Oxygen-Carrying Plasma Hemoprotein "Albumin-Heme": Nitric Oxide Binding and Physiological Responses after Administration in vivo

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Summary: Recombinant human serum albumin complexed with tetraphenylporphinatoiron(II) derivative, "albumin-heme (rHSA-FeP)", is a synthetic oxygen (O_2)-carrying plasma hemoprotein, which becomes a new class of red blood cell substitute. The UV-vis. absorption and ESR spectroscopy revealed that rHSA-FeP formed six-coordinate nitrosyl complex after exposure of nitric oxide (NO) gas. Although the NO-binding affinity of rHSA-FeP ($P_{1/2}^{NO}$: 1.7×10^{-6} Torr, pH 7.3, 25 °C) is 9-fold higher compared to that of hemoglobin (Hb), the administration of this artificial hemoprotein solution into anesthetized rat does not induce an acute increase in blood pressure (hypertension), which is often observed in Hb-based O_2-carriers due to the depletion of NO (endothelial derived relaxing factor).

Keywords: albumin-heme; hemoprotein; hypertension; nitric oxide; oxygen carrier

Introduction

Recently, one of the hemoglobin(Hb)-based oxygen(O_2)-carriers, which consists of polymerized bovine Hb, was approved for human use in South Africa as the first artificial red cell substitute.[1,2] It shows some superior characteristics compared to stored human blood, for instance, compatibility with all blood type and two-year room temperature stability. However, the administration of such Hb-solutions often elicit an acute increase in blood pressure, because the Hb molecules extravasuate through the vascular endothelium and depletes nitric oxide (NO; endotherial-derived relaxing factor), thus inducing vasoconstriction. Although the precise mechanism of this bradycardia is still controversial, the unfavorable hemodynamic alterations may limit the use of these modified-Hb solutions as blood replacement compositions.

We have shown that the recombinant human serum albumin (rHSA) incorporating a tetraphenylporphinatoiron(II) derivative with a covalently linked proximal base, 2-[8-{N-(2-

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methylimidazolyl)}octanoyloxymethyl]-5,10,15,20-tetrakis(α,α,α,α-*o*-pivalamido)phenylporphiphinatoiron(II) (FeP) [rHSA-FeP, Figure 1], can reversibly bind and release O_2 under physiological conditions (in aqueous media, pH 7.3, 37 °C) in the same manner as Hb and myoglobin (Mb).[3] The exchange transfusion tests with hemorrhagic rats demonstrated that rHSA-FeP satisfies the initial clinical requirements for an O_2-carrying resuscitative fluid.[4] The only source of our present concern was that the small rHSA-FeP molecule (8 × 3 nm) injected into the blood vessels would be eliminated from the circulations, and contributes to the significant consumption of NO in the interstitial space between the endothelium and vascular smooth muscle.

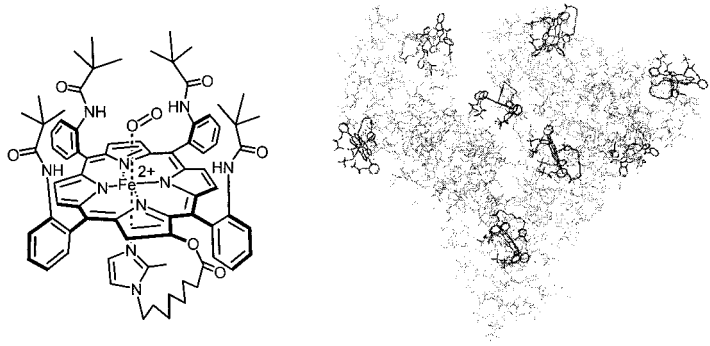

Fig. 1. Oxygenated FeP and predicted structure of rHSA complexed with eight molecules of FeP. The rHSA molecule is colored gray and FePs are shown in dark gray. The figure was made with insight II (Molecular Simulations).

