Electrochemistry of (Protoporphyrinato IX) Iron(III) and its Imidazole Complexes in Liposomes of L-α-Phosphatidylcholine

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Abstract — The electrochemical behaviour of hemin [(3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionate)iron(III)] and its complexes with imidazole and 1-methyl imidazole in aqueous liposomes of L-α-phosphatidylcholine (L-α-Lecithin) was studied by cyclic voltammetry (CV) and Square Wave Voltammetry (SWV) techniques. The redox potential versus Ag-AgCl reference electrode was found to be -339 mV, -390 mV and -355 mV for hemin complexes with ligand -OH, imidazole (ImH) and 1-methyl imidazole (1-MelmH), respectively. Compared to aqueous solutions where hemin exists as aggregated species, hemin encapsulated in liposomes shows a large positive shift of the redox potential of ca. 100 mV. The apolar nature of the local heme environment stabilizes the Fe(II) oxidation state relative to the Fe(III) state. Electron transfer at the heme is influenced by the uptake/release of protons with ca. -59 mV shift of potential per unit change of pH (ΔE/ΔpH).

Keywords : Hemin, Micelles, Liposomes, Cyclic Voltammetry, Square Wave Voltammetry.

INTRODUCTION

The heme proteins, which contains an iron porphyrin at the active site, are widely distributed in cellular systems and they catalyse a variety of biological processes, most notably the electron transfer reactions in respirations in the mitochondrial membrane [1,2]. In the proteins, the redox potential of hemin is influenced by several factors such as the local heme environment [2-5], and the ionization of a functional group near the heme [2-4]. Obtaining a precise physical and chemical description of electron transfer in biological membranes, particularly the pH dependence of redox potentials [2-4], is of current research interest.

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Another area of significance is the aggregation of hemin and the involvement of lipids in heme polymerization in the treatment of malaria [6-8]. Membrane associated hemin catalyses formation of highly oxidizing reactive species which are toxic to the parasites and thus provide protection against malaria [9-10]. Hemes are also used as sensitizers in the photodynamic therapy of cancer [11]. Liposomes may be promising drug delivery systems in various treatments where hemin may be used.

Surfactant micelles and liposomes (where lipids are arranged in concentric bilayer) are known to mimic biological membranes [12,13], and are attractive systems to study electron transfer under physiological conditions. Previous studies on hemin show that hydrophobic local heme environment impart a large positive shift on the redox potential and proton coupled electron transfer in aqueous surfactants [14-18]. We believe that electrochemical study on hemin encapsulated in liposomes may be worthwhile in order to compare the redox behaviour of hemin in various environment. In this paper, we report the redox potentials of hemin and its bis imidazole complexes encapsulated in phospholipid vesicles. The pH dependence of redox potentials of hemin is also reported.

**EXPERIMENTAL**

Hemin (bovin) and L-α-phosphatidylcholine (Type IV-S from soyabean), Imidazole (ImH) and 1-methyl imidazole (1-MeImH) were purchased from Sigma Chemical Co., USA. The phosphatidylcholine was washed several times with acetone and dried before use. Liposomes were prepared by the injection method [19] where an ethanolic solution of phospholipids (1 mM) was injected by a syringe into 10 mL of magnetically stirred 0.1 M sodium phosphate buffer solution (pH 7.4) containing 0.1 M NaNO₃ and thermostated at 55 ºC. The injection rate was 5 × 10⁻² mL per minute. The procedure has no degrading effect on phospholipids and gives reasonably homogeneous preparation of unilamellar vesicles [19] with a diameter of 26.5 nm. A slightly alkaline solution of hemin chloride in 1 : 1 (v/v) ethanol-water was added to the liposomes and the mixture was allowed to equilibrate in the dark at 40-50 ºC for 1 hour. The final concentration of hemin in liposome is 1 mM for electrochemical studies and 10⁻⁵ M for electronic spectral studies. Few sample solutions were prepared using sonication method [20] also but no major changes were observed in spectrochemical and electrochemical data.

