

Detection of Octaethylverdohaem π -Neutral Radical by Electrochemical-Optical Absorption and -ESR Measurements

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Optical absorption and ESR spectra ascribed to the octaethylverdohaem π -neutral radical species, $[\text{Fe}^{\text{II}}\text{octaethylverdohaem}(\text{pyridine})_2]^{\cdot}$, were recorded by electrochemical reduction of $[\text{Fe}^{\text{II}}\text{octaethylverdohaem}(\text{pyridine})_2]$ chloride under nitrogen.

It has been well established that aerobic reduction of verdohaemochrome¹ with ascorbic acid or phenylhydrazine results in formation of biliverdin analogues.^{2,3} The presence of a radical species derived from verdohaemochrome has frequently been proposed in the processes of decomposition of verdohaemochrome, however, a detailed electronic structure of the radical species has not been available until now. Here, we report the optical absorption and ESR spectra of the paramagnetic species generated by electrochemical reduction of verdohaemochrome under nitrogen.

Verdohaemochrome (yield 20%) was obtained using column chromatography from a dimethylformamide (DMF) solution of $\text{Fe}^{\text{III}}\text{octaethylporphyrin}$ chloride ($\text{Fe}^{\text{III}}\text{OEPCl}$; 1.0 mmol dm^{-3} , 50 ml), pyridine (5 ml) and an aqueous solution of ascorbic acid sodium salt (0.5 mmol dm^{-3} , 4 ml).⁴ Based on the results of the mass spectrum [$\text{M} - \text{Cl} - (\text{C}_5\text{H}_5\text{N})_2$; $m/z = 591$] and on satisfactory elemental analysis, the verdohaemochrome was formulated to be $[\text{Fe}^{\text{II}}\text{octaethylverdohaem}(\text{pyridine})_2]$ chloride **1**. The optical absorption spectrum of **1** [λ_{max} (ϵ , $\text{dm}^3 \text{ mmol}^{-1} \text{ cm}^{-1}$), in pyridine; 387 (50.0), 494 (6.5), 527 (12.0) and 653 (40.0)], and ¹H NMR spectrum [(270 MHz in [²H₅]pyridine); δ 1.60 (24 H, m Me), 3.50 (16 H, m CH₂), 9.09 (1 H, s CH) and 9.55 (2 H, s CH)], were consistent with those of $[\text{Fe}^{\text{II}}\text{octaethylverdohaem}(\text{pyridine})_2]^+$ reported by Hirota and Itano.⁵ The electrochemical-optical absorption, -ESR and cyclic voltammetry (CV) measurements were carried out on a DMF-pyridine (6:4) solution of **1** (0.2 mmol dm^{-3}), in the presence of tetrabutylammonium perchlorate (TBAP; 0.1 mol dm^{-3}).

CV measurements were made with a Yanagimoto P-1000H Voltammetric Analyser, equipped with Pt disk working electrode (diameter 2.2 mm), Pt wire counter electrode and Ag/Ag^+ reference electrode, with a scan speed 10 mV s^{-1} at 298 K. Fig. 1(a) shows a nearly reversible voltammogram (110 mV peak separation) observed for **1** under a nitrogen atmosphere. The formal reduction potential of **1** was evaluated to be $-1060 \text{ mV}/(\text{Ag}/\text{Ag}^+) (\pm 10 \text{ mV})$, as the mean value

of the oxidation and reduction peak potentials. Interestingly, the oxidation peak of the voltammogram completely disappeared, after flushing the solution with oxygen gas (ca. 5.0 ml), as shown in Fig. 1(b). On changing the atmosphere to nitrogen, a nearly reversible voltammogram was again recorded, and the formal redox potential agreed with that before oxygen flushing. The voltammetric changes, observed before and after oxygen flushing, show that the

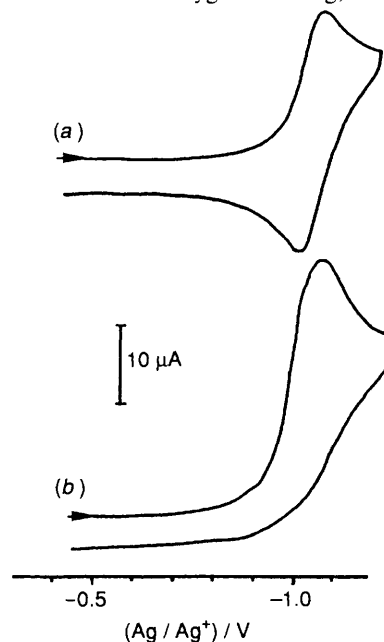


Fig. 1 Cyclic voltammograms recorded at 298 K for **1** (0.2 mmol dm^{-3}) in DMF-pyridine mixed solution (6:4), in the presence of TBAP (0.1 mol dm^{-3}) as an electrolyte at 298 K: (a) recorded in anaerobic conditions and (b) recorded in aerobic conditions. Scan rate is 10 mV s^{-1} .

