Oxygen-binding Rate Parameters for a Liposome-embedded Heme under Physiological Conditions

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Oxygen-binding rate parameters for a liposome-embedded heme were measured under physiological conditions by the stopped flow method; the binding rate constant (*ca.* 10^4 dm^3 mol^{-1} s^{-1}) was similar to that of red blood cells in suspension.

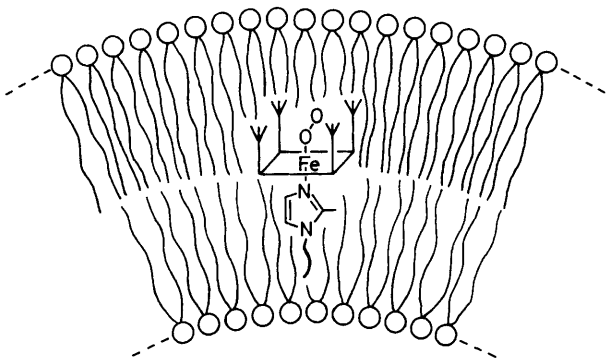
The determination of the rate parameters for oxygen-binding with iron(II) porphyrin complexes is directly relevant to the elucidation of the mechanisms of oxygen uptake and release by naturally occurring heme proteins. The oxygen-binding parameters for iron porphyrins have been widely reported,[1-3] but they were measured using flash photolysis in organic solvents or in aqueous media protected with CO.

Recently we have found that the 5,10,15,20-tetra(*o*-pivalamidophenyl)porphinato iron(II) complex of mono(1-dodecyl-2-methylimidazole) embedded in a liposome of phosphatidylcholine (abbreviated as 'liposome-embedded heme', Scheme 1) binds molecular oxygen reversibly under physiological conditions [in aqueous media (pH 7.0) at 37 °C].[4] The life-time of the oxygen adduct of the liposome-embedded heme was 12 h and the oxygen-binding affinity was similar to that of hemoglobin (Hb) in blood. In the present communication, the oxygen-binding rate parameters of the liposome-embedded heme were estimated by the stopped flow method. The reversible oxygen-binding reaction of the heme, which is protected by incorporation into a liposome is

Table 1. Oxygen-binding rate parameters for liposome-embedded hemes at 25 °C compared with literature values for other hemes.

Heme	Solvent	Measurement method[a]	k_{on}/dm^3 mol^{-1} s^{-1}	k_{off}/s^{-1}	K/dm^3 mol^{-1}	$P_{1/2}$/mmHg[b]	$P_{1/2}$*/mmHg[b]
DMPC[c]	pH 7.0[d]	s	7.9×10^3	0.32	2.5×10^4	24	22
,,	,,	f	5.4×10^3	1.6	—	—	—
DPPC[c]	,,	s	2.4×10^3	0.11	2.2×10^4	27	25
Burst-DMPC[c,e]	pH 7.0/H_2O (1/5)[e]	s	1.2×10^4	0.29	4.2×10^4	14	—
Red blood cell suspension[f]	pH 7.4[f]	s	1.1×10^4	0.16	6.8×10^4	8.8	10
Heme-$C_3N_2Me_2H_2$[g]	Toluene	f	1.1×10^8	46000	2.3×10^3	262	38
Chelated-heme[h]	pH 7.3[h]	f	2.6×10^7	47	5.5×10^5	1.0	5.6
Myoglobin[i]	pH 7.0—7.4	f,s	$1.0—2.0 \times 10^7$	10—30	$0.67—1.0 \times 10^6$	0.55—0.82	0.50—1.0
Hemoglobin[j]	,,	f,s	3.3×10^7	12—13	$2.5—2.7 \times 10^6$	0.20—0.22	0.22—0.36

[a] s = Stopped flow method, f = flash photolysis method. [b] $P_{1/2}$ values calculated from K and $P_{1/2}$* values determined from the oxygen-binding equilibrium curves. [c] Liposome-embedded heme composed of DMPC (dimyristoylphosphatidylcholine) or DPPC (dipalmitoylphosphatidylcholine). [d] Phosphate buffer solution at pH 7.0. [e] The liposome-embedded heme in higher concentration was poured into water and diluted. The liposome is expected to be burst by osmotic pressure and to have a lamellar structure. [f] The red blood cell suspension was prepared as in ref. 5. [g] From refs. 3, 6, heme-$C_3N_2Me_2H_2$ = 5,10,15,20-tetra(*o*-pivalamidophenyl)porphinato iron(II) complex of mono(1,2-dimethylimidazole). [h] From ref. 1, chelated-heme (pH 7.3) = protoheme *N*-(3-imidazol-1-ylpropyl)amide methyl ester solubilized by myristyltrimethylammonium bromide at 20 °C. [i] From ref. 7, at 20 °C. [j] From refs. 7, 8, at 20 °C, hemoglobin = stripped hemoglobin. The oxygen-binding in organic solvents (toluene and benzene) was so fast that the rate parameters (k_{on} and k_{off}) could not be measured with this stopped flow apparatus.



Scheme 1. Liposome-embedded heme.

discussed and compared with that for red blood cells, in which Hb is encapsulated in a cell membrane.