The optical spectra were recorded on a Hitachi (U 3210) spectrophotometer. Electrochemical measurements were performed on a BAS 100B electrochemical analyzer using a three electrode cell assembly with nitrogen gas pursing lines. A gold disc was used as the working electrode and Ag-AgCl (3 M aqueous NaCl)
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electrode as the reference. The working electrode was polished and cleaned by sonication [5, 16]. A background voltammogram of the liposome containing 0.1 M NaNO₃ at the Au disc electrode showed that liposomes were free from redox interferences in the potential range of interest. In the Square Wave Voltammetry, the square wave amplitude was 25 mV, frequency 15 Hz and the potential height for base stair case wave front 4 mV [15, 16]. The diffusion co-efficient (Dₒ) were measured by chronocoulometry and the heterogeneous electron transfer rate constant were measured as reported previously [15, 21].

RESULTS AND DISCUSSION

The electronic spectrum of hemin (Scheme 1) in liposomes at pH 7.4, showed λ_max at 396, 567 and 596 nm. The spectrum is similar to that of a six coordinated high spin ferric hemin [17, 18] which is distinctly different from the spectrum of hemin in aqueous sodium dodecylsulphate solutions where iron is probably penta coordinated with OH⁻ ion as the fifth ligand [22]. Sixth coordination of hemin in liposomes may be due to the binding of a lipid molecule [23] at the vacant coordination site. Spectral and electrochemical data of hemin in liposomes indicate

![Scheme 1](image-url)

Structure of Protoporphyranato IX iron (III) or Hemin
that these are grossly different from those of aggregated hemin in aqueous solutions and compare well with those previously reported for monomeric hemins in aqueous micelles [15,24]. Monodispersion of hemin is essentially due to encapsulation in liposomes where hemin is buried in surface monolayers of the vesicles with the carboxylate ion of the propionic acid in contact with the solvent [25].

At lower pH the electronic spectrum showed $\lambda_{\max}$ values at 384, 510, 540 and 638 nm. A plot of absorbance at 390 nm as a function of pH was analysed by a non linear least squares fitting of the data [16] which indicated a $pK_a$ value of 7.1±0.1 with the ionization of one proton as in surfactant micelle [22,26].

Gradual addition of 1-methyl imidazole gave spectral changes with isosbestic points at ca. 510 nm and ca. 570 nm. Analysis of the data as per reported procedure [27] indicated that two 1-methyl imidazoles were bound to iron in hemin and that the binding constant ($\beta$) is $9.0 \times 10^2$ M$^{-1}$. Thus the coordination of imidazole to hemin in liposomes is very weak as compared to that in aqueous and organic solvents [27].

Fig. 1 shows reversible cyclic voltammogram of Hemin with H$_2$O and OH$^-$ as axial ligands at gold electrode without any promoter and mediator. The redox

![Cyclic voltammogram](image)

Fig. 1. Cyclic voltammogram of Protoporphyrinato IX iron(III) with H$_2$O and OH$^-$ as axial ligands in liposomes of L-\(\alpha\)-phosphatidylcholine (Type IV-S from soyabean) in phosphate Buffer solutions at pH 7.0. Working electrode is Gold with Ag-AgCl as reference electrode.
potential was found at -340 mV versus Ag-AgCl with a peak to peak separation of 60 mV. The plot of $i_{pa}$ and $i_{pc}$ versus square root of scan rate is linear and passes through the origin indicating that the process is diffusion controlled [28-31]. Similar reversible cyclic voltammograms were obtained for the bis imidazole and the bis 1-methyl imidazole complexes (not shown) with redox potential values, -390 mV and -355 mV, respectively. In the liposomes hemin is quite stable to hydrolysis and reproducible results are obtained when experiments were repeated several times. Since both hydrolysis to form the μ-oxo species and aggregation of heme require intermolecular interactions, encapsulation in liposome would prevent such processes relative to those in ordinary aqueous media. Redox potential of -340 mV versus Ag-AgCl corresponds to a situation where hemin exists as monomeric species in hydrophobic environment [15,16].

