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POLYMERIZED LIPOSOME AS THE CARRIER OF HEME. A PHYSICALLY STABLE OXYGEN CARRIER UNDER PHYSIOLOGICAL CONDITIONS

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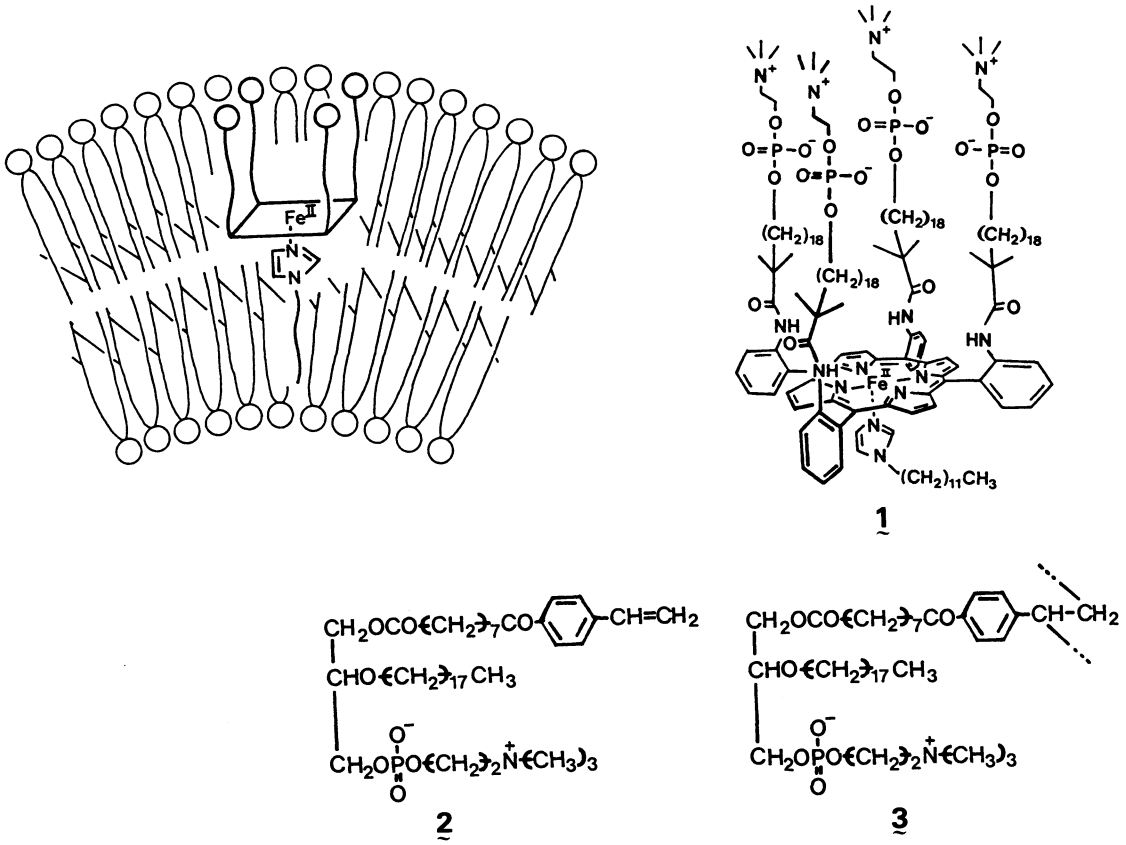
By incorporating the lipid-heme in the polymerized liposome, conc. and mechanically stable heme solution ([heme] = 10 mM) was prepared; this solution (100 ml) could bind molecular oxygen (ca. 23 ml) reversibly under physiological conditions even in a high speed flowing system.

Recently we have found that the 5,10,15,20-tetra(α,α,α,α-o-pivalamidophenyl)porphinato iron(II) complex of mono(1-lauryl-2-methylimidazole) embedded in a liposome of phosphatidylcholine binds molecular oxygen reversibly under physiological conditions (pH 7.0 aqueous media, 37 °C)[1] and that the oxygen-binding affinity and the rate parameters were similar to those of hemoglobin in blood.[1,2] We further synthesized an amphiphilic heme derivative having four phosphocholine groups, 5,10,15,20-tetra{α,α,α,α-o-[2',2'-dimethyl-20'-(2"-trimethylammonioethyl)phosphonatoxyeicosanamido]phenyl}porphinato iron(II) (lipid-heme)[3] and a lipophilic heme derivative, 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)porphinato iron(II),[4] which increased compatibility of the heme with a phospholipid and the oxygen-binding ability.

Next we intended to improve stability of the liposome as the carrier of the heme, which will bring about a highly concentrated, physically and mechanically stable and storageable solution of heme just like as blood. To accomplish this intention, new concept[5-7] which makes liposomes stable by the polymerization of the lipid bilayer would be applied to our system. In the present communication, the lipid-heme complex of 1-laurylimidazole (1) (Scheme 1) was incorporated in polymerized 1-[9-(p-vinylbenzoyl)nonanoyl-2-O-octadecyl-rac-glycerol-3-phosphocholine[8] (2) (abbrebiated as "polymerized liposome/heme", Scheme 1). The oxygen-binding and physical properties were measured and discussed in comparison with those of blood.