Nitrosyl Complex

We have shown that covalently attaching the imidazolyl group directly to the porphyrin periphery inhibits the proximal base elimination, which is triggered by the NO-binding to the trans side.[5] The UV-vis. absorption spectrum of the rHSA-FeP solution showed maxima at 425 and 546 nm after the exposure of NO gas (1% in N_2) [Figure 2 (A)]. The carbonyl rHSA-FeP (λ_{max}: 428, 541 nm) also moved slowly to the same species after flowing the NO. Further addition of the 100% CO gas to these solutions led to the spectral changes in the carbonyl complexes. These results indicated that (i) the nitrosylation of FeP is reversible and (ii) the central iron was not oxidized during the reaction. ESR spectroscopy was also employed to

confirm the coordination structure of this nitrosyl rHSA-FeP. The absorption curve of the frozen solution at 77 K showed the characteristic shape of rhombic symmetry around the paramagnetic center and very similar to those of the nitrosyl Hb and Mb [Figure 2 (B)].[6] The obtained g values (g_1, g_{II-1}, g_3) are also in good agreement with those of the six-coordinate nitrosyl Hb, Mb, as well as FeP in toluene.[5a,6,7] If the imidazolyl moiety is dissociated from the central iron(II) of the FeP, the spectrum of the five-coordinate nitrosyl complexes become a sharp intense triplet associated with g_3; this was not observed. Based on these findings, it can be concluded that FeP forms the six-coordinate nitrosyl complex with an intramolecularly bound 2-methylimidazolyl arm in the albumin structure.

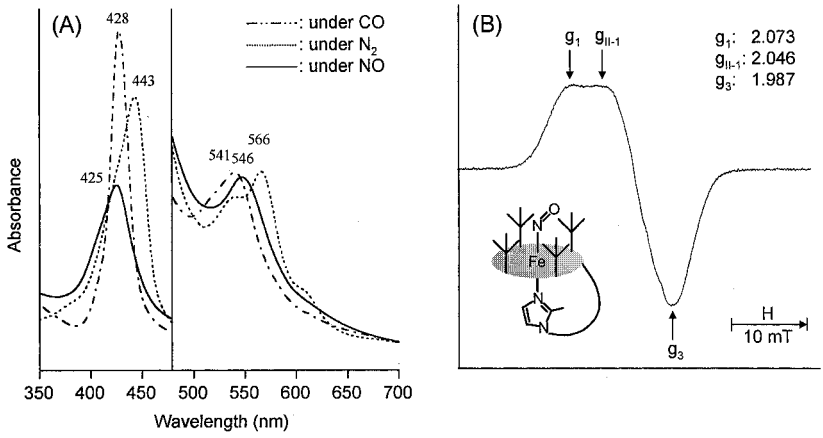

Fig. 2. (A) UV-vis. Absorption spectral changes of rHSA-FeP in phosphate buffer solution (pH 7.3) at 298 K and (B) ESR spectrum of nitrosyl rHSA-FeP in frozen phosphate buffer (pH 7.3) at 77K.

NO-Binding Parameters

The NO-association and -dissociation rate constants (k_{on}^{NO}, k_{off}^{NO}), and NO-binding affinity ($P_{1/2}^{NO}$) of rHSA-FeP are summarized in Table 1.[5b] Kinetically, the low NO-binding affinity (high $P_{1/2}^{NO}$ value) of rHSA-FeP compared to that of FeP in toluene arises from the decreased association rate constant. It has been widely recognized that the NO-binding to Fe(II)porphyrins and natural hemoproteins are diffusion controlled.[8-10] Actually, Hb and Mb have identical

k_{on}^{NO} values (1.7–1.8 × 10^7 $M^{-1}s^{-1}$) which are 10-fold lower than that of the naked Fe(II)protoporphyrin (1-methylimidazole) complex.[9] Interestingly, a similar decrease is observed in our artificial hemoprotein, *i.e.*, incorporation of FeP into the albumin matrix restricts the NO access to the central iron which led to a 60-fold reduction in its NO-association rate.

Table 1. NO-Binding parameters of rHSA-FeP in phosphate buffer solution (pH 7.3) at 25 °C.

	Solvent	10^{-8} k_{on} ($M^{-1}s^{-1}$)	10^4 k_{off} (s^{-1})	10^6 $P_{1/2}$ (Torr)
rHSA-FeP	pb[a]	0.15	0.67	1.7
FeP	toluene	8.9	2.3[b]	0.018
Hb(T-state)[c]	ab[d]	0.18	40	15
Mb[e]	pb[f]	0.17	1.2	2.7
FePP(1-MeIm)[g]	water[h]	1.8	2.9	0.57

[a]pb: Phosphate buffer (30 mM, pH 7.3).
[b]The dissociation rate of NO was determined by k_{on}^{NO}/K^{NO}.
[c]From ref. 9.
[d]ab: Aqueous buffer (pH 8.0).
[e]At 20 °C. From ref. 11.
[f]pb: Phosphate buffer (50 mM, pH 7.0).
[g]FePP(1-MeIm): Fe(II)protoporphyrin(1-methylimidazole) complex, at 22 °C. From ref. 9.
[h]20% N-Methylimidazole solution (pH 9.0).

