Characteristic O_2-Binding of Lipidprotoheme in Phospholipid Bilayer Membrane

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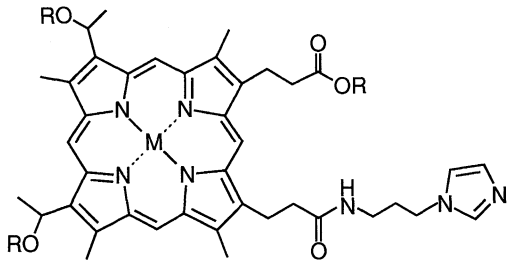
A protoporphyrin IX derivative having three long alkyl chains and an axial imidazole (lipidprotoporphyrin) was homogeneously embedded into the bilayer membrane of the phospholipid vesicle. The O_2-binding affinity of the lipidprotoheme was affected by the phase transition of the bilayer membrane, reflecting the change in the O_2-association rate constant.

It is well known that the multi-step O_2-binding to protohemes in hemoglobin (Hb) is conjugated with the structural transition from the "tense (T)" to "relaxed (R)" state of the globin chains; the O_2-binding affinity of the R-state Hb is, in fact, 180-fold higher than that of the T-state. Several hemoprotein models have been synthesized so far and their performances were widely studied, *e.g.*, the micelle or phospholipid vesicle containing metalloporphyrins.[1-4] However, to date, it is difficult to regulate the O_2-binding equilibrium of the protoheme derivative using the synthetic molecular environment around the porphyrin moiety.

We have recently found that a protoporphyrin IX derivative having three long alkyl chains and an axial imidazole [lipidprotoporphyrin (**1b**, **1c**)] is homogeneously embedded into the bilayer membrane of the phospholipid vesicle and its O_2-binding affinity is affected by the phase transition of the membrane. We describe herein the characteristic O_2-binding property of the lipidprotoheme embedded in the phospholipid vesicle.



R: $-(CH_2)_{11}CH_3$ M: 2H a
Zn(II) b
Fe(III)Cl c'
Fe(II) c

Lipidprotoporphyrin (**1**)

The synthetic routes for the lipidprotoporphyrin are as follows. 8,13-Bis(1-dodecanoxyethyl)-3,7,12,17-tetramethyl-2,18-bis(2-dodecanoxycarbonylethyl)-21H,23H-porphine (DDP) was synthesized according to a previous procedure.[5] DDP was dissolved in 4N hydrochloric acid/THF solution and stirred for 14 h at room temperature. After evaporation, the residue was extracted with CH_2Cl_2 and the mixture was separated by column chromatography; the second elution band was the porphyrin mono-ester mono-acid (28%). This porphyrin was dissolved in anhydrous THF including 4-(N-dimethylamino)pyridine under an argon atmosphere. The mixed anhydride was then quantitatively formed by adding pivaloyl chloride and was reacted with 1-(3-aminopropyl)imidazole. The obtained crude product was chromatographed on a silica gel column affording **1a** (45%). Zinc or iron insertion into **1a** was carried out using $Zn(AcO)_2$ or $FeCl_2$, respectively. All porphyrin derivatives were characterized by IR, UV-vis and ^{1}H-NMR spectroscopics.[6]

1b or **1c'** was sonicated with a phospholipid molecule [1,2-bis(parmytoyl)-*sn*-glycero-3-phosphocholine (DPPC)] [**1b** or **1c'** : DPPC = 1:50 (molar ratio), [Por]= 1×10^{-5} M] in deionized water to give a phospholipid vesicle embedding the lipidprotoporphyrin solution. Incorporation of the lipidprotoporphyrin in the vesicle was confirmed by gel-permeation chromatography using Sepharose CL-4B and monitored at 419 nm and 255 nm based on the porphyrin derivatives and phospholipid, respectively.[7] The curves coincided with each other meaning that the porphyrin is completely included in the bilayer membrane of the phospholipid vesicle. From the TEM of the hybrid assembly containing the lipidprotoporphyrin, only small unilamellar vesicles with a diameter of 40-50 nm were detected.

The coordination structure and dispersing behavior of **1b** in the phospholipid bilayer were observed using its UV-vis absorption spectral pattern. The λ_{max} of the DPPC vesicle embedded **1b** appeared at 419, 548, and 582 nm, which are identical with those of the five N-coordinated species of **1b** in $CHCl_3$. This result indicated that **1b** was homogeneously dispersed in the hydrophobic environment in the phospholipid vesicle as a monomeric form and intramolecularly formed five N-coordinated species.

