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OXYGEN-BINDING AFFINITY OF LIPOSOMAL HEME UNDER SEMI PHYSIOLOGICAL CONDITIONS

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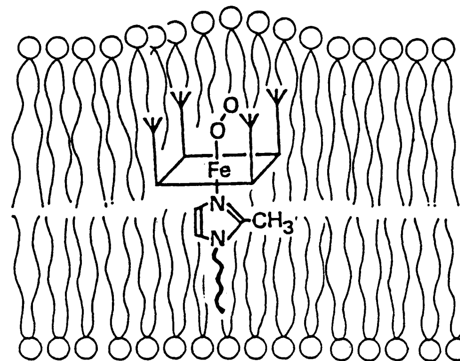
Oxygen-binding affinity ($P_{1/2}$) of the meso-tetra-(α,α,α,α-(o-pivalamidophenyl))porphinato iron(II)-mono-(1-lauryl-2-methylimidazole) complex incorporated in liposome of phosphatidylcholine was measured at pH 7.0 and 15~37 °C. The $P_{1/2}$ values at 37 °C were similar to that of hemoglobin in blood (*ca.* 49 mmHg) and dependent on the phase state of phospholipid.

The authors have previously reported the meso-tetra-(α,α,α,α-(o-pivalamidophenyl))porphinato iron(II) complex of mono-(1-lauryl-2-methylimidazole) incorporated in liposome of phosphatidylcholine (abbreviated as "liposomal heme", Scheme 1) which bound molecular oxygen reversibly under semi physiological conditions (in pH 7.0 aqueous media at room temperature).[1] It was considered that the iron porphyrin complex was embedded in a bilayer of liposome and that the hydrophobic environment of the inner region of liposome protected the oxygen adduct from its proton-driven oxidation.[2] The oxygen-binding and -dissociation proceeded very rapid, and their kinetic constants were comparable to those of hemoglobin (Hb).[3] The present communication describes the oxygen-binding equilibrium study on the liposomal heme. Oxygen-binding affinity ($P_{1/2}$: oxygen pressure at half oxygen-binding) was estimated in pH 7.0 phosphate buffer solution at 15~37 °C for the liposomal heme composed of dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and egg yolk lecithin (EYL).

Meso-tetra-(α,α,α,α-(o-pivalamidophenyl))-porphinato iron(III) bromide (picket fence iron porphyrin[4]) was prepared as in lit.[5] Picket-fence iron porphyrin (1 μmol), 1-lauryl-2-methylimidazole (50 μmol), and phosphatidylcholine were mixed in dichloromethane. By evaporating the solution under reduced pressure, thin film was prepared on the glass wall of a round flask. 20 ml of oxygen-free M/15 phosphate buffer (pH 7.0) was added, and



Scheme 1. Liposomal heme

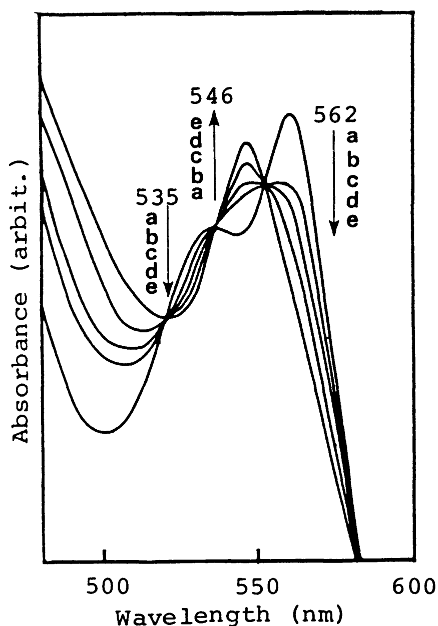


Fig. 1. Visible absorption spectra of the liposomal deoxy-heme and its oxygen adduct. a: saturated with N_2, b: P_{O_2}= 25, c: 50, d: 100 mmHg, e: saturated with O_2, [Fe] = 50 μM, in pH 7.0 at 37 °C.

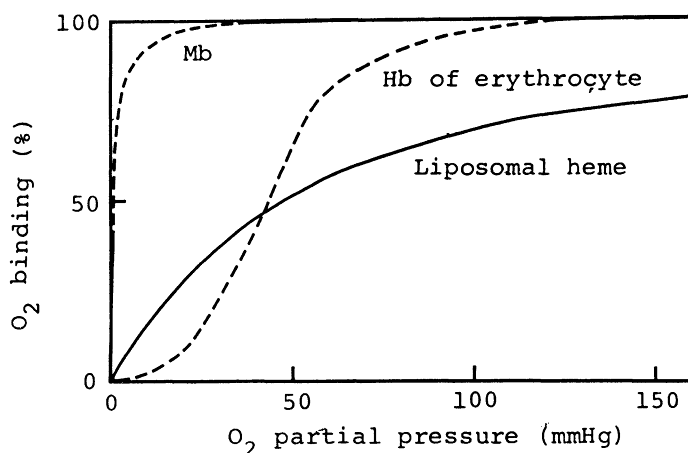


Fig. 2. Oxygen-binding and -dissociation curve. pH 7.0 phosphate buffer solution, at 37°C.

the mixture was ultrasonicated and homogenized in an ice-water bath. Then a small excess of L-ascorbic acid (20-fold mol of iron(III)) was added under nitrogen atmosphere, and the mixture was incubated at room temperature for 2 hrs. The red and transparent solution showed the visible absorption spectrum with maxima at 535 and 562 nm which agreed with the pentacoordinate deoxy complex of the liposomal heme.[1)] An oxygen adduct (λ_{max} = 546 nm) of the liposomal heme was obtained by bubbling oxygen through the solution. The spectral change in the visible region was measured with a spectrophotometer (Hitachi Co. UV-320) and oxygen concentration in the solution was monitored with an oxygen probe (Yellow Springs Instrument Co. YSI 5331) at the same time, after equilibrium was reached.