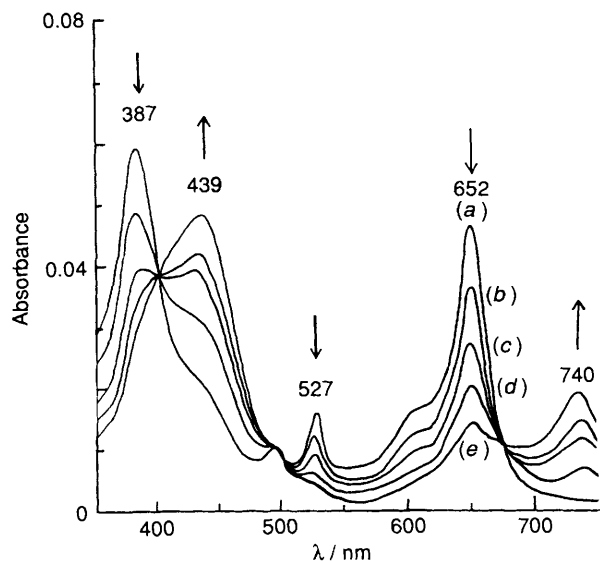


Fig. 2 Electrochemical optical absorption spectra observed for DMF-pyridine mixed solution (6:4) of **1** (0.2 mol dm^{-3}) in the presence of tBAP (0.1 mol dm^{-3}), by using an optically transparent thin-layer electrode cell at 289 K under nitrogen atmosphere. Applied potentials: (a) before electrolysis, (b) -1033 mV , (c) -1055 mV , (d) -1082 mV and (e) -1105 mV (Ag/Ag^+).

reduced substance of **1** has a short life-time under the oxygen atmosphere.

By using an optically transparent thin-layer electrode,⁶ in which a Pt-mesh working electrode was folded in the light-path with 0.2 mm thickness, the optical spectra generated on reduction of **1** were followed. As shown in Fig. 2, three sets of isosbestic points can be identified during potential variation from -1033 to -1105 mV (Ag/Ag^+) under nitrogen. After supplying -1105 mV (Ag/Ag^+) for ca. 30 s, absorption coefficients at 439 and 740 nm reached a maxima. While, absorption maxima due to **1**, at 387, 527 and 652 nm, had almost disappeared. The supplied potential was then increased from -1105 to -1033 mV (Ag/Ag^+) and the same optical spectrum of **1** was again observed. In addition, the coulometric analysis supported the fact that the observed spectral changes arose from a reversible one-electron redox process of **1** under nitrogen condition.

A simultaneous electrochemical-ESR cell, consisting of a Au helical working electrode, Au wire counter electrode and Ag wire reference electrode,⁷ was used for ESR measurements in a JEOL FE2XG X-band spectrometer. Before electrolysis, the diamagnetic species **1** gave no ESR signal in the magnetic field range 0–500 G ($1 \text{ G} = 10^{-4} \text{ T}$). On supplying -1150 mV (Ag/Ag^+) to the working electrode for ca. 10 min at 253 K under nitrogen, an isotropic ESR signal (denoted as **2**; $g = 1.981$) was detected, as shown in Fig. 3(a). This reaction mixture gave an anisotropic ESR signal ($g_{\perp} = 2.001$ and $g_{\parallel} = 1.935$) at 77 K [Fig. 3(b)]. Since, an averaged g -value of the anisotropic ESR signal (1.98) agreed well with the isotropic g value ($g = 1.981$) at 253 K. These observations demonstrated that the one-electron reduction of **1** [-1105 mV (Ag/Ag^+)], under nitrogen, resulted in the formation of the paramagnetic species **2** (λ_{max} : 439, 650 and 740 nm, $g_{\text{iso}} = 1.981$). Similar ESR spectra of **2** were also recorded for a well-degassed reaction mixture composed of **1** and phenylhydrazine, which is a well known reducing reagent to promote decomposition of **1**.