Change of Blood Pressure after Administration

The rHSA-FeP strongly binds NO; the NO-binding affinity is still 9-fold higher compared to that of Hb and enough high to react 1 μM NO in the wall of the vasculator, therefore one can anticipate that the injection of this O_2-carrying plasama hemoprotein into the blood vessels may induce unfavorable hypertension. In order to clarify the hemodynamic behavior after the administration of rHSA-FeP, we tested a top-load dose of this solution in anesthetized rats.[12] Contrary to our expectations, only a negligibly small change in the mean arterial pressure (MAP) was observed after the administration (Figure 3). If anything, the difference from the baseline (ΔMAP) slowly decreased to –6.8 ± 3.4 Torr within 20 min and remained constant during the monitoring period. The response is quite similar observed following infusion with an equivalent volume of rHSA (5 g/dL) in this experimental setup. In contrast, the administration

of extracellular Hb solution elicited an acute increase in blood pressure (ΔMAP: 16±1.9 mmHg), followed a graduated decrease throughout the 60 min period of observation. Why does rHSA-FeP not induce the hypertension? The molecular size is almost the same as Hb, and its association rate constant for NO binding (k_{on}^{NO}: 1.5×10^7 $M^{-1}s^{-1}$) is high for the rapid scavenging of NO. The answer probably lies in the negatively charged molecular surface of the albumin vehicle. One of the unique characteristics of serum albumin is its low permeability through the muscle capillary pore, which is less than 1/100 that for Hb due to the electrostatic repulsion between the albumin surface and the glomerular basement membrane around the endothelial cells.[13] The isoelectric point of rHSA-FeP (pI = 4.8) is exactly the same as that of rHSA itself, because the FeP molecule without any ionic residue interacts non-specifically with the hydrophobic cavity of rHSA.[3a,b]

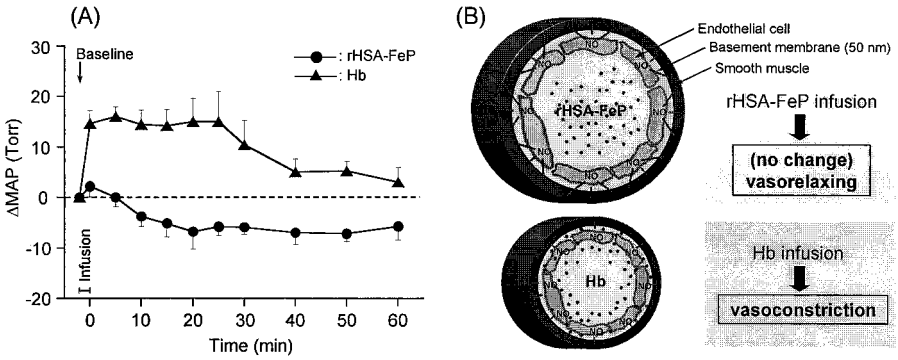

Fig. 3. (A) Changes of mean arterial pressure (MAP) before and after the infusion of rHSA-FeP solution ([rHSA]: 5 g/dL, FeP/rHSA: 4 (mol/mol), 300 mg/kg) and extracellular Hb solution (5 g/dL, 300 mg/kg) in the anesthetized rats (significant difference from baseline: $p < 0.05$, n=5). All data are shown as changes from the basal values (ΔMAP) just before the infusion and expressed as mean ± standard error. Basal value is 90.1 ± 3.0 Torr. (B) Schematic illustration of section of aorta and changes of diameter of the vessels after administration of rHSA-FeP and Hb solutions.

Conclusion

While more research is required for a full understanding of the biological and pharmacological properties of this rHSA-FeP, the present results obviously indicate that the albumin-based synthetic O_2-carrying hemoprotein excludes unfavorable hemodynamic responses observed in the modified-Hb solutions. Thus, rHSA-FeP can be utilized not only as a safe and effective red blood cell substitute, but also as an O_2-carrying medicine which will be adopted for use in several clinical applications, such as myocardial infraction, tracheal blockade, preservation of organs for transplantation, etc.

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