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OXYGEN-BINDING PROFILE OF THE IRON PORPHYRIN COMPLEX EMBEDDED IN POLYMERIZED LIPOSOME: EFFECT OF THE POLYMERIZED LIPOSOME ON THE STABILITY AND THE OXYGEN-BINDING ABILITY OF THE IRON PORPHYRIN COMPLEX

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ABSTRACT

5,10,15,20-Tetra(α,α,α,α-*o*-(2′,2′-dimethyl-20′-(2″-trimethylammonioethyl)phosphonatoxyeicosanamido)phenyl)porphinatoiron(II) (lipid-heme) complex embedded in polymerized liposome was prepared by polymerizing 1-(9-(*p*-vinylbenzoyl)nonanoyl)-2-*O*-octadecyl-*rac*-glycero-3-phosphocholine in the presence of lipid-heme under ultraviolet irradiation. The polymerization proceeded rapidly, and the reduction of the hemin to the heme occurred spontaneously during the polymerization. The lipid-heme complex embedded in the polymerized liposome bound molecular oxygen reversibly under physiological conditions (pH 7, 37°C) and was chemically, physically, and mechanically stable during storage for a long period and even in a high-speed flow system. The oxygen-binding affinity was not affected by the type of medium due to the effect of the rigid polymerized liposome.

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INTRODUCTION

Hemoglobin (Hb) and myoglobin (Mb), which are composed of iron-porphyrin complexes (hemes) and globin proteins, serve to transport and store molecular oxygen in a living body. If their oxygen-binding sites, the iron-porphyrin complexes, are isolated from the proteins and exposed to oxygen, the iron(II)-porphyrin complexes are immediately and irreversibly oxidized and no longer act as oxygen carriers. Globin protein forms the five-coordinate iron-porphyrin complex and "tucks" it separately, i.e., the globin protein protects the iron-porphyrin complex from irreversible oxidation via μ-dioxo dimer by embedding it separately in the macromolecule, and the hydrophobic domain of the globular protein excludes water molecules and suppresses the proton-driven oxidation.

Much effort has been exerted to mimic oxygen carriers like Hb by synthesizing various modified iron-porphyrin derivatives [1, 2]. These synthetic iron-porphyrin complexes were successful in binding oxygen in aprotic solvents or in the solid state, but in aqueous media they were irreversibly oxidized.

In order to construct a hydrophobic environment in aqueous solution and to occlude a complex separately, liposome, a macromolecular assembly of phospholipids, is a possible replacement of the globin protein. Recently, we found and reported that heme derivatives, 5,10,15,20-tetra(α,α,α,α-*o*-pivalamidophenyl)porphinatoiron(II), 5,10,15,20-tetra(α,α,α,α-*o*-(2′,2′-dimethyl-20′-(2″-trimethylammonioethyl)phosphonatoxyeicosanamido)phenyl)porphinatoiron(II) (lipid-heme), 2-mono(1′-(*N*-(3″-(2‴-methylimidazol-1‴-yl)-propyl)carbamoyl)-2′-(*N*-hexadecylcarbamoyl)ethyl-*trans*-acrylamido)-5,10,-15,20-tetra(α,α,α,α-*o*-pivalamidophenyl)porphinatoiron(II), and tetradecyl-substituted diporphinatocopper-iron, embedded in natural, synthetic, and polymerized liposome (liposome/heme), could bind molecular oxygen reversibly under physiological conditions (pH 7, 37°C) [3-7]. The oxygen-binding affinity and rate were similar to those of red blood cell (RBC) or Hb in blood [3-10]. In the present paper, lipid-heme embedded in polymerized 1-(9-(*p*-vinylbenzoyl)nonanoyl-2-*O*-octadecyl-*rac*-glycero-3-phosphocholine (lipid monomer) (polylipid liposome/lipid-heme) was prepared by polymerizing the lipid monomer in the presence of lipid-heme complex. The effects of the polymerized liposome or the polylipid on the stability and the oxygen-binding ability of the lipid-heme complex were studied.