It has been well established that d^7 metal ions, such as low-spin Co^{II} and Ni^{III} complexes, give g -values much larger than 2.⁸ However, Goodman and Raynor⁹ suggested that six-coordinate nominally d^7 spin-paired ion species would give

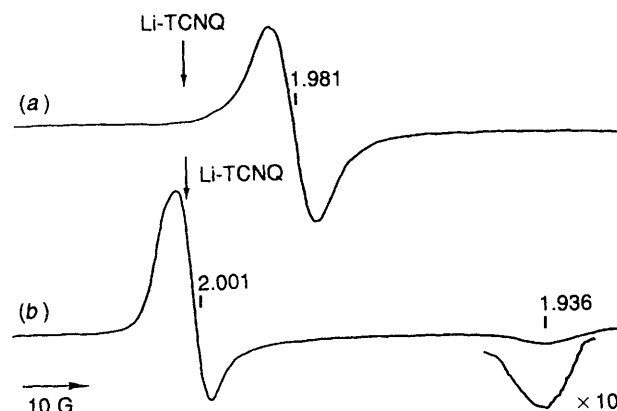
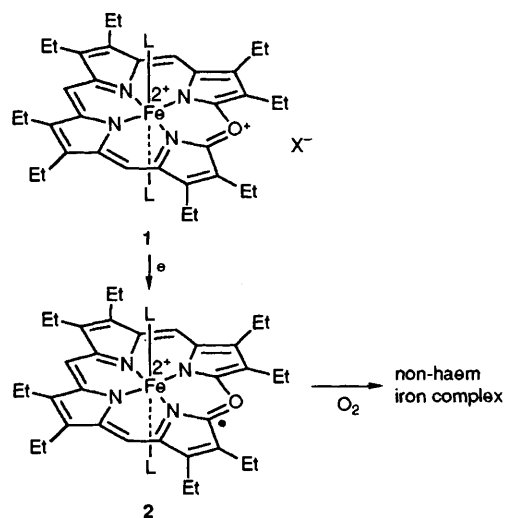


Fig. 3 ESR spectra recorded for **1** using electrochemical-ESR cell: (a) recorded at 253 K for **1**, after supplying -1150 mV (Ag/Ag^+) to the working electrode for about 10 min *in vacuo* and (b) recorded at 77 K for the same sample at 77 K (TCNQ = tetracyanoquinodimethane)



Scheme 1 Probable mechanism for one-electron reduction process of **1** to **2**. (L is axially ligating pyridine, x^- is counter anion)

a small g -value if suitable p-bonded ligands were present with low-lying π^* orbitals available. Tanaka *et al.*¹⁰ have reported that the one-electron reduced species of $\text{Fe}^{\text{II}}(\text{bipyridine})_3$ gave a small g value (1.995). In the present case, it seems reasonable to consider that the central iron of **2** takes a d^6 low-spin state ($S = 0$) and an additional one-electron would be dominantly localized on the π -orbital of verdohaemochrome. Taking into account that **1** possesses a positive charge, the electronic structure of **2** is tentatively formulated to be the π -neutral radical, $[\text{Fe}^{\text{II}}\text{octaethylverdohaem}(\text{pyridine})_2]^{\cdot}$.

It was noted that removal of oxygen from the reaction solution was necessary for observation of the optical absorption and ESR spectra due to the π -neutral radical **2**. When the electrochemical-optical absorption measurements were carried out under an oxygen atmosphere, the optical absorption spectrum of **1** was irreversibly changed to the broadened line, having the absorption maxima at 778 nm with shoulders at 370 and 440 nm. The new absorbing species was assumed to be the open-chained polypyrrole iron complex. Since, ESR signals ascribable to the non-haem iron complex ($g = 10, 4.3$ and 2)¹¹ were recorded by electrochemical reduction of **1** at 1150 mV (Ag/Ag^+) under oxygen atmosphere. These observations were consistent with the results that the oxidation peak in the voltammogram of **1** completely disappeared after oxygen flushing [Fig. 1(b)]. These findings described herein provide

experimental evidence that **2** would be one of the intermediate species in the decomposition processes of **1** to the biliverdin iron complex, as illustrated in Scheme 1.

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