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Stabilization effect of tocopherol and catalase on the life-time of liposome-embedded heme as an oxygen carrier

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Combination of the liposome-embedded heme with tocopherol and catalase much prolonged the life-time of liposome-embedded heme as an oxygen carrier under physiological conditions.

Recently we reported that a synthetic heme derivative embedded in the bilayer of natural, synthetic and polymerized lipid liposomes (abbreviated as the liposome-embedded heme), forms its reversible oxygen adduct and carries oxygen under physiological conditions [1–5]. Oxygen-binding capability of the liposome-embedded heme was comparable to that of hemoglobin in red blood cell [1–5]. The heme-oxygen adduct was slowly degraded during the oxygen-binding under physiological conditions. On degradation of the heme-oxygen adduct it is considered that slight amounts of superoxide and/or hydrogen peroxide are formed, oxidize phospholipids and weaken the liposome. Thus the life-time of the liposome-embedded heme as the oxygen carrier is limitted to about 1 day under physiological conditions. It is well known that α-tocopherol and catalase act as antioxidizing agents against hydrogen peroxide and superoxide. Furthermore, the addition effect of α-tocopherol and catalase on the life-time of liposome-encapsulated hemoglobin has been also reported [6,7]. In this note, we report that an antioxidizing agent such as tocopherol and a hydrogen peroxide remover such as catalase much prolong life time of the liposome-embedded heme as an oxygen carrier under physiological conditions. The effect of these additives on the liposome-embedded heme was discussed in comparison with their antioxidizing ability.

The liposome-embedded heme was prepared from the heme derivative, 5,10,15,20-tetra(α,α,α,α-*o*-(2′,2′-dimethyl-20′-(2′-trimethylammonioethyl)phosphonatoxyeicosanamido)phenyl)-porphinato iron(II) [4,8] complex of 1-laurylimidazole [1] and phospholipid. As the phospholipid, egg yolk phosphatidylcholine (PC) and poly(1,2-bis(2′,4′-octadecadienoyl)-*sn*-glycero-3-phosphocholine) [9,10] were used. α-Tocopherol, ascorbate, arachidonic acid, gluthatione, and catalase were purchased from Sigman (special grade). The liposome-embedded heme with the antioxidizing reagent or the enzyme was prepared as follows. The methanol/chloroform solution of heme (1 μmol), 1 laurylimidazole (3 μmol), phospholipid (50 μmol) and α-tocopherol (0.01–10 μmol) was evaporated in vacuo to given thin film or powder in a round flask. The mixture was dispersed in 0.1 M phosphate (pH 7.0) buffer solution (20 ml) by a Vortex mixer. The solution was homogenized and ultrasonicated (32 W × 20 min, a prove type sonicator, Nihonseiki Co. Ltd.,

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US-600) in ice water bath under nitrogen atmosphere to give transparent and red-colored and single-walled type (SUV) liposome-embedded heme solution. In the case of other anti-oxidizing reagents, the dried mixture of heme, 1-laurylimidazole and phospholipid was added to the buffer solution (20 ml) of ascorbate, arachidonic acid or gluthatione (0.01–10 μmol), and then the mixture solution was dispersed and ultrasonicated as mentioned above to give the liposome-embedded heme solution. For the enzyme coexisted system, catalase (0.01–10 μmol) was finally added to the liposome-embedded heme solution. The heme embedded in the bilayer of polymerized 1,2-bis(2′,4′-octadecadienoyl)-*sn*-glycero-3-phosphocholine liposome (the poly-lipid liposome-embedded heme) was prepared as follows: The liposome solution of the heme, ligand and phospholipid with α-tocopherol, ascorbate, arachidonic acid, gluthatione or catalase was prepared and allowed under nitrogen atmosphere and ultraviolet irradiation (32 W, 1 h) to give the poly-lipid liposome-embedded heme solution.

Visible absorption spectrum of the deoxy liposome-embedded heme (λ_{max}; 426, 535 and 562 (sh) nm) changed to that assigned to the oxygen adduct (λ_{max}; 422 and 546 mm) on exposure to oxygen in the presence of α-tocopherol under physiological conditions (pH 7, 37°C) as well as that in the absence of the α-tocopherol (Fig. 1(a)). The oxy-spectrum changed to that of the carbon monoxide (CO) adduct (λ_{max}; 423 and 540 nm) on bubbling CO through the solution of the oxygen adduct and returned to that of deoxy-heme on bubbling nitrogen through the solution. It was spectroscopically confirmed that the oxygen adduct is not reduced and not returned to starting the deoxy derivative by α-tocopherol. The oxygen concentration in the solution phase was kept constant after the oxygen adduct formation (measurement with oxygen electrode) which denies a rapid oxygen reduction by α-tocopherol catalyzed with the heme.