EXPERIMENTAL

Materials

Lipid-heme [4, 11], lipid monomer [12], and the ligand, 1-laurylimidazole (LI), of lipid-heme [3] were synthesized as previously reported.

Preparation of Polylipid Liposome/Lipid-Heme

The polylipid liposome/lipid-heme was prepared as follows. The liposome of lipid-heme, LI, and lipid monomer (molar ratio lipid-heme/LI/lipid monomer = 1/3/50) was prepared by the normal thin-film method and subsequent treatment under nitrogen with an ultrasonicator. Liposome thus prepared was allowed to polymerize under nitrogen and ultraviolet irradiation (32 W, 40°C, 1 h) to give the polylipid liposome/lipid-heme. The reduction of the Fe(III) derivative of lipid-heme to the Fe(II) or deoxy derivative occurred spontaneously during the polymerization. The polylipid liposome/lipid-heme was concentrated by ultrafiltration.

Measurements

The reversible oxygen-binding of the polylipid liposome/lipid-heme was measured with ultraviolet (UV) and visible (vis) spectrophotometry (Shimadzu MPS-2000) and the pulse flash photolysis method (Unisoku FP-2000) [8]. The oxygen-binding rate and affinity were measured by the stopped-flow method (Unisoku SF-1000) [9] and oxygen-binding and -dissociation equilibrium curve measurement, respectively [10]. The turbidity of the liposome solution was measured as reported in Refs. 13-15. The mechanical stability was evaluated by letting the polylipid liposome/lipid-heme flow through a capillary system at a high rate.

RESULTS AND DISCUSSION

UV and vis spectra of the lipid monomer and the polylipid liposome/lipid-heme before and after the polymerization are shown in Fig. 1. Complete polymerization was confirmed by UV absorption and ^{13}C-NMR spectrscopy, i.e., disappearance of the absorbance due to the vinyl group (λ_{max} 265 nm, Fig. 1a) and of the characteristic signals due to the vinyl carbons of the lipid

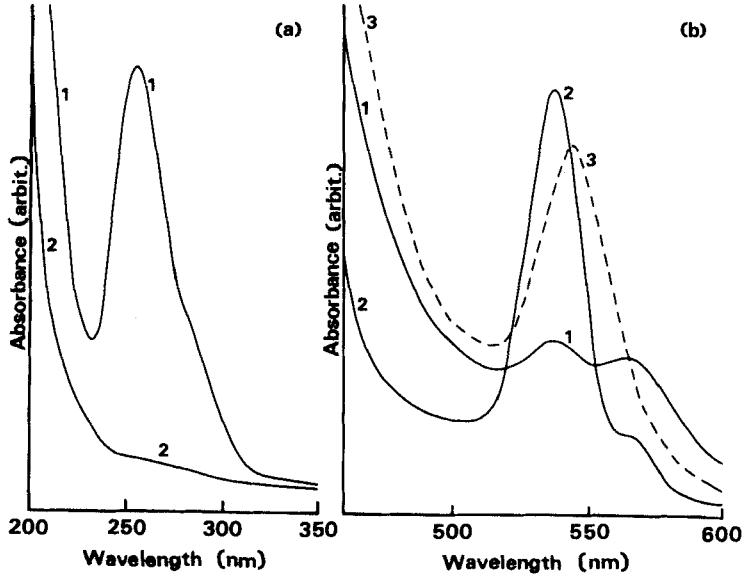

FIG. 1. UV and vis spectra of (a) the lipid monomer and (b) the lipid-monomer liposome/lipid-heme before and after UV-initiated polymerization. (1) Before UV irradiation, (2) after UV irradiation, (3) bubbled oxygen gas through the solution.

monomer (δ_C 116.8 and 136.1 ppm). Reduction of Fe(III) of the lipid-heme to Fe(II) or the deoxy derivative occurred spontaneously during the polymerization. Complete reduction of the lipid-heme was confirmed by vis absorption spectra (λ_{max} 429 ($\epsilon = 8.4 \times 10^5$ M^{-1} cm^{-1}), 535 (8.7×10^4), and 562 (2.8×10^4) nm) (Fig. 1b).

