Electrochemical-optical and -ESR Investigation of the Reaction between Oxygen and π-Neutral Radicals of $\mathrm{Zn^{II}}$- and $\mathrm{Fe^{II}}$-Protoverdohaem Dimethylester

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Electrochemically generated π-neutral radicals of $\mathrm{Zn^{II}}$- and $\mathrm{Fe^{II}}$-protoverdohaem dimethylesters are concluded to be the intermediate species in the process of the biliverdin formation.

Verdohaemochrome has been assumed to be decomposed into biliverdin derivatives by a reductant, *e.g.* ascorbic acid,[1] phenyl hydrazine,[2] under aerobic conditions. Recently, we[3] have reported that the one electron reduction of $\mathrm{Fe^{II}}$ octaethylverdohaem ($[\mathrm{Fe^{II}}\mathbf{OEV}]^+$) gave a π-neutral radical species, which was deduced to be the transient intermediate species in verdohaem decomposition. Here, the chemical reactivity of π-neutral radicals of $\mathrm{Zn^{II}}$- and $\mathrm{Fe^{II}}$-protoverdohaems towards molecular oxygen is studied in order to obtain information about the role of the central iron atom in verdohaem decomposition.

According to ordinary methods,[4] $\mathrm{Zn^{II}}$-protoverdohaem dimethylester† ($[\mathrm{Zn^{II}}\mathbf{PV}]^+$) was prepared from α-protobiliverdin. By the same method, $\mathrm{Fe^{III}}$-verdohaem complex was synthesized and purified as a bis-pyridine complex $[\mathrm{Fe^{II}}\mathbf{PV}(\mathrm{Py})_2]^+$.[4] The purity of these verdohaems was clarified by elemental analysis, optical absorption and ^{1}H NMR (270 MHz) measurements.‡ The electrochemical-optical absorption, ESR and cyclic voltammogram measurements were carried out in a DMF–pyridine (6:4) solution, containing TBAP (0.1 mol $\mathrm{dm^{-3}}$) as a supporting electrolyte. A Yanagimoto P-1000H Voltammetric Analyser equipped with Pt-disk (2.0 mm diameter), Pt-wire counter and (Ag/0.1 mol $\mathrm{dm^{-3}}$ $\mathrm{Ag^+}$)§ reference electrodes was used for CV measurements. $[\mathrm{Zn^{II}}\mathbf{PV}]^+$ (0.2 mmol $\mathrm{dm^{-3}}$) gave a nearly reversible CV with 110 mV peak separation. The formal redox potential of $[\mathrm{Zn^{II}}\mathbf{PV}]^+$ was estimated to be −847 mV *vs.* $\mathrm{Ag/Ag^+}$. The formal redox potential of $[\mathrm{Fe^{II}}\mathbf{PV}(\mathrm{Py})_2]^+$ was also estimated to be −903 mV *vs.* $\mathrm{Ag/Ag^+}$ (Table 1). No detectable oxidation peak current, supporting the formation of $[\mathrm{Fe^{III}}\mathbf{PV}(\mathrm{Py})_2]^+$ or $[\mathrm{Fe^{II}}\mathbf{PV}(\mathrm{Py})_2]^{2+\cdot}$, has been recorded for the complex in DMF–pyridine. In addition, the ferrous complex was very stable towards molecular oxygen, and did not decompose to biliverdin under aerobic conditions. Thus, the possibility of a redox couple occurring between the ferrous complex and molecular oxygen, forming the ferric complex and superoxide anion, can be safely ruled out.

By utilizing an optically transparent thin layer electrode,[5] the optical absorption spectra of $[\mathrm{Zn^{II}}\mathbf{PV}]^+$ (0.2 mmol $\mathrm{dm^{-3}}$) were recorded by a JASCO UVDEC-1 spectrometer. As shown in Fig. 1(*a*), absorption coefficients at 418, 464 and 804 nm showed concomitant increases with variation of the supplied potential from −650 to −800 mV *vs.* $\mathrm{Ag/Ag^+}$. On the other hand, the absorption maxima due to $[\mathrm{Zn^{II}}\mathbf{PV}]^+$ at 400 and 677 nm, have almost disappeared after *ca.* 10 min. with a supply of −800 mV. Three sets of isosbestic points, 415, 514 and 718 nm, can be identified during the electrolysis of $[\mathrm{Zn^{II}}\mathbf{PV}]^+$. The optical spectrum due to the $[\mathrm{Zn^{II}}\mathbf{PV}]^+$ was again recorded, when the supplied potentials were increased to −650 mV *vs.* $\mathrm{Ag/Ag^+}$. In addition, coulometric analysis provided experimental evidence that the observed spectral change arose from a reversible one-electron reduction of $[\mathrm{Zn^{II}}\mathbf{PV}]^+$.

