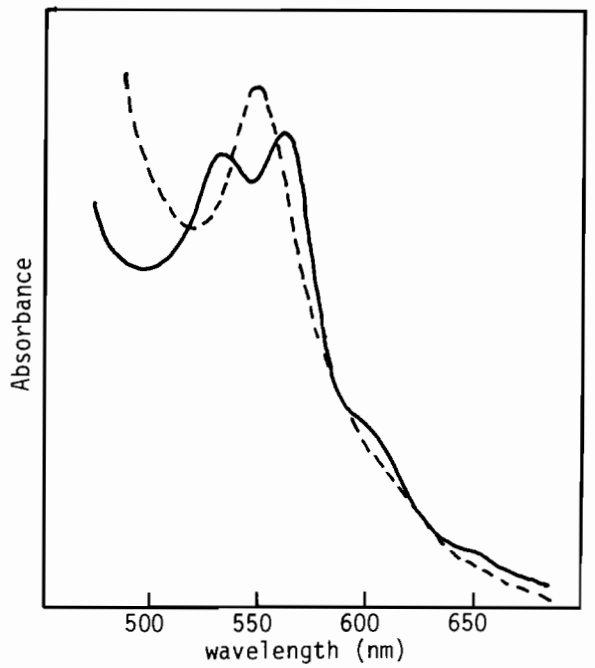

Fig. 3. Visible absorption spectra of the heme **1** embedded in micelle formed by Triton X-100 under nitrogen (A, ——) and under oxygen (B, – – –) at 25 °C [heme] = 40 μM, [1-lauryl-2-methylimidazole] = 2 0 mM, [Triton X-100] = 1 0 wt/vol%.

spectrometry. Figure 2 shows the elution pattern of the heme-surfactant mixture determined on Sepharose 4B (Pharmacia Fine Chemicals). The elution curve determined by monitoring the absorbance at 415 nm based on **1** was consistent with that of the micelle itself (detected at 250 nm). The average molecular weight of the aggregates formed was calculated by the k_{av} value (0.74) to be 3×10^4 [10]. The incorporation of **1** in the hydrophobic environment of micelle was confirmed using the fluorescence probe, *meso*-tetra{α,α,α,α-*o*-[20-(2′-trimethylammonioethoxy)-phosphinatoxy-2,2-dimethyleicosanamido] phenyl}-porphinato zinc(II) **3**. The fluorescence spectrum (λ_{ex}: 418 nm), with maxima at 620 and 669 nm, agrees with those in aprotic organic solvents like propanol. The fluorescence intensity is stronger than in aprotic organic solvents. This result also indicates that the metalloporphyrin is molecularly dispersed in micelle and surrounded by an environment similar to that in an organic solvent.

The micelle-incorporated heme was then studied for its oxygen binding ability at 25 °C. The visible absorption spectra of the micellar heme **1** are shown in Fig. 3. The initial absorption spectrum (A) under N_2 (λ_{max}: 532 and 561 nm) is characteristic of the five coordinated deoxy heme complex [2, 5, 6]. Bubbling oxygen gas through the deoxy solution caused a rapid spectral change to give the new absorption spectrum (B) (λ_{max}: 547 nm), which agrees with that of the oxygen adduct [2, 5, 6]. The spectrum changed reversibly from B to A dependent on oxygen pressure. These results indicate the formation of the oxygen complex without irreversible oxidation. The life time of the oxygen complex ($t_{1/2}$) was determined by measuring the time-change of absorbance at 547 nm. From the first order plot, $t_{1/2}$ was calculated to be longer than 5 hours at 25 °C, while the complex with 1,2-dimethylimidazole in place of 1-lauryl-2-methylimidazole gave an unstable oxygen adduct ($t_{1/2}$ 0.2 h). The complex of **1** with 1-laurylimidazole, however, formed a very stable oxygen complex against oxidation when it was embedded in liposome [11]. The fact that **1** binds oxygen reversibly even in micellar aqueous solution shows the importance of its amphiphilic property

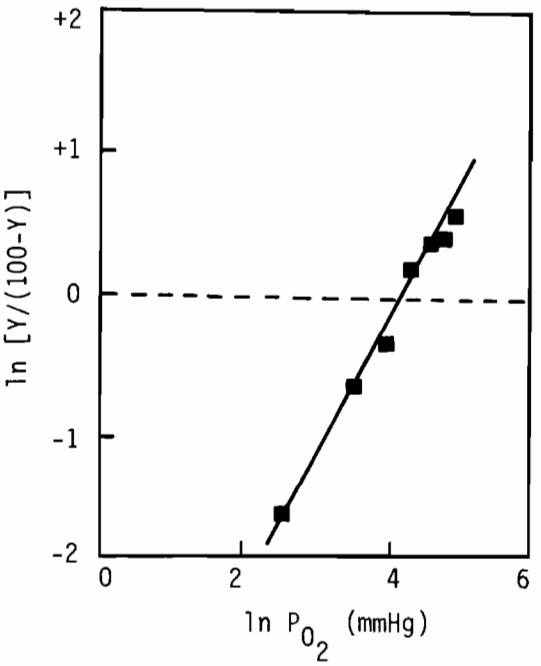

Fig. 4. The Hill's plot of the oxygen binding equilibrium of the heme 1-mono(1-lauryl-2-methylimidazole) complex embedded in micelle formed by Triton X-100 at 37 °C (Conditions shown in Fig 3).

which makes it more compatible with the micelle aggregate.

The oxygen binding equilibrium curve of the complex with 1-lauryl-2-methylimidazole was determined and the Hill's plot [12] is shown in Fig. 4. The Hill's equation for an oxygenation reaction

$$Fe + O_2 \overset{K}{\rightleftharpoons} FeO_2$$

where K means an equilibrium constant is represented by the following equation:

$$\ln[Y/(100 - Y)] = n \ln P_{O_2} + \ln K$$

where Y, n, and P_{O_2} mean the degree of oxygenation (%), the Hill's coefficient, and the partial oxygen pressure (mm Hg). Figure 4 shows a straight line with a slope equal to 1.0, indicating the Langmuir type oxygen binding reaction without any cooperative interaction between hemes. The oxygen pressure for half oxygenation of heme at 37 °C was calculated to be 64 mm Hg, which agrees with the value for **2** embedded in liposome (49 mm Hg) [6]. The micellar heme **1** has a value close to that of hemoglobin in red blood cells (27 mm Hg) [13], but considerably different to that of myoglobin (0.9 mm Hg) [12].

Thus it was found that the novel amphiphilic heme **1** embedded in micelle can bind oxygen reversibly under semiphysiological conditions. Details of the oxygenation reaction will be reported.

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