The liposome-embedded heme was prepared as described in the literature.[4] The oxygen-binding reaction was studied using a stopped flow spectrophotometer equipped with a kinetic data processer. After rapid mixing of the liposome-embedded heme with the buffer solution saturated with oxygen used above, the spectrum (λ_{max} 438 nm) of the deoxy-heme became that of the oxygen adduct (λ_{max} 422 nm). On bubbling carbon monoxide through the mixed solution, the oxy-spectrum changed to that of the CO adduct (λ_{max} 423 nm). By changing the monitoring wavelength from 390 to 460 nm, a differential spectrum before and after rapid mixing was obtained. The negative and positive extremes in the differential spectrum, 422 and 438 nm, were selected as the monitoring wavelengths. These wavelengths agreed with the absorption maxima of the oxygen adduct and the deoxy-heme, respectively. The time–reaction curves measured at 422 and 438 nm were symmetric, and the absorbance at 430 nm remained constant before and after mixing, consistent with the isosbestic point which exists between the spectrum of the oxygen adduct and of the deoxy-heme (Figure 1a). These results supported the validity of the measurement. The reproducibility of the measurement was confirmed by using several batches of the sample.

The time–reaction curve for the oxygen-binding of the liposome-embedded heme was that of a multi-phase system, as the heme complex was dissolved heterogeneously with the liposome in the aqueous medium. However, the curve could be approximated by mono-phase kinetics within experimental error (Figure 1b). Binding and dissociation rate constants (k_{on} and k_{off}) are summarized in Table 1. The k_{on} value of the DMPC-liposome-embedded heme agreed with that determined by flash photolysis.[9] The k_{off} value is compatible with that estimated from the reaction in which the solution of the oxygen adduct was mixed rapidly with an argon-saturated solution and the reverse reaction from the oxygen adduct to the deoxy-heme took place. The $P_{1/2}$ (oxygen pressure at half oxygen-saturation for the heme) value calculated from the K ($= k_{on}/k_{off}$) value is consistent with that determined from the oxygen-binding equilibrium curve. These results support the validity of this kinetic treatment. The k_{on} values of the liposome-embedded hemes are similar to those of the red blood cell suspension. This means that the oxygen-binding reactions for the liposome-embedded heme and the red blood cell suspension show the same features.

The oxygen-binding rate parameters given in the literature for homogeneous systems, *i.e.* heme–$C_3N_2Me_2H_2$ in toluene,[3] chelated-heme at pH 7.3,[1] myoglobin,[7] and stripped Hb,[7,8] are also listed in Table 1 and they are about 10^3 times larger than those of the liposome-embedded hemes and the red

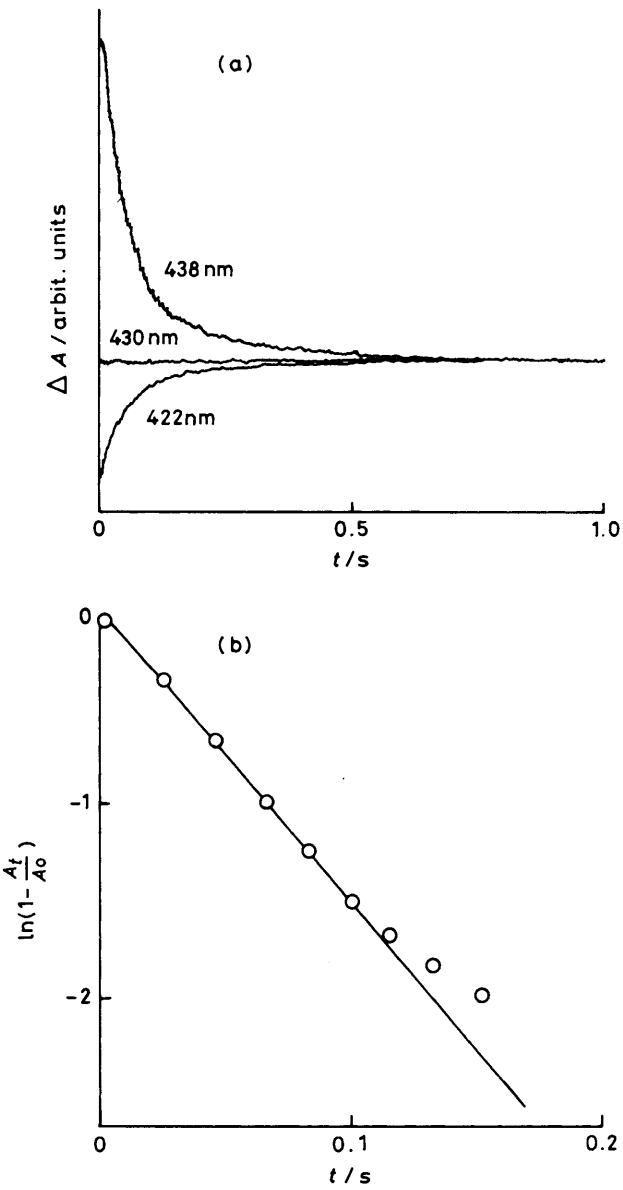


Figure 1. (a) Time–reaction curve for oxygen-binding of the liposome-embedded heme; (b) approximation to mono-phase kinetics. ΔA = differential absorbance; A_0 and A_t = absorbance at 438 nm (time = 0 s and t, respectively).

blood cell suspension. It is thought that the oxygen-binding reaction is retarded by the need for oxygen to diffuse in and through the phospholipid membrane.

The oxygen-binding rate was larger for the DMPC- than for the DPPC-liposome-embedded heme. This may be explained as follows. The DPPC-liposome is below its T_c (gel–liquid crystal phase transition temperature: 41 °C) and the DMPC-liposome is above (23 °C)[10] under these experimental conditions (25 °C), so that membrane fluidity, and probably oxygen permeability, of the DMPC-liposome is larger than that of DPPC. The largest oxygen-binding rate was observed for the burst DMPC-liposome-embedded heme. It is assumed that this liposome has a lamellar structure or a non-closed (non-vesicle) bilayer structure, and this is advantageous for the supply of oxygen to the heme. The liposome-embedded heme is clearly a suitable model with which to study the mechanism of oxygen uptake and release by red blood cells.

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

Received, 23rd September 1983; Com. 1260

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