# Redox Behavior of Human Serum Albumin–Heme Hybrid on Graphite Electrode Modified with Didodecyldimethylammonium Bromide

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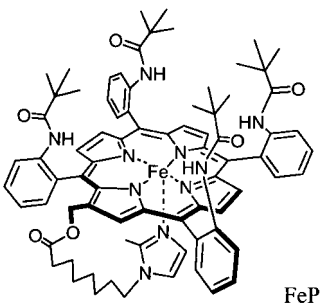
The redox behavior of a synthetic hemoprotein, the recombinant human serum albumin (rHSA), incorporating a tetraphenylporphinatoiron derivative (FeP) [rHSA-FeP] was first evaluated using a graphite electrode modified with didodecyldimethylammonium bromide as a promoter. Compared to that of the naked FeP, the redox potential of the Fe(III)/Fe(II) couple in rHSA-FeP shifts in the positive direction, indicating that the central ferrous ion of FeP becomes more difficult to be oxidized by incorporation into the albumin structure.

We have recently found that the incorporation of 2-[8-{*N*-(2-methylimidazolyl)}octanoyloxymethyl]-5,10,15,20-tetrakis(*α,α,α,α-o*-pivalamidophenyl)porphinatoiron(II) (FeP) into recombinant human serum albumin (rHSA) provides a totally synthetic hemoprotein (rHSA-FeP), which can reversibly bind and release dioxygen under physiological conditions (pH 7.3, 37 °C) like hemoglobin (Hb) and myoglobin (Mb).[1] The obtained rHSA-FeP shows a relatively long half-life time for the dioxygenated complex compared with the micellar solution of FeP, which is solubilized by sodium dodecyl sulfate in water. This indicates that the molecular environment around FeP in albumin plays a crucial role in extending the stability of the $O_2$ adduct. We tried to elucidate its mechanism by electrochemistry. However, hemoproteins usually tend to denature on an electrode surface,[2] and it is not easy to directly measure their redox behavior. In order to prevent this denaturing, promoter molecules were introduced onto the electrode, which can efficiently act as electron commuters between the electrode and the immobilized hemoproteins.[3–6] Using a surfactant modified electrode, we can first study the redox behavior of rHSA-FeP in aqueous media and the effect of rHSA on stabilization of dioxygenated FeP complex. Concomitantly, the effects of scan rate, pH and counter anions were carefully evaluated.




FeP

Didodecyldimethylammonium bromide (DDAB) was selected as a promoter for the edge plane pyrolytic graphite (EPPG) electrode. After a DDAB chloroform solution was cast on the EPPG surface, the thin film that probably shows a liquid-crystal like structure was formed.[5] Further casting of the aqueous solution of rHSA-FeP ([rHSA] = 0.2 mM, FeP/rHSA = 1:1) led to a good immobilization of the synthetic hemoproteins on the modified electrode. Cyclic voltammograms of this hybrid in aqueous buffer solutions (pH 4.1) are shown in Figure 1. rHSA-FeP directly cast onto the electrode without a DDAB film shows no evident redox waves, suggesting that rHSA-FeP was probably denatured,[2] therefore, electrons could not commute between the electrode surface and central iron of FeP in rHSA. In contrast, the redox waves corresponding to the Fe(III)/Fe(II) couple could be well identified and showed good reversibility using the EPPG electrode modified with the DDAB thin film, which can efficiently act as a promoter and prevent the denaturing of rHSA.

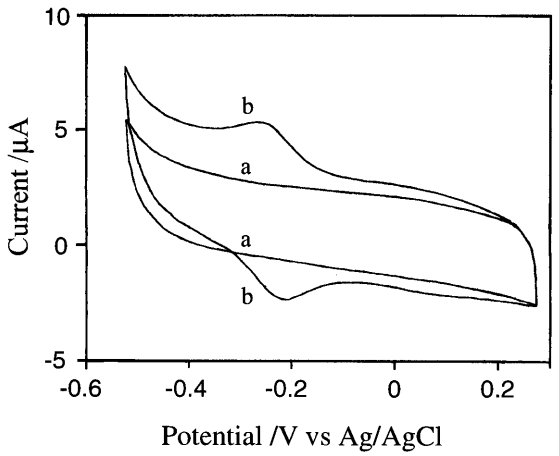



**Figure 1**. Cyclic voltammograms of rHSA-FeP, (a) on the EPPG electrode coated with 15 μL 0.2 mM aqueous rHSA-FeP solution, and (b) on the EPPG electrode first modified with 5 μL 0.1 M DDAB chloroform solution and further coated with 15 μL 0.2 mM aqueous rHSA-FeP solution. Platinum wire counter electrode and Ag/AgCl (saturated KCl solution) reference electrode was used in pH 4.1 acetate buffer solution (0.1 M) containing KBr (0.1 M). Scan rate was 0.1 V/s under an argon atmosphere at room temperature.

