Dynamics of Oxygen Binding to Modified Iron(II)–Porphyrin Complexes embedded in the Bilayer of Phospholipid Liposome†

Makoto Yuasa, Hiroyuki Nishide, and Eishun Tsuchida *
Department of Polymer Chemistry, Waseda University, Tokyo 160, Japan

Activation parameters have been determined for the binding of oxygen to 5,10,15,20-tetra{α-*o*-[2′,2′-dimethyl-20′-(2″-trimethylammonioethylphosphonatoxy)eicosanamido]phenyl}porphyrinatoiron(II) (lipid-heme) and 5,10,15,20-tetra(α-*o*-pivalamidophenyl)porphyrinatoiron(II) (heme) complexes of 1-lauryl-2-methylimidazole embedded in the bilayer of phospholipid liposome (abbreviated liposome-embedded lipid-heme and heme, respectively). Activation parameters, $\Delta H^{\ddagger}_{on}$ and $\Delta S^{\ddagger}_{on}$, for the liposome-embedded lipid-heme were consistent with those of hemoglobin and myoglobin, but those for the liposome-embedded heme were similar to those of red blood cells. A potential energy diagram and binding pathway for the oxygen-binding reaction to the liposome-embedded lipid-heme and to the liposome-embedded heme are discussed in comparison with those of hemoglobin.

Hemoglobin (hb) and myoglobin (mb), which are composed of iron(II)–porphyrin complexes and globin proteins,[1] serve to transport and store molecular oxygen in a living body. If their oxygen-binding site, the iron(II)–porphyrin complex, is taken out and set in a solution it is irreversibly oxidized and does not function as an oxygen carrier. For reversible oxygen binding to hb and mb it is considered that the globin protein of hb and mb 'tucks' the iron(II)–porphyrin complex separately in the macromolecule to prevent its irreversible oxidation through dimer formation, and the hydrophobic domain of the globular protein excludes water molecules to suppress its proton-driven oxidation.

The crystal structure around the iron(II)–porphyrin or the oxygen-binding site of hb and mb has been well established.[2] The skeleton structure of the protein forms a pocket above the iron(II)–porphyrin plane thereby maintaining the oxygen-binding site vacant for penetration of gaseous molecules. The pocket is constructed with hydrophobic amino acid residues which also contribute to suppressing oxidation of the iron(II). A pathway for oxygen binding to hb and mb has been proposed, and the effect of the protein on the dynamics of the oxygen binding has been discussed by use of data measured extensively with stopped-flow, flash-photolysis, and T-jump techniques.[3,4] The protein forms a dense wall between the external medium and the iron(II)–porphyrin complex, and a calculated three-dimensional map of the protein conformation suggested that fluctuation of the protein conformation reduces the energy barrier for oxygen penetration and provides a pathway for oxygen binding.[5–7]

Recently we succeeded in achieving reversible oxygen binding. Thus 5,10,15,20-tetra(α-*o*-pivalamidophenyl)porphyrinatoiron(II) (heme) and 5,10,15,20-tetra{α-*o*-[2′,2′-dimethyl-20′-(2″-trimethylammonioethylphosphonatoxy)eicosanamido]-phenyl}porphyrinatoiron(II) (lipid-heme) complexes of 1-lauryl-2-methylimidazole (lmi) (lauryl = dodecyl) embedded in the bilayer of phospholipid liposome (abbreviated as liposome-embedded heme and lipid-heme) bind molecular oxygen reversibly under physiological conditions (in neutral aqueous media at 37 °C).[8,9] It was considered that the heme complex was included and dispersed in the hydrophobic environment of the liposome which protected the oxygen adduct of the heme from its irreversible oxidation.[8] The lifetime (half-life) of the oxygen adduct of the liposome-embedded heme was several days and its oxygen-binding affinity close to that of hb in blood.[10] Oxygen-binding rate parameters of the liposome-embedded heme were similar to those of red blood cells.[11]

In this note, kinetic and activation parameters for oxygen binding to the heme and the lipid-heme embedded in the bilayer of phospholipid liposome have been estimated by stopped-flow and flash-photolysis methods. A potential energy diagram for the oxygen-binding reaction has been drawn and the oxygen-binding pathway to the liposome-embedded heme and lipid-heme discussed in comparison with those of hb.