The time course of the polymerization for the lipid-monomer liposome/lipid-heme under UV irradiation is shown in Fig. 2 together with that for the lipid monomer itself. The polymerization was complete after 1 h irradiation, which is faster than that of the lipid monomer with the lipid-heme in homogeneous $CHCl_3$ solution under the same conditions. Evidently the polymerization of a vinyl monomer in a liposome state or in an oriented structure proceeds rapidly as a zipping-up reaction due to a favorable frequency factor [16, 17]. The polymerization rate of the lipid-monomer liposome/lipid-heme was higher than that of the lipid-monomer liposome although the former accompanied the reduction of the Fe(III) complex or a termination reaction by

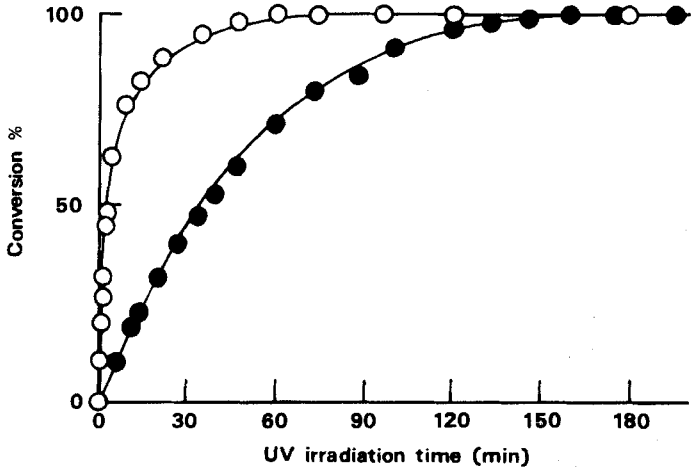

FIG. 2. Time-conversion curves for the UV-initiated polymerization of the lipid-monomer liposome/lipid-heme (○) and the lipid-monomer liposome (●).

capturing the propagation radical. It is considered that the lipid-heme acts as a photosensitizer for the polymerization and accelerates the polymerization further although its reductive reaction retards the polymerization.

The vis absorption spectrum of the red and transparent solution of the polylipid liposome/lipid-heme is shown in Fig. 1(b): The spectrum of the deoxy-heme complex changed to one with the maximum at 546 nm assigned to the oxygen adduct on exposure to oxygen. The oxy spectrum changed to that of the CO adduct (540 nm) on bubbling CO gas through the solution and returned to that of deoxy-heme on bubbling nitrogen gas. The oxy-deoxy cycle could be repeated more than a hundred times with isosbestic points (Fig. 3a). The reversible oxy-deoxy cycle was also observed by pulse flash irradiation which dissociates the bound oxygen (Fig. 3b).

Kinetic and equilibrium parameters for the oxygen-binding to the polylipid liposome/lipid-heme were measured by the stopped-flow method and by oxygen-binding and -dissociation equilibrium curve measurement, respectively. The oxygen-binding rate of the polylipid liposome/lipid-heme in pH 7 phosphate buffer solution was $\sim 10^4\ M^{-1}s^{-1}$, which was similar to that of RBC and that of the corresponding nonpolymerized liposome/lipid-heme. The oxygen-binding rate constant was not affected by the polymerization of liposome.