ESR measurements were performed by a JEOL FE2XG X-band spectrometer equipped with a simultaneous electrochemical-ESR cell[6] with a Au-helix working electrode. An isotropic ESR signal (g = 2.003), having a broad doublet splitting (3 gauss), was observed [inset of Fig. 1(*a*)] at −30 °C by reduction of $[\mathrm{Zn^{II}}\mathbf{PV}]^+$ on supplying −850 mV ($\mathrm{Ag/Ag^+}$). Based on the observed g-value,¶ the paramagnetic species can be ascribed to be a π-neutral radical of $\mathrm{Zn^{II}}$-protoverdohaem-IX dimethylester, $[\mathrm{Zn^{II}}\mathbf{PV}]^{\cdot}$.

On changing the potential from −720 to −900 mV ($\mathrm{Ag/Ag^+}$), the one electron reduced species of $[\mathrm{Fe^{II}}\mathbf{PV}(\mathrm{Py})_2]^+$ showed absorption maxima at 451, 554 and 762 nm [Fig. 1(*b*)], with four sets of isosbestic points. The electrochemical-ESR spectrum observed at −30 °C for the one-electron reduced $[\mathrm{Fe^{II}}\mathbf{PV}(\mathrm{Py})_2]^+$ revealed an isotopic ESR signal (g = 1.985), as shown in the inset of Fig. 1(*b*). The frozen solution at −110 °C gave an anisotropic ESR signal ($g_\perp = 2.002$, $g_{/\!/} = 1.937$). The observed ESR parameters agreed well with that recorded for π-neutral radical[5] species of $[\mathrm{Fe^{II}}\mathbf{OEV}(\mathrm{py})_2]^{\cdot}$, Table 1. This paramagnetic species is therefore assigned to be a π-neutral radical, $[\mathrm{Fe^{II}}\mathbf{PV}(\mathrm{Py})_2]^{\cdot}$, in which the central iron atom takes a ferrous low-spin state.

Under aerobic conditions, electrochemical-optical absorption measurements were carried out for $[\mathrm{Zn^{II}}\mathbf{PV}]^+$. During the potential variation from −650 to −800 mV ($\mathrm{Ag/Ag^+}$), absorption coefficients of $[\mathrm{Zn^{II}}\mathbf{PV}]^+$ at 400, 521, 556 and 679 nm showed a stepwise decrease (Fig. 2). After supplying −830 mV for *ca.* 10 min., absorption maxima due to $[\mathrm{Zn^{II}}\mathbf{PV}]^+$ had almost disappeared, while new absorption maxima appeared at 387, 457 and 646 nm, Fig. 1(*b*). The optical spectrum due to $[\mathrm{Zn^{II}}\mathbf{PV}]^+$ was not observed by applying −650 mV again. The new absorbing species was assumed to be a $\mathrm{Zn^{II}}$–biliverdin complex, in terms of the observed optical parameters.[7] The characteristic absorption maxima due to the π-radical species

Table 1 The formal redox potentials and spectroscopic properties of $\mathrm{Zn^{II}}$- and $\mathrm{Fe^{II}}$-protoverdohaems and their π-neutral radicals

Complexes	Verdohaem complexes Optical property/nm	Redox potential/ mV *vs.* ($\mathrm{Ag/Ag^+}$)	π-Neutral radicals Optical property/nm	ESR parameters g_{iso}	$g_\perp$	$g_{/\!/}$
$[\mathrm{Zn^{II}}\mathbf{PV}]^{+a}$	400 521 556 677	−737	451 464 697(sh) 804	2.002	2.002	2.000
$[\mathrm{Fe^{II}}\mathbf{PV}(\mathrm{Py})_2]^{+a}$	392 504 534 673	−903	451 554 675(sh) 762	1.985	2.002	1.937
$[\mathrm{Fe^{II}}\mathbf{OEV}(\mathrm{Py})_2]^{+b}$	387 494 527 652	−1060	439 650 740	1.982	2.000	1.934

[a] Present study. [b] Ref. 3.

(464 and 804 nm) were scarcely detected. In fact, no detectable ESR signal due to the [Zn^{II}**PV**]· was observed by reduction of [Zn^{II}**PV**]$^+$ in the presence of oxygen. An analogous spectral change was also observed during the electrolysis of [Fe^{II}**PV**(Py)$_2$]$^+$ at −950 mV under aerobic conditions. In this case, the absorption spectrum of [Fe^{II}**PV**(Py)$_2$]$^+$ was fully changed to an iron–biliverdin complex [Fig. 2(*b*)], as shown by absorption maxima at 390 and 850 nm.[8] In addition, an ESR signal assigned as [Fe^{II}**PV**(Py)$_2$]· was not detected under these reaction conditions. These observations suggest that the [Zn^{II}**PV**]· and [Fe^{II}**PV**(Py)$_2$]· have very short life-times and are rapidly changed to the corresponding biliverdin species by reaction with molecular oxygen.