In a double potential step chronocoulometry experiment, the charge versus square root of time ($t^{1/2}$) response decreases sharply in the reverse step. From Anson plot a large intercept [15,21] for the reverse step of 3.4 μC for the bis 1-methylimidazole complex was found. From this, the amount adsorbed at the electrode [21,32] was calculated to be ca. $1.3 \times 10^{-7}$ mol cm$^{-1}$ for hemin and ca. $4.2 \times 10^{-7}$ mol cm$^{-1}$ for the bis imidazole complexes of hemin. These results indicate that hemin is weakly adsorbed at the electrode and that the electrochemical response of imidazole complexes at the Au electrode may be due to those of hemin adsorbed in the thin films of lipids at the electrodes similar to those reported in surfactant films [33,34].

The diffusion co-efficient of hemin and its complexes in liposomes ($D_o = 1.2-2.5 \times 10^{-7}$ cm$^2$ s$^{-1}$) are much smaller than those in ethanol and in aqueous media [15] ($D_o = 14.1-20.0 \times 10^{-7}$ cm$^2$ s$^{-1}$) which may be due to slow diffusion of encapsulated hemin species to the electrode [15,34].

The redox potential of hemin in liposomes as a function of pH was measured by SWV technique (Fig. 2). The data was analysed following the method described by Moore et al. [35] by a weighted non linear least squares fit [16] of the potential to a theoretical curve described by the equation (1),

$$E_m = E_o + \frac{RT/nF}{\ln(K_{a_{II}} + [H^+])/(K_{a_{III}} + [H^+]以外の numerator部分)}$$

The $pK_{a_{III}}$ and $pK_{a_{II}}$ are the $pK_a$ s of the ionisable functional ferric and ferrous state of hemin, respectively. The best fitted theoretical curve corresponds to one electron ($n=1$) and one proton ionization with $pK_{a_{III}} = 7.1 \pm 0.1$ and $pK_{a_{II}} = 8.0 \pm 0.1$. When the operating pH is in the range $pK_{a_{III}} < pH < pK_{a_{II}}$, the redox potential shift cathodically, the change in potential per unit change in pH being -59 mV. This indicates proton coupled electron transfer involving one proton and
one electron [34] and that the pKₐ s of the ionizations are widely separated (ΔpKₐ is ca. 1.0) between the Fe(III) and the Fe(II) oxidation states in the heme in liposomes. The values of pKₐ (ca. 7.1) agree quite well with that found from the measurement of absorbance of the Soret band by the electronic spectral measurement.

CONCLUSION

Liposomes are found to be a suitable medium for spectroscopic and electrochemical studies on hemin and its imidazole complexes. Compared to aqueous solutions where aggregated hemin shows large negative potentials [15], monomeric hemin in liposomes show a large positive potential. The shift of the potential on bringing a hemin from an aqueous solution to an essentially hydrophobic environment in liposomes corresponds to the effective relative permittivity of the local heme environment to be approximately [2,16] around 16-20 mV. The apolar nature of the local heme environment stabilizes the Fe(II) oxidation state relative to the Fe(III) state in liposomal model membranes. Moreover, the electron transfer at the heme is further influenced by the uptake/release of protons. Liposome encapsulated hemin is likely to be an excellent model system for the study of electron transfer and redox reactions in biological membranes.

Fig. 2. Plot of redox potential (E₁/₂) versus pH of Protoporphyrinato IX iron(III) with H₂O and OH⁻ as axial ligands in liposomes of L-α-phosphatidylcholine. Working electrode is Gold with Ag-AgCl as reference electrode.
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