Experimental

The heme and the lipid-heme were synthesized as reported in previous papers.[9,12] Phospholipids, L-α-dimyristoylphosphatidylcholine (dmpc) and L-α-dipalmitoylphosphatidylcholine (dppc), were purchased from Sigma (special grade). The liposome-embedded heme and lipid-heme were prepared as described in previous papers.[8,9] A suspension including the heme or lipid-heme, lmi, and the phospholipid (molar ratio 1:20—50:50—200) was ultrasonicated and homogenized in 0.1 mol dm^{-3} phosphate buffer solution (pH 7.0) to give red and transparent solutions of the liposome-embedded heme and lipid-heme.

Oxygen-binding rate constants for the liposome-embedded heme and lipid-heme in aqueous medium and for the heme– and lipid-heme–lmi complexes in organic solvent (toluene or methanol) at 10—40 °C were measured using stopped-flow and flash-photolysis spectrophotometers equipped with kinetic data processors (Unisoku SF-1000 and FP-2000, respectively). After rapid mixing of the liposome-embedded heme or lipid-heme solution with the buffer solution saturated with oxygen, the spectrum ($\lambda_{max.}$ at 438, 535, and 562 nm for the liposome-embedded heme; 437, 535, and 563 nm for the liposome-embedded lipid-heme) of the deoxy-complex changed to that of the oxygen adduct ($\lambda_{max.}$ at 422 and 546 nm for the liposome-embedded heme; 422 and 546 nm for the liposome-embedded lipid-heme). On bubbling carbon monoxide through the mixed solutions, the oxygen adduct changed to the carbon monoxide adduct ($\lambda_{max.}$ at 423 and 540 nm for the liposome-embedded heme; 523 and 541 nm for the liposome-embedded lipid-heme).

† *Non-S.I. unit employed:* cal = 4.184 J.

Table. Activation parameters for the oxygen-binding reactions of liposome-embedded heme and lipid-heme at 25 °C and pH 7.0

Heme	Solvent	$10^{-4}k_{on}$/ dm^3 mol^{-1} s^{-1}	$\Delta H^{\ddagger}_{on}$/ kcal mol^{-1}	$\Delta S^{\ddagger}_{on}$/ cal K^{-1} mol^{-1}	Ref.
Liposome-embedded heme					
dmpc[a] (below T_c)	H_2O	—	3.8	−32	This work
dmpc[a] (above T_c)	H_2O	0.79	10	−7.3	This work
dppc[a]	H_2O	0.24	4.2	−31	This work
Liposome-embedded lipid-heme, dmpc (above T_c)	H_2O	9 800	10	13	This work
Red blood cell suspension	H_2O	1.1	11	−4.0	This work
mb[b]	H_2O	1 000—2 000	—	—	*b*
hb[b]	H_2O	3 300	—	—	*b*
Heme–Imi[c]	Toluene	11 000	10	11	This work
Lipid-heme–Imi	Methanol	10 000	10	12	This work
Chelated-heme[d]	H_2O	3 900	10	11	*d*

[a] Liposome composed of dmpc, (dimyristoylphosphatidylcholine) or dppc (dipalmitoylphosphatidylcholine). [b] At 20 °C, from ref. 4. [c] Heme–Imi = 5,10,15,20-tetra(α-*o*-pivalamidophenyl)porphyrinatoiron(II) complex of 1-lauryl-2-methylimidazole. [d] Chelated-heme = the iron(II) complex of the *N*-[3-(imidazol-1-yl)propylamide] methyl ester of 3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionic acid, at pH 7.3, T. G. Traylor and A. P. Berizinic, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3175.

The oxygen adducts of the liposome-embedded lipid-heme in aqueous medium and of the heme and lipid-heme complexes in toluene or methanol were so stable and reversible that flash photolysis on the oxygen adducts could be carried out without using the carbon monoxide protection method.[13] The oxygen adduct was irradiated with a pulse flash to cause it to photodissociate. Quantum yields of the photodissociation were 0.06 and 0.04 for the liposome-embedded heme and lipid-heme, and 0.03 and 0.03 for the heme and lipid-heme complexes, which were similar to those of 'oxy-hb' and 'oxy-mb' (0.008 and 0.03, respectively[4]). After photodissociation, the deoxy complex rebound oxygen and this rapid re-binding reaction was monitored with the spectrophotometer.