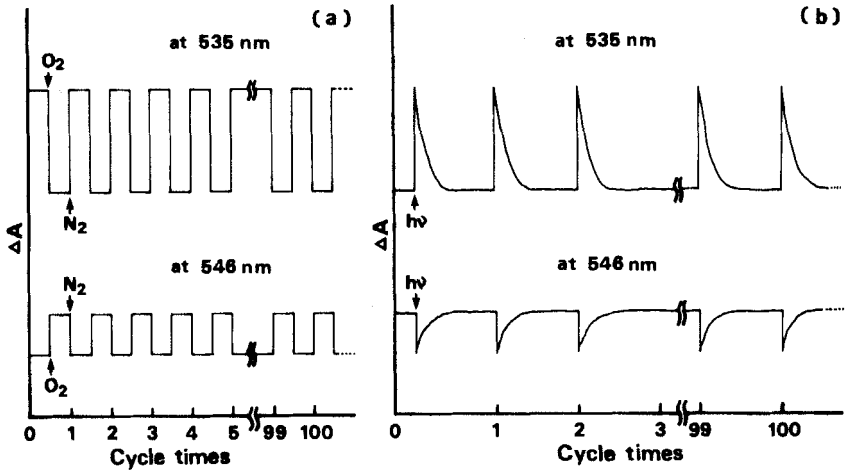

FIG. 3. Deoxy-oxy cycle of the polylipid liposome/lipid-heme (a) by oxygen-pressure change and (b) by pulse flash irradiation (pH 7, 37°C).

TABLE 1. Oxygen-Binding Affinities of Polylipid Liposome/Lipid-Heme in Various Media at 37°C

Heme	$p_{1/2}$, torr
Polylipid liposome/lipid-heme:	
In phosphate buffer solution	40
In carbonate buffer solution	45
In physiological salt solution	38
In dextran expander	37
In human serum	42
Hemoglobin in blood[a]	27
Myoglobin[b]	0.1

[a]From Ref. 18.
[b]From Ref. 19.

Oxygen-binding affinity ($p_{1/2}$) of the polylipid liposome/lipid-heme in various media is shown in Table 1. The $p_{1/2}$ values in various media were close to those of Hb and RBC [18] but far from that of Mb [19]. The oxygen-binding affinity was also not influenced by the type of medium. The rigid polylipid matrix of the polylipid liposome/lipid-heme protects the oxygen-binding ability of the lipid-heme from environmental conditions.

The stability of the polylipid liposome/lipid-heme was estimated by measuring the effect of added ethanol on the liposome structure. The transparency or opalescence of the polylipid liposome/lipid-heme solution remained even after ethanol addition, while the turbidity of nonpolymerized liposome/lipid-heme was much decreased by ethanol addition, implying destruction of the liposome structure (Fig. 4). This means that the polylipid liposome/lipid-heme keeps the liposome structure and is very stable even in alcoholic solution.

The solution of the polylipid liposome/lipid-heme was stable without precipitation and change of particle size, i.e., without aggregation and fusion of the liposome, after storing at room temperature for a year, after

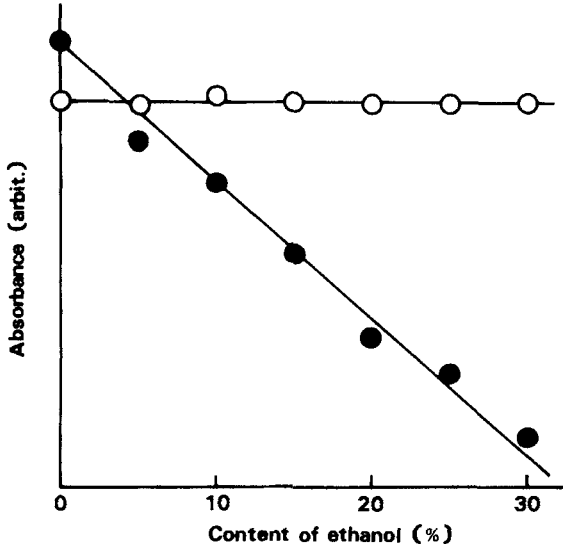

FIG. 4. Turbidity changes of polylipid liposome/lipid-heme (○) and liposome/lipid-heme (●) after addition of ethanol.