Equilibrium between the deoxy heme and the oxygen adduct was shown in Fig. 1; the visible absorption spectrum changes through isosbestic points at 520, 536, 552, and 589 nm by bubbling oxygen and nitrogen gas.

Measurements of differential absorbance at 562 nm and oxygen pressure at the same time gave an oxygen-binding and -dissociation curve, as shown in Fig. 2. The curve for the DMPC-liposomal heme is hyperbolic as that of myoglobin (Mb). The $P_{1/2}$ value is 49 mmHg at 37 °C, which agrees with the $P_{1/2}$ value (= 38 mmHg at 25 °C) of the picket-fence iron(II) porphyrin complex with 1,2-dimethylimidazole in toluene.[6)] Fig. 2 shows that the $P_{1/2}$ value of the liposomal heme in aqueous medium is close to $P_{1/2}$ (= 43 mmHg[7)]) of Hb in blood but situates fairly apart from that of Mb. This suggests that the liposomal heme has a potential to act as an oxygen carrier like hemoglobin under physiological conditions.

The oxygen-binding and -dissociation behaviors of the DPPC- and EYL-

liposomal hemes were the same as that of the DMPC-liposomal heme except for $P_{1/2}$ of the DPPC-liposomal heme at 37 °C.

Temperature-dependence of $P_{1/2}$, i.e. van't Hoff plots: $\ln(760/P_{1/2})$ *vs.* 1/T, showed a linear relationship (Fig. 3). Enthalpy change (ΔH) and entropy change (ΔS) for the oxygen-binding equilibrium were determined and given in Table 1. ΔH and ΔS for the EYL-liposomal heme and DMPC-liposomal heme (above 24 °C) were estimated to be *ca.* -15 kcal/mol and *ca.* -45 eu, respectively. These values were comparable to that of picket-fence iron(II) porphyrin complex in toluene[8] and to those of Hb and Mb.[7]

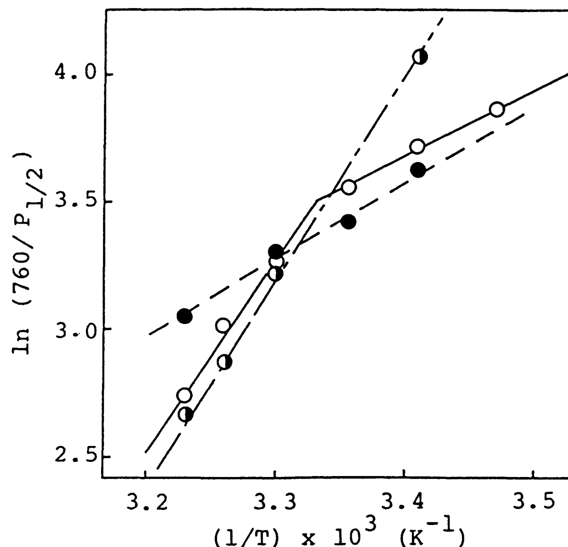


Fig. 3. van't Hoff plots.
the liposomal heme composed of ○ : DMPC, ● :DPPC, ◑ :EYL.

One notices in Table 1 that both ΔH and ΔS values for the DPPC-liposomal heme are much larger than those of the others. The phospholipid environment gives a large effect on the oxygen-binding affinity of heme through its enthalpy contribution. The liposome of DPPC has its transition temperature(Tc) between gel and liquid crystal phase of phospholipid at 41 °C.[9] It is reasonable to assume that the DPPC-liposomal heme is situated below its Tc under this experimental condition. Below Tc phospholipid molecules are in the crystal state, which probably induces an orientation of the laurylimidazole ligand and a structural distortion of the heme complex: This may be one of the reasons for the low oxygen-binding affinity of the DPPC-liposomal heme.

Fig. 3 indicates also that the temperature dependence of oxygen-binding for the DMPC-liposomal heme has a breaking point at *ca.* 26 °C, which agrees with the Tc degree of the DMPC-liposomal heme.[10] The ΔH and ΔS values of the DMPC-

Table 1. $P_{1/2}$ and thermodynamic parameters

Heme	$P_{1/2}$ (at 37°C) (mmHg)	ΔH (kcal/mol)	ΔS (eu)
Liposomal heme			
DMPC (<Tc)	—	- 5.0	-10
(>Tc)	49	-15	-43
DPPC	35	- 5.9	-14
EYL	51	-16	-46
Heme-Me_2Im/Tol*[8]	38**	-14	-41
Hemoglobin[7]	43	-14 ~ -15	—
Myoglobin[7]	0.9	-14 ~ -21	—

*Heme-Me_2Im/Tol = meso-tetra-(α,α,α,α-(o-pivalamidophenyl))-porphinato iron(II)-mono-1,2-dimethylimidazole in toluene. **at 25 °C.

liposomal heme above Tc were similar to those of the EYL-liposomal heme and, below Tc, to those of the DPPC-liposomal heme. From these results, we can say that the oxygen-binding affinity of the liposomal heme is dependent on the phase state of phospholipid. Further study will be reported in a subsequent paper.

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