With an increase in the scan rate from 0 to 2.0 V/s, the redox potential ($E_{1/2}$) was constant, but the peak current and the peak separation significantly increased. Below 0.2 V/s, the peak current demonstrates a linear correlation with the scan rate since diffusion is sufficient enough to bring all the required materials to the electrode (Figure 2). On the other hand, it becomes lower than the linear correlation but is proportional to the square root of the scan speed above 0.5 V/s. This behavior

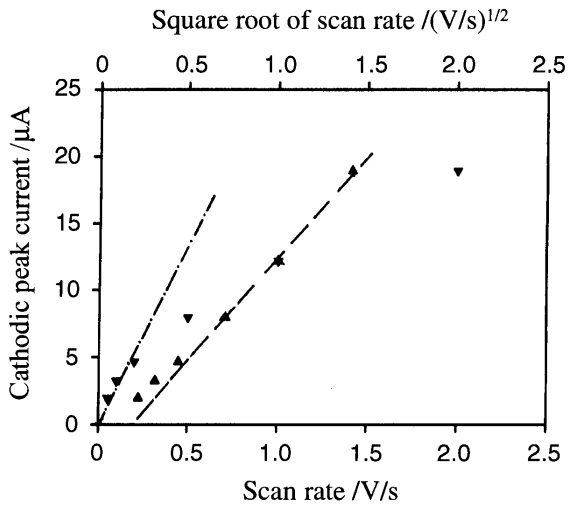



**Figure 2**. Change of cathodic peak current with the scan rate (▼) and its square root (▲).

can be explained in terms of thin-film electrochemistry,[7] i.e., the electron transfer becomes diffusion controlled in this region.

The effects of the counter anions ($Cl^-$, $Br^-$, $I^-$) and their concentration on the $E_{1/2}$ of FeP in the hybrid are given in Table 1. The value of $E_{1/2}$ decreases with the sequence $Cl^- > Br^- > I^-$, suggesting that the large electronegativity of the counter anions in aqueous solution makes the central ferrous ion of FeP more difficult to be oxidized. The $E_{1/2}$ decreased almost linearly with the log of the counter anions' concentration following the Nernst equation. These results indicate that the anions take part in this redox reaction, which is quite similar to the observation in hemoproteins.[8]

**Table 1**. Redox potentials ($E_{1/2}$) of the Fe(III)/Fe(II) couple for rHSA-FeP in aqueous solution at scan rate 0.1 V/s by thin film method

| System | pH | Supporting electrolyte | $E_{1/2}$ /mV *vs* Ag/AgCl |
|---|---|---|---|
| rHSA-FeP | 4.1 | 0.1 M KBr | -246 |
| | | 0.3 M KBr | -267 |
| | | 0.5 M KBr | -282 |
| | | 0.5 M KCl | -266 |
| | | 0.5 M KI | -333 |
| | 7.6 | 0.1 M KBr | -274 |
| | 9.0 | 0.1 M KBr | -286 |
| | 7.3 | 0.15 M NaCl | -253 |
| FeP | 4.1 | 0.1 M KBr | -273 |
| | 9.0 | 0.1 M KBr | -281 |
| | 7.3 | 0.15 M NaCl | -274 |

Furthermore, the $E_{1/2}$ of rHSA-FeP shifts in the negative direction with an increase in the pH. This is in contrast to the fact that the naked FeP on the DDAB modified electrode shows a small $E_{1/2}$ difference in our measured pH range (4.1–9.0). Although the imidazolyl moiety in FeP is basic ($pK_a$: 7.85), a proton does not interact with the FeP molecule because the imidazole nitrogen donates an electron to coordinate with the central iron and becomes almost neutral.[9] This is also true for FeP in albumin, therefore, we can conclude that this pH dependence of $E_{1/2}$ results from the change in the electron density of rHSA. It is known that the isoelectric point (pI) of rHSA is 4.8 and the tertiary structure deforms with the change in pH.[1,10] That is, a high pH increases the number of dissociated $-COO^-$, which results in the corresponding increase in the electron density of rHSA. As a result, $E_{1/2}$ shifts in the negative direction and the central ferrous ion becomes more easily oxidized.

Compared with that of the naked FeP at pH 4.1, where the rHSA surface is almost neutral, the $E_{1/2}$ of rHSA-FeP is shifted in the positive direction from –273 to –246 mV (vs Ag/AgCl). The hydrophobic microenvironment around FeP presumably makes the ferrous ion difficult to be oxidized, namely a polarity effect. We found that the change in the $E_{1/2}$ of FeP in physiological solutions (pH 7.3, 0.15 M NaCl) is consistent with the above results and shifts from –274 to –253 mV after incorporation into rHSA (Table 1).

In conclusion, the direct electrochemistry of the rHSA-FeP hybrid on a DDAB modified electrode first provides the redox potential of Fe(III)/Fe(II) couple, which shifts in the positive direction compared to that of the naked FeP in physiological solution. This indicates that the central ferrous iron of FeP becomes more stable against oxidation by incorporation into the albumin.

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† CREST investigator, JST.

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