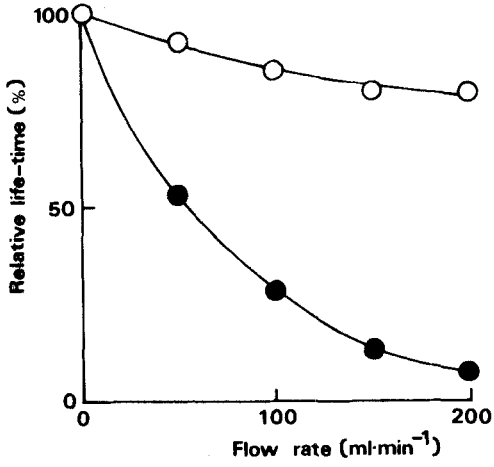

FIG. 5. Relative lifetimes of polylipid liposome/lipid-heme (○) and liposome/lipid-heme (●) in a high-speed flow system at pH 7 and 37°C.

storing at freezing temperature, and after the repreparation from freeze-dried powder.

The polylipid liposome/lipid-heme solution was passed through capillary tubes (hollow fiber, $\phi < 0.1$ mm) at flow rates of 20-200 mL/min. The relative lifetime of the oxygen adduct of the polylipid liposome/lipid-heme hardly decreased on standing compared to that of the solution. The polylipid liposome/lipid-heme is also expected to be active during passage through the capillaries of the human body. (See Fig. 5.)

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REFERENCES

[1] R. D. Jones, D. A. Summerville, and F. Basolo, *Chem. Rev., 79,* 139 (1979).

[2] E. Tsuchida, *J. Macromol. Sci.–Chem., A13,* 545 (1979).

[3] E. Tsuchida, H. Nishide, M. Yuasa, E. Hasegawa, and Y. Matsushita, *J. Chem. Soc., Dalton Trans.*, p. 1147 (1984).
[4] E. Tsuchida, *Ann. N. Y. Acad. Sci., 466,* 429 (1985).
[5] K. Eshima, M. Yuasa, H. Nishide, and E. Tsuchida, *J. Chem. Soc., Chem. Commun.*, p. 130 (1985).
[6] H. Nishide, H. Maeda, S.-G. Wang, and E. Tsuchida, *Ibid.*, p. 573 (1985).
[7] E. Tsuchida, E. Hasegawa, Y. Matsushita, K. Eshima, M. Yuasa, and H. Nishide, *Chem. Lett.*, p. 969 (1985).
[8] E. Tsuchida, H. Nishide, M. Sekine, M. Yuasa, T. Iizuka, and Y. Ishimura, *Biochem. Biophys. Res. Commun., 109,* 858 (1982).
[9] E. Tsuchida, M. Yuasa, and H. Nishide, *J. Chem. Soc., Dalton Trans.*, p. 65 (1985).
[10] E. Tsuchida, H. Nishide, M. Yuasa, and M. Sekine, *Bull. Chem. Soc. Jpn., 57,* 776 (1983).
[11] Y. Matsushita, E. Hasegawa, K. Eshima, and E. Tsuchida, *Chem. Lett.*, p. 1387 (1983).
[12] E. Hasegawa, Y. Matsushita, K. Eshima, H. Nishide, and E. Tsuchida, *Makromol. Chem., Rapid Commun., 5,* 779 (1984).
[13] C. D. Tran, P. L. Klahn, A. Romero, and J. H. Fendler, *J. Am. Chem. Soc., 100,* 1622 (1978).
[14] S. L. Regen, A. Singh, G. Oehme, and M. Singh, *Ibid., 104,* 791 (1982).
[15] Y. Iino, Y. Ogata, K. Shigehara, and E. Tsuchida, *Makromol. Chem., 186,* 923 (1985).
[16] P. Tundo, D. J. Kippenberger, P. L. Klahn, N. E. Prieto, T.-C. Jao, and J. H. Fendler, *J. Am. Chem. Soc., 104,* 456 (1982).
[17] W. Reed, L. Guterman, P. Tundo, and J. H. Fendler, *Ibid., 106,* 1897 (1984).
[18] J. W. Severinghaus, *J. Appl. Physiol., 21,* 1108 (1966).
[19] E. Antonini and M. Brunori, *Hemoglobin and Myoglobin in Their Reactions with Ligands,* North-Holland, Amsterdam, 1971.