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Lipid-Heme/Microsphere. A New Totally Synthetic Oxygen-Carrier under Physiological Conditions

Teruyuki KOMATSU, Eriko MATSUBUCHI, Hiroyuki NISHIDE, and Eishun TSUCHIDA*

Department of Polymer Chemistry, Waseda University, Shinjuku-ku, Tokyo 169

Triglyceride microsphere emulsified with 5,10,15,20-tetrakis[α,α,α,α-*o*-[2,2-dimethyl-20-[2-(trimethylammonioethoxy)phosphonatoxy]eicosanamido]phenyl] porphyrinatoiron (lipid-heme) provides a new totally synthetic oxygen-carrier (lipid-heme/microsphere) under physiological conditions . Oxygen-binding property of the lipid-heme/microsphere is comparable with that of red blood cell.

Phospholipid derivatives of tetraphenylporphyrinatoiron have been synthesized by us to prepare totally synthetic oxygen-transporter under physiological conditions (pH 7.4, 37 °C).[1] A typical example is 5,10,15,20-tetrakis[α,α,α,α-*o*-[2,2-dimethyl-20-[2-(trimethylammonioethoxy)phosphonatoxy]eicosanamido]phenyl] porphyrinatoiron (lipid-heme). The lipid-heme has a good hydrophobic and hydrophilic balance and forms a bilayer aggregate with phospholipids in an aqueous medium. The phospholipid vesicle embedded lipid-heme with diameters of 40-50 nm transports O_2 reversibly under physiological conditions and in blood stream.[2]

Oil-in-water (O/W) lecithin emulsions (lipid microsphere), *e.g.* formulated from soybean oil and stabilized by egg york lecithin, has already been used in clinics for parenteral nutrition and a carrier for a variety of lipophilic drugs.[3-6] Unlike phospholipid vesicles, the O/W emulsions are easy to manufacture on an industrial scale and have a high colloidal stability, which makes it possible to store the emulsions for a long period (several months) at room temperature without any change in their physicochemical properties. Furthermore, lipid microspheres are well tolerated by the body since they resemble chylomicrons.

Recently we have found that an O/W lipid microsphere emulsified with the lipid-heme, as surfactant, give red-colored dispersion which is able to bind O_2 reversibly in aqueous medium (Fig. 1). This paper reports the preparation and oxygen-binding properties of a new oxygen-carrier, lipid-heme/microsphere, under physiological conditions.

The lipid-heme/microsphere was prepared as follows; lipid-hemin (iron(III)), 1-dodecylimidazole (DIm)

having a good compatibility with lipid molecules, and trioctanoylglyceride (TG)[7] (1/0.2/4 (wt ratio), lipid-hemin/DIm=1/2.5 (mol ratio)) were mixed and then phosphate buffer (pH 7.4, 0.03 mol dm^{-3}) was added to the mixture. The solution was homogenized by ultrasonic generator under nitrogen to give a red-colored O/W emulsion ([heme]=0.01-30 mmol dm^{-3}).

From SEM and TEM, the lipid-heme/microsphere looked like spherical particles with diameters of 100-150 nm (Fig. 2). The average particle size of the lipid-heme/microsphere was also measured by dynamic light scattering method using a submicron particle analyzer; the diameter of the particle is 110±32 nm.

The formation of O/W emulsion covered with lipid-hemin was confirmed by gel permeation chromatography (Sepharose CL-4B, 3.5 cmϕ x 60 cm) monitored by absorption at 425 nm and 295 nm, based on the lipid-hemin and the α-tocopherol added for the probe in the oil phase. The curves coincided with each other, meaning that the lipid-hemin serves as effective surfactant for the obtained lipid microsphere. Further, the surface lipid-hemin molecules around the microparticle (110 nmϕ, TG/heme=4 (wt ratio)) agreed with those calculated by assuming a base area of the porphyrin.

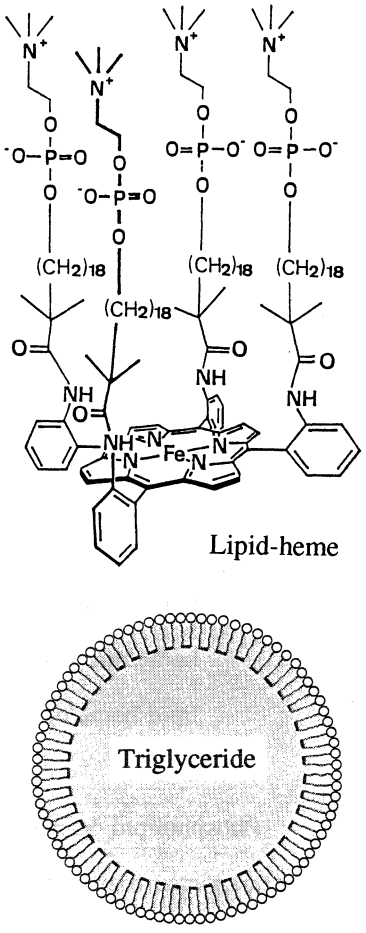

Fig. 1.
Lipid-heme/microsphere.