The lipidprotohemin (**1c'**) in the phospholipid vesicle was reduced by the addition of a small excess of aqueous $Na_2S_2O_4$ under an argon atmosphere giving a Fe(II) **1c** complex. The absorption spectrum of **1c** embedded in the DPPC vesicle (λ_{max}: 422, 523 (shoulder), and 553 nm) showed the formation of a five N-coordinated deoxy species. The spectrum of the deoxy form changed to that of a CO adduct upon exposure to CO (λ_{max}: 414, 533, and 561 nm).

The O_2-binding equilibrium constant (K) and O_2-association and -dissociation rate constants (k_{on}, k_{off}) were estimated using the CO protect method with laser flash photolysis (Table 1).[8] The K of **1c** embedded in 1,2-bis(myristoyl)-*sn*-glycero-3-phosphocholine (DMPC) or 1,2-bis(octadecadienoyl)-*sn*-glycero-3-phosphocholine (DODPC) vesicle at 25°C was lower than that of the DPPC vesicle. This was kinetically attributed to the reduced O_2-association rate constant. It is well known that the bilayer membrane of the phospholipid vesicles have a clear gel-(liquid crystal) phase transition temperature (Tc); DPPC: 41°C, DMPC: 23°C, DODPC: 18°C.[9] Therefore, the obtained parameters showed that the O_2-binding equilibrium constant of **1c** in the phospholipid vesicle was changed above and below the Tc of the bilayer membrane. Concerning the DMPC vesicle embedding

Table 1. O_2-Binding paramaters of **1c** embedded in the phospholipid vesicle in aqueous medium at 25 °C

Lipid	Tc[a] / °C	10^{-3} K / M^{-1}	10^{-7} k_{on} / $M^{-1}s^{-1}$	10^{-3} k_{off} / s^{-1}
DPPC	41	2.2	7.0	3.2
DMPC	23	0.36	1.3	3.7
DODPC	18	0.34	1.1	3.2
poly-DODPC	–	0.51	1.9	3.7

[a]From Ref. 9.

1c, a van't Hoff plot (*ln* K vs. 1/T) and Arrhenius plot (*ln* k_{on} vs. 1/T) for the O_2-association are shown in Figure 1. In both cases, the temperature dependencies were drastically changed at ca. 23°C, which just corresponds to the Tc of the DMPC vesicle. The enthalpy and entropy changes (ΔH, ΔS) for the O_2-binding equilibrium and activation energy (E_a) of k_{on} were estimated above and below the Tc, respectively (Figure 1). The values of the thermodynamic parameters below the Tc are comparable to those of a chelated heme in toluene,[10] however, the values above the Tc are higher than those of the others. Thus, the characteristic O_2-binding behavior of **1c** embedded in the phospholipid vesicle can be explained as follows. Below the Tc,

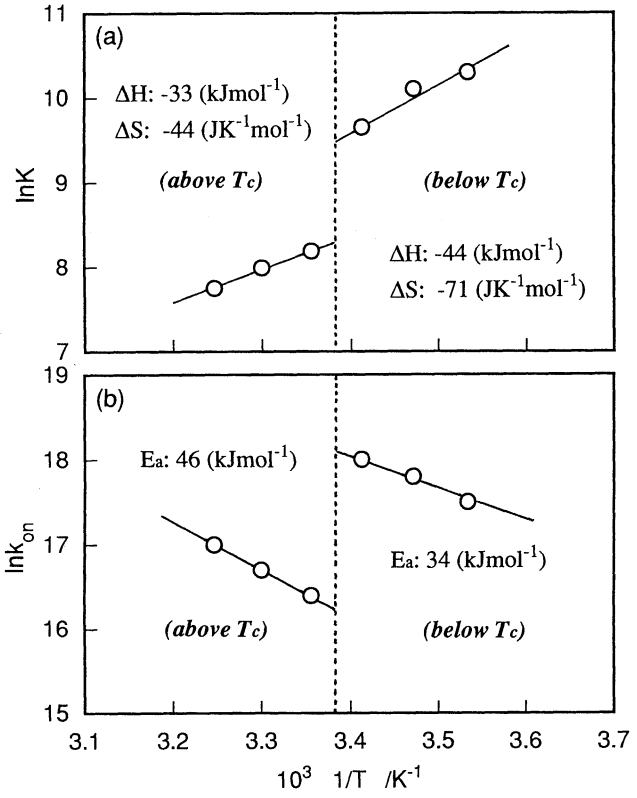


Figure 1. (a) van't Hoff plot of K and (b) Arrhenius plot of k_{on} of **1c** embedded into DMPC vesicle.