The oxygen-binding rate constant (k_{on}) was determined by pseudo-first-order kinetics.[3,4] Activation parameters ($\Delta H^{\ddagger}_{on}$, $\Delta S^{\ddagger}_{on}$) for the oxygen binding were calculated from Arrhenius plots of the k_{on} values (10—40 °C).

Results and Discussion

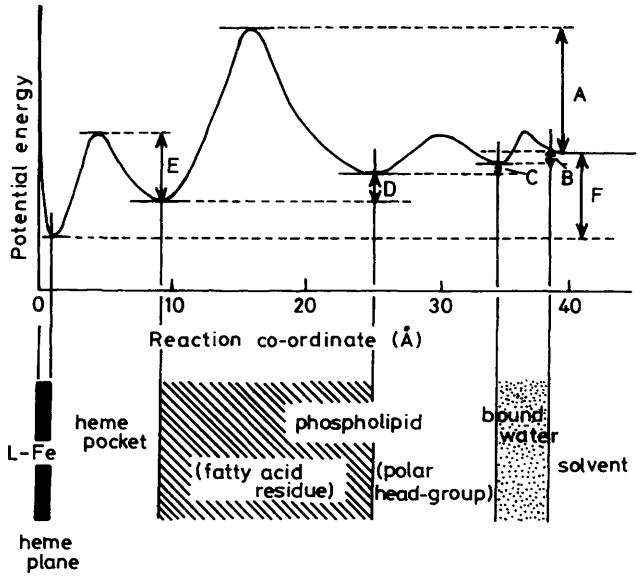
Oxygen-binding rate constants (k_{on}) of the liposome-embedded heme and lipid-heme are summarized in the Table. The values of k_{on} for the liposome-embedded heme were a little larger than or similar to that of the red blood cell suspension. The oxygen-binding rate constants for the homogeneous systems such as mb and hb were *ca.* 1 000 times larger than those of the lipsome-embedded heme and the red blood cell suspension, thus showing that the oxygen-binding reactions for the liposome-embedded heme and the red blood cell suspension are comparable. The oxygen-binding reaction is assumed to be largely retarded by the diffusion process of oxygen in and through the phospholipid membrane.

Values of k_{on} for the liposome-embedded lipid-heme were 1 000 times larger than those of the liposome-embedded heme and the red blood cell suspension, were consistent with those of the corresponding lipid-heme and heme complexes in homogeneous solution, and were similar to those of hb and mb. The lipid-heme molecule has four long alkanephosphocholine groups on the porphyrin plane and is embedded in the phospholipid bilayer with high compatibility. Thus it is assumed that the lipid-heme embedded in the phospholipid bilayer forms an oxygen-binding pathway from the outside water phase to the oxygen-binding site of the heme in a similar manner to the heme pocket of hb and mb.

Enthalpy changes ($\Delta H^{\ddagger}_{on}$) and entropy changes ($\Delta S^{\ddagger}_{on}$) for the oxygen binding are also listed in the Table. Values of $\Delta H^{\ddagger}_{on}$ and $\Delta S^{\ddagger}_{on}$ for the dmpc liposome-embedded heme above the gel–liquid crystal phase transition temperature (T_c) were similar to those of the red blood cell suspension. On the other hand, activation parameters for the dppc liposome-embedded heme and the dmpc liposome-embedded heme below T_c differed significantly from those mentioned above. Thus, the lipid bilayer of the liposome makes a significant favourable entropic contribution to the oxygen binding above T_c. The oxygen binding above T_c is not favoured enthalpically, compared with that below T_c, but this is compensated by a further favourable change in the entropy. The entropy factor is considered to correspond to the solubility and permeability of oxygen in and through the lipid membrane. The Gibbs free energy change of oxygen solubility in the fatty acid residue region of a lipid bilayer was reported by Kimmich and Peters.[14,15] The free energy change standardized with the oxygen solubility of water is 1.8 kJ mol^{-1} and −3.2 kJ mol^{-1} below and above T_c, respectively. This large oxygen solubility in the lipid membrane compared to that in the aqueous medium is considered to affect the rapid oxygen-binding rate and the favourable entropic contribution to the oxygen binding to the liposome-embedded heme above T_c.