The lipid-hemin was reduced to iron(II) by an addition of small excess of aqueous ascorbic acid under nitrogen atmosphere. The solution of the lipid-heme/microsphere is stable and can be stocked for a few months without precipitation and change of the particle size at 25 °C. The viscosity of this emulsion ([heme]=10 mmol dm^{-3}) was determined to be 1.5 cP, which is much lower than that of human blood (4.5-5.0 cP).

The stability of the lipid-heme/microsphere was also examined by ^{31}P-NMR spectoroscopy. The NMR signal of the lipid-heme/microsphere ([heme]=16.5 mmol dm^{-3}, pH 7.4) is obtained with a line width of 400 Hz, which corresponds to that of the stable lecithin emulsions.[8]

The visible absorption spectrum of the deoxy complex (λ_{max}: 429, 536 and 562(sh) nm) changed to that of O_2 adduct rapidly on exposure to O_2 (λ_{max}:424 and 539 nm). The spectrum changed reversibly dependent on O_2 pressures. The O_2 adduct changed to the corresponding CO adduct on bubbling CO gas through the solution (λ_{max}: 426 and 540 nm). The oxygen-binding affinity ($P_{1/2}(O_2)$; the O_2 partial pressure at half oxygenation for the heme) of the lipid-heme/microsphere was determined to be 46 Torr at 37 °C. This suggests that

the lipid-heme/microsphere has a potential to act as an efficient oxygen-carrier under physiological conditions which transports O_2 from the lungs ($P(O_2)$=*ca.* 110 Torr) to Mb in muscle tissue ($P(O_2)$=*ca.* 40 Torr) as Hb does. Half-life time ($\tau_{1/2}$) of the O_2 adduct of the lipid-heme/microsphere is 12 h in an aqueous solution.

The kinetics of the reaction with O_2 and CO were measured for the lipid-heme/microsphere[9] by laser flash photolysis technique (Eq. 1 (B:Axial imidazole derivative, L:O_2 or CO)).[10] Gaseous ligand association and dissociation rate constants (k_{on}, k_{off}) were determined by pseudo-first-order approximation. The O_2 and CO binding parameters are summarized in Table 1. O_2 and CO binding behaviors of the lipid-heme/microsphere are similar to those of Hb, Mb and phospholipid vesicle (*e.g.* dipmyrystoylphosphocholine) embedded lipid-heme.[10-12]

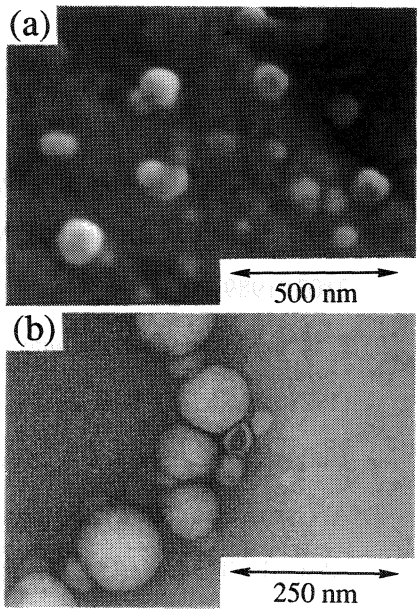

Fig. 2. (a)SEM and (b)TEM of lipid-heme/microsphere.

A most important feature of the lipid-heme/microsphere is to solubilize the heme at high concentration in an aqueous medium.

$$\mathrm{BFeL} \xrightarrow{h\nu} \mathrm{BFe} \underset{k_{off}(L)}{\overset{k_{on}(L)}{\rightleftharpoons}} \mathrm{BFeL} \qquad (1)$$

Table 1. O_2 and CO Binding Affinities and Rate Constants for Lipid-heme/Microsphere in an Aqueous Medium (pH 7.4, 25 °C)