e.g., in the case of the DPPC vesicle at 25°C, the phospholipid molecules surrounding the **1c** complex are in gel state, which causes an orientation of the porphyrin plane parallel to the acyl chains in the bilayer membrane. Therefore, the O_2-binding reaction of **1c** is not retarded by the crystallized alkyl moieties of the phospholipid bilayer. On the other hand, above the Tc, *e.g.*, in the DMPC or DODPC vesicle at 25°C, **1c** is located in the liquid-crystal state. It is assumed that a high mobility of the acyl chains in the phospholipid bilayer sterically hindered the O_2-association to **1c**, resulting in a reduction of the k_{on}.

Furthermore, in the case of the poly-DODPC vesicle (polymerization conversion: 55%),[5] k_{on} was slightly increased in comparison with that before polymerization. This suggested that the polymerization of phospholipid component reduces the fluidity of the bilayer membrane, so the steric hindrance during O_2-association to **1c** is somewhat relieved.

In conclusion, the O_2-binding affinity of the lipidprotoheme embedded in the phospholipid vesicle can be regulated by the phase state of the bilayer membrane. That is, the T- and R-states of Hb were mimicked using the phase transition behavior of the phospholipid vesicle.

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References and Note

1 E. Tsuchida and H. Nishide, *Top. Curr. Chem.*, **132**, 64 (1986); E. Tsuchida, T. Komatsu, K. Arai, and H. Nishide, *J. Chem. Soc., Dalton Trans.*, **1993**, 2465.
2 J. T. Groves and R. Neumann, *J. Am. Chem. Soc.*, **111**, 2900 (1989).
3 M. Nango, A. Mizusawa, T. Miyake, and J. Yoshinaga, *J. Am. Chem. Soc.*, **112**, 1640 (1990).
4 N. Nishino, H. Mihara, K. Kobata, and T. Fujimoto, *J. Chem. Soc., Chem. Commun.*, **1993**, 162.
5 T. Komatsu, K. Yamada, S. Yanagisawa, H. Nishide, and E. Tsuchida, *Chem. Lett.*, **1994**, 1953.
6 **1a**; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ -3.8 (2H, s, inner H), 0.8-1.8 (71H, m, $-CH_2-$), 2.2 (6H, d, $-CH(OCH_2-)\underline{CH_3}$), 3.0 (2H, t, $-CH_2$-Im), 3.3 (4H, t, $-CH_2COO-$), 3.4 (2H, t, $-CONHCH_2-$), 3.7 (12H, m, pyrrole β-CH_3), 3.8 (4H, m, $-CH_2O-$), 4.2 (4H, t, $-COOCH_2-$), 4.5 (4H, t, $-\underline{CH_2}CH_2-$COO-), 6.1 (2H, m, $-\underline{CH}(OCH_2-)CH_3$), 6.1, 6.5, 6.8 (3H, Im H), 10.0, 10.6 (4H, d, *meso*-H). FABMS: 1210.8 M^+. IR (NaCl): ν 1730 (C=O (ester)), 1664 (C=O (amide)) cm^{-1}. UV-vis ($CHCl_3$): λ_{max} = 623, 568, 533, 499, and 402 nm.
7 A small amount of DODPC (see text) was added as the UV absorption probe.
8 J. P. Collman, J. I. Brauman, B. L. Iverson, J. I. Sessler, R. M. Morris, and Q. H. Gibson, *J. Am. Chem. Soc.*, **105**, 3052 (1983); T. G. Traylor, D. Campbell, M. Mitchel, D. Stynes, and N. Koga, *J. Am. Chem. Soc.*, **107**, 604 (1985); E. Tsuchida, T. Komatsu, S. Kumamoto, K. Ando, and H. Nishide, *J. Chem. Soc., Perkin Trans. 2*, **1995**, in press.
9 S. Takeoka, H. Iwai, H. Ohno, and E. Tsuchida, *Bull. Chem. Soc., Jpn.*, **62**, 102 (1989).
10 T. G. Traylor, A. P. Berzins, *Proc. Natl. Acad. Sci. USA*, **77**, 3171 (1980); F. C. Mills, G. K. Ackers, H. T. Gaud, S. J. Gill, *J. Biol. Chem.*, **254**, 2875 (1979).