The activation entropy for the liposome-embedded lipid-heme was much larger than those of the liposome-embedded heme and the red blood cell suspension, and similar to those of hb, mb, and the hemes in homogeneous solution. This suggests that the oxygen-binding pathway from the outside water phase to the embedded or internal heme formed for the liposome-embedded lipid-heme increased the activation entropy for the oxygen binding.

The total potential energy profile for the oxygen-binding reaction was constructed for the liposome-embedded heme above and below T_c (Figure) by using the values of $\Delta H^{\ddagger}_{on}$ and $\Delta S^{\ddagger}_{on}$ in the Table, values of ΔH and ΔS from previous papers,[9,10,16] and potential energy values for passage of an oxygen molecule through a phospholipid membrane.[17] For the oxygen-binding reaction for the liposome-embedded heme, the oxygen molecule encounters a potential barrier when it approaches from the solvent phase to the internal heme. The potential barrier is composed of four steps, *i.e.* the bound water region at the surface of the liposome, the polar head-group region and fatty acid residue region (oriented alkyl chain) of the liposome phospholipid, and the pocket over the heme plane (internal heme). The highest barrier is the oxygen penetration step through the oriented alkyl chain phase of the phospholipid bilayer. On the other hand, for the liposome-embedded lipid-



Heme	Potential energy (kcal mol^{-1}) A	B	C	D	E	F
Liposome-embedded heme						
dmpc (below T_c)	13	−0.57[17]	−0.50[17]	−0.76[17]	6.7	−2.5[10]
dmpc (above T_c)	12	−0.57[17]	−0.50[17]	−0.76[17]	6.7	−7.4[10]
Liposome-embedded lipid-heme						
dmpc (above T_c)			6.1*			−7.0

Figure. Potential energy profile for the oxygen binding reaction for the liposome-embedded heme above and below T_c. * Corresponds to potential energy for the heme pocket

heme the heme pocket above the heme plane cancelled out the potential barrier observed for the liposome-embedded heme.

The oxygen-binding profiles of the liposome-embedded heme and the liposome-embedded lipid-heme were compared with those of hb and mb. The former is a model with which to discuss oxygen binding to red blood cells, and the latter is a model for hb and mb.

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research on the priority area of macromolecular complexes from the Ministry of Education, Science and Culture, Japan.

References

1 M. F. Perutz, *J. Mol. Biol.*, 1965, **13**, 646.
2 M. F. Perutz, *Nature (London)*, 1970, **228**, 726.
3 Q. H. Gibson, *J. Physiol. (London)*, 1956, **134**, 112.
4 E. Antonini and M. Brunori, 'Hemoglobin and Myoglobin in their Reactions with Ligands,' North-Holland, Amsterdam, 1971.
5 R. H. Austin, K. W. Beeson, L. Eisenstein, H. Frauenfelder, and I. C. Gunsalus, *Biochemistry*, 1975, **24**, 5355.
6 D. A. Case and M. Karplus, *J. Mol. Biol.*, 1983, **132**, 343.
7 J. A. McGammon and M. Karplus, *Acc. Chem. Res.*, 1983, **16**, 187.
8 E. Tsuchida, H. Nishide, M. Yuasa, E. Hasegawa, and Y. Matsushita, *J. Chem. Soc., Dalton Trans.*, 1984, 1147.
9 E. Tsuchida, H. Nishide, M. Yuasa, E. Hasegawa, Y. Matsushita, and K. Eshima, *J. Chem. Soc., Dalton Trans.*, 1985, 275.
10 E. Tsuchida, H. Nishide, M. Yuasa, and M. Sekine, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 776.
11 E. Tsuchida, M. Yuasa, and H. Nishide, *J. Chem. Soc., Dalton Trans.*, 1985, 65.
12 J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Lang, and W. T. Robinson, *J. Am. Chem. Soc.*, 1975, **97**, 1427.
13 R. W. Nobel and Q. H. Gibson, *J. Biol. Chem.*, 1969, **244**, 3905.
14 R. Kimmich and A. Peters, *Chem. Phys. Lipids*, 1975, **14**, 350.
15 A. Peters and R. Kimmich, *Biophys. Struct. Mechanism*, 1978, **4**, 67.
16 E. Tsuchida, H. Nishide, and M. Yuasa, unpublished work.
17 R. Kimmich, A. Peters, and K-H. Spohn, *J. Membr. Sci.*, 1981, **9**, 313.

Received 31*st December* 1986; *Paper* 6/2492