The polymerized liposome/heme was prepared as follow. The liposome of 1 and 2 (molar ratio; hemin/ligand/lipid = 1/3/50) was prepared by normal thin film method and the subsequent treatment under nitrogen with ultrasonicator. Thus prepared liposome was allowed under nitrogen atmosphere to polymerize under ultraviolet irradiation (32 W, 50 °C, 1 h) to give the polymerized



Scheme 1. Polymerized liposome/heme.

liposome/heme. The reduction of the Fe(III) derivative of 1 to the deoxy was spontaneously occured during the polymerization. Complete polymerization was confirmed by UV absorption and ^{13}C-NMR spectroscopical measurement; disappearance of the absorbance (λ_{max}; 265 nm) based on the vinyl group and of the characteristic signals (δ_C; 116.8 and 136.1 ppm) based on the vinyl carbons of 2. Complete reduction of the Fe(III) derivative of 1 was also confirmed by visible absorption spectra (λ_{max}: 429 (ε = 8.4 x 10^5 l·mol^{-1} ·cm^{-1}), 535 (8.7 x 10^4) and 562 (2.8 x 10^4) nm. The polymerized liposome/heme was concentrated by an ultrafiltration method.

The red and transparent solution of the polymerized liposome/heme was changed to its oxygen adduct solution on exposure to oxygen (λ_{max}: 422 and 544 nm). The oxygen adduct of the polymerized liposome/heme was stable, and the adduct formation was rapid and reversible under physiological conditions even at high concentration ([heme] = 10 mM, being equal to that of human blood).

The oxygen-binding affinity ($p_{1/2}$; oxygen pressure at half oxygen-binding for the heme) and oxygen-binding and -dissociation rate constants (k_{on} and k_{off}) of the polymerized liposome/heme were determined by the oxygen-binding and -dissociation equilibrium curve measurement ([heme] = 2.0 mM) and the stopped-flow method ([heme] = 0.50 mM), respectively; $p_{1/2}$ = 40 mmHg at 37 °C,

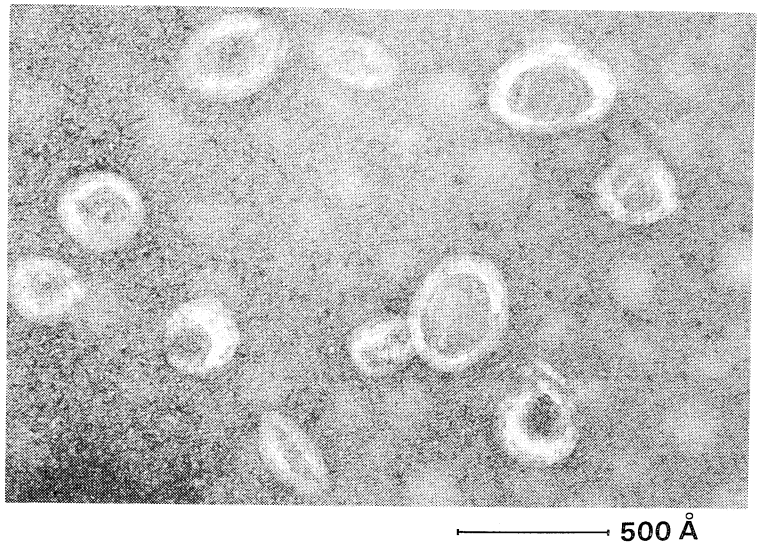


Fig. 1. Transmitting electron micrograph of the polymerized liposome/heme.

k_{on} = 1.3 x 10^4 l·mol^{-1}·s^{-1}, k_{off} = 0.42 s^{-1} at 25 °C. These values were similar to those of blood ($p_{1/2}$ = 27 mmHg at 37 °C, k_{on} = 1.1 x 10^4 l·mol^{-1}·s^{-1}, k_{off} = 0.16 s^{-1} at 25 °C).

Transmitting electron microscopy was carried out by the negative staining method using uranyl acetate. By the photo of the polymerized liposome/heme (Fig. 1) the diameter was ca.350 Å and it did not change before and after the polymerization. The gel permiation chromatography on Shepharose 4B (Pharmacia Fine Chem.) and the ultracentrifugation of the polymerized liposome/heme showed that all of 1 was entrapped within the liposome: The elution curves based on the polymerized phospholipids 3 and the heme 1 coincided with each other, and the supernatant by ultracentrifugation did not contain both the phospholipid 3 and the heme 1.

Solution properties of the polymerized liposome/heme were almost same as those of human blood as summarized in Table 1. The solution of the polymerized liposome/heme was stable and could be stocked for months without precipitation and change of particle size, i.e. without aggregation and fusion of the the liposome, at ambient temperature. This result is in contrast to the fact that

Table 1. Physical properties of the polymerized liposome/heme

System	Polymerized liposome/heme[a)]	Human blood
Specific gravity	1.012	1.050-1.063
Viscosity (cp)	3.75-4.12	4.50-5.00
Osmotic pressure (mOsm)	334	280-290

a)20% lipid, 0.9 wt/vol% physiological salt solution.

the homogeneous solution of the liposome/heme could not be prepared by using e.g. egg yolk lecithin (EYL) at the above mentioned condition and even the EYL liposome/heme prepared at lower concentration aggregated and fused after a few days.

The polymerized liposome/heme solution was also stable in physiological salt solution, plasma expander or human serum (pH 6.6-7.4) and gave the same stable oxygen adduct with the same life time and the oxygen-binding affinity ($p_{1/2}$ = 39-43 mmHg at 37 °C). The polymerized liposome/heme was flowed through a teflon tube (ϕ = 1 mm) with high space velocity (50 $ml \cdot min^{-1}$). The polymerized liposome/heme was mechanically stable under strong share and acted as the oxygen carrier: It is expected to be a candidate for blood substitutes.

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