Complex	$P_{1/2}(O_2)$ / Torr	$k_{on}(O_2)$ / $dm^3mol^{-1}s^{-1}$	$k_{off}(O_2)$ / s^{-1}	$P_{1/2}(CO)$ / Torr	$k_{on}(CO)$ / $dm^3mol^{-1}s^{-1}$	$k_{off}(CO)$ / s^{-1}
Lipid-heme/microsphere	20	7.0×10^7	9.3×10^3	1.2×10^{-2}	1.7×10^6	9.9×10^{-2}
Lipid-heme/DMPC vesicle[a]	53[b]	9.8×10^7	3.3×10^3	7.4×10^{-3}	1.0×10^6	9.3×10^{-3}
Hb(R-state)[c]	0.22	3.3×10^7	13.1	1.4×10^{-3}	4.6×10^6	9.0×10^{-3}
Mb[d]	0.37	$(1.0\text{-}2.0)\times10^7$	10-30	$(1.4\text{-}2.5)\times10^{-2}$	$(3.0\text{-}5.0)\times10^5$	$(1.5\text{-}40)\times10^{-3}$

a)pH 7.0. From Ref. 10. b)At 37 °C. C)pH 7.0-7.4. From Ref. 12. d)pH 7.0-7.4. From Ref. 11.

That is, the lipid-heme/microsphere suspension (the concentration of TG is 10 wt%) is able to uptake O_2 gas up to 23 ml/100 ml medium, which is equal to that of human blood. Furthermore, the lipid-heme/microsphere dispersion is also stable in physiological saline solution or blood plasma and give stable O_2 adduct with the same $P_{1/2}(O_2)$.

Our results show that the lipid-heme/microsphere acts as efficient oxygen-carrier under physiological

conditions.

References

1) E. Tsuchida and H. Nishide, *Top. Curr. Chem.*, **132**, 64 (1986); E. Tsuchida, *Nippon Kagaku Kaishi*, **1988**, 845; E. Tsuchida, H. Nishide, M. Yuasa, E. Hasegawa, K. Eshima and Y. Matsushita, *Macromolecules*, **22**, 2103 (1989).

2) E. Tsuchida and H. Nishide, "Liposome as Drug Carrier", ed by G. Gregoriadis, John Wiley, New York (1988), p.569; T. Komatsu, E. Matsubuchi, H. Nishide, E. Tsuchida, M. Watanabe, K. Kobayashi, and T. Ishihara, *Jpn. J. Artif. Organ.*, **210**, 299 (1992).

3) A. Carlsson, T. Sato, and J. Sunamoto, *Bull. Chem. Soc., Jpn.*, **62**, 791 (1989); S. Yamaguchi, Y. Fukui, K. Akiyoshi, T. Sato, and J. Sunamoto, *Nippon Kagaku Kaishi*, **1992**, 186.

4) T. Handa, H. Saito, and K. Miyajima, *Biochemistry*, **29**, 2884 (1990); T. Handa, Y. Asai, K. Miyajima, Y. Kawashima, M. Kayano, K. Ida, and T. Ikeuchi, *J. Colloid Interface Sci.*, **143**, 205 (1991).

5) Y. Mizushima, T. Hamano, and K. Yokohama, *Annal. Rheu. Diseases*, **41**, 263 (1982).

6) S. S. Davis, C. Washington, P. West, L. Illum, G. Liversidge, L. Sternson, and R. Kirsh, *Ann. N.Y. Acad. Sci.* **507**, 75 (1987).

7) TG was a special grade material gifted from Nippon Oil & Fats Co., Ltd. Its acid value and iodine number are less than 0.1 and 1.0, respectively.

8) S. Hayakawa and R. Nakamura, Nippon Kagaku Kaishi, **1988**, 859.

9) Lipid-heme/microsphere composed by lipid-heme, 1-dodecyl-2-methylimidazole (DMIm) and TG (1/2.7/4 (wt ratio), lipid-heme/DMIm=1/20 (mol ratio)).

10) M. Yuasa, H. Nishide, E. Tsuchida, *J. Chem Soc., Dalton Trans.*, **1987**, 2493; T. Komatsu, S. Kumamoto, H. Nishide and E. Tsuchida, *ibid.*, **1991**, 3281.

11) F. Antonini and M. Brunori "Hemoglobin and Myoglobin and Their Reactions with Ligands", North Holland Pub. Co., Amsterdam (1971); M. Brunori and T. M. Schuster, *J. Biol. Chem.*, **244**, 4046 (1969).

12) Q. H. Gibson, *J. Biol. Chem.*, **245**, 3285 (1970); J. S. Olson, M. E. Anderson, and Q. H. Gibson, *ibid.*, **246**, 5919 (1971).

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