The oxygen adduct was slowly degraded to the iron(III)-porphyrin (hemin) complex (Fig. 1(b)) and this time-degradation curve obeyed first-order kinetics [11,12]. Life-time (half-life, τ), of the oxygen adduct of the egg yolk PC liposome-embedded heme with α-tocopherol was a week (Table I), which was much prolonged in comparison with that without α-tocopherol. The life-time was also prolonged with catalase, but was not with gluthatione, arakidonic acid or ascorbate (Table I). On degradation of the oxygen adduct it is considered that slight amounts of superoxide and hydrogen peroxide was formed [13]. Although hydrogen peroxide and superoxide ion could not be detected by spectroscopic method due to deep color of the

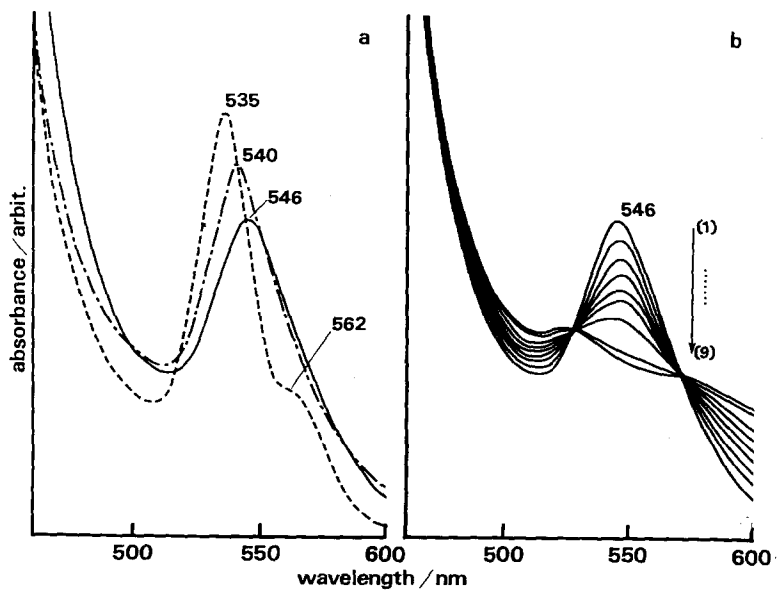

Fig. 1. Visible absorption spectra of the egg yolk PC liposome-embedded heme with α-tocopherol (a) and degradation of its oxygen adduct (b) in pH 7.0 aqueous solution at 37°C. (a)- - - - -, deoxy; ———, oxy; ·–·–·, CO adduct. (b) (1) at 0, (2) 2, (3) 4, (4) 6, (5) 8, (6) 10, (7) 15, (8) 30, and (9) 60 days.

TABLE I

LIFE-TIME OF THE LIPOSOME-EMBEDDED HEME WITH ANTI-OXIDIZING REAGENTS OR CATALASE IN pH 7.0 AT 37 °C

[heme]/[lipid] = 1/50, [heme] = 50 μM, under oxygen atmosphere.

System	Half-life (day)
Egg yolk PC liposome-embedded heme	
without additive	1.0
α-tocopherol	8.0
gluthatione	1.1
arachidonic acid	0.9
ascorbate	1.2
catalase	7.5
Poly-lipid liposome-embedded heme	
without additive	4.0
α-tocopherol	21
catalase	18

heme solution and by electrode (peroxide probe) method probably due to their low concentration, the life-time of the liposome-embedded heme was decreased by slight amount addition of hydrogen peroxide: added amount 0 (τ = about 1 day), 50 μM (5 h), 200 μM (5 h), 2 mM (0.5 h) and 20 mM (< 1 min). This inhibiting effect of hydrogen peroxide was cancelled in the presence of tohcopherol: added amount of hydrogen peroxide 0 (τ = 8 day), 50 μM (7 day) and 2 mM (7 day).

Lipids are known to be easily oxidized by hydrogen peroxide and superoxide [14,15]. It is also reported that oxidation of phospholipid induces an destabilization of its liposome [6,7,15]. The authors have elucidated that for the liposome-embedded heme the liposome structure protects the oxygen adduct from its degradation and that of the oxygen adduct is much decreased by destroying the liposome structure with lyso phosphatidylcholine or a synthetic surfactant [1]. Thus it is assumed that the oxidation of phospholipid by hydrogen peroxide or superoxide brings about the degradation of the oxygen adduct. It has been reported that tocopherol is incorporated in liposome bilayer and depress effectively the oxidation of lipids [16,17]. For the liposome-embedded heme tocopherol is expected to be incorporated in the liposome with the heme and stabilize the liposome. The effect of catalase is also assumed to be caused by its removing ability of hydrogen peroxide and other peroxide from the liposome solution.

Combination of the heme embedded in the bilayer of the polymerized lipid liposome (poly-lipid liposome-embedded heme, details; see Experimental Part) with α-tocopherol or catalase prolonged life-time of the oxygen adduct to a few week under physiological conditions. This means that the liposome-embedded heme with tocopherol or catalase satisfies one of the requisites for an artificial blood.

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