On the basis of the results described, a possible reaction mechanism is proposed for verdohaem degradation (Scheme 1). The first reaction is the one electron reduction of verdohaem to the verdohaem π-radical, [Zn^{II}**PV**]· and [Fe^{II}**PV**(Py)$_2$]·. In the second stage, the verdohaem radical is oxygenated by molecular oxygen. The verdohaem-peroxide radical or -ozonide species (Scheme 1), are probable transient intermediate species. From comparison of the chemical reactivity of [Zn^{II}**PV**]· and [Fe^{II}**PV**(Py)$_2$]· towards molecular oxygen, it is, therefore, concluded that the central iron atom does not take part in the

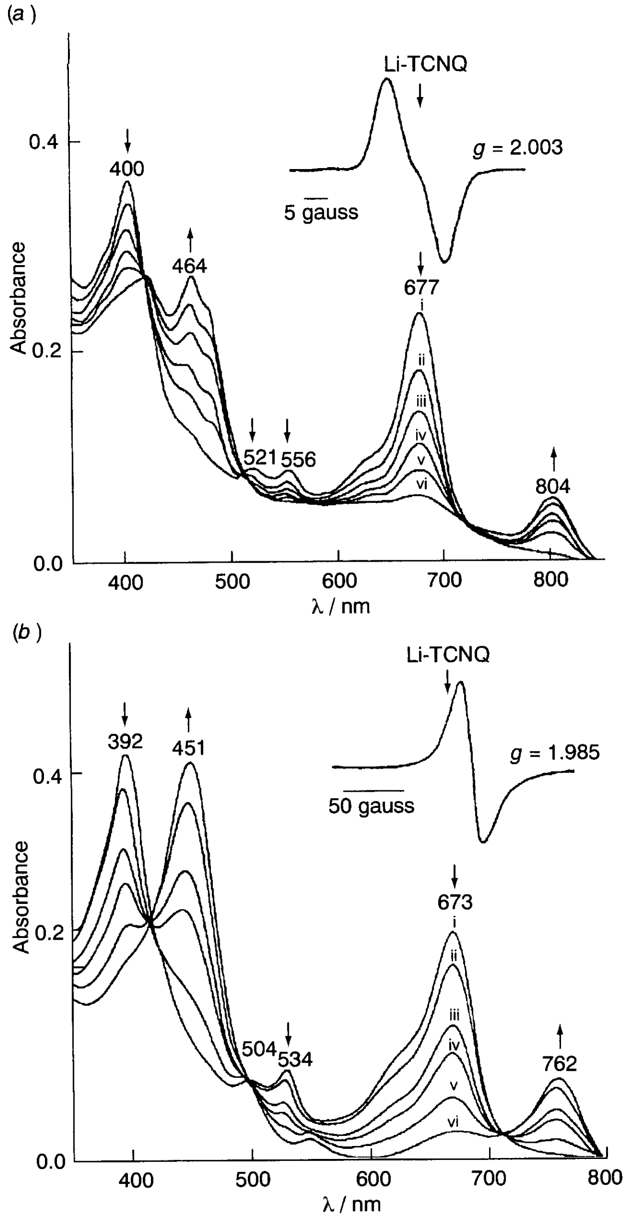


Fig. 1 Electrochemical optical absorption and ESR spectra observed for (*a*) [Zn^{II}**PV**]$^+$ and (*b*) [Fe^{II}**PV**(Py)$_2$]$^+$ complexes in DMF–pyridine (6 : 4) under nitrogen at 25 °C. (*a*) Applied potential for reduction of [Zn^{II}**PV**]$^+$; i, before reduction, ii, −650; iii, −680; iv, −710; v, −760 and vi, −800 mV *vs.* Ag/Ag$^+$. Inset: ESR spectrum of [Zn^{II}**PV**]· recorded by electrochemical ESR cell [−850 mV (Ag/Ag$^+$)] at −30 °C *in vacuo*. (*b*) Applied potential for reduction of [Fe^{II}**PV**(Py)$_2$]$^+$; i, before reduction; ii, −720; iii, −780; iv, −820; v, −860 and vi, −900 mV *vs.* Ag/Ag$^+$. Inset: ESR spectrum of [Fe^{II}**PV**(Py)$_2$]· recorded by electrochemical ESR cell [−900 mV (Ag/Ag$^+$)] at −30 °C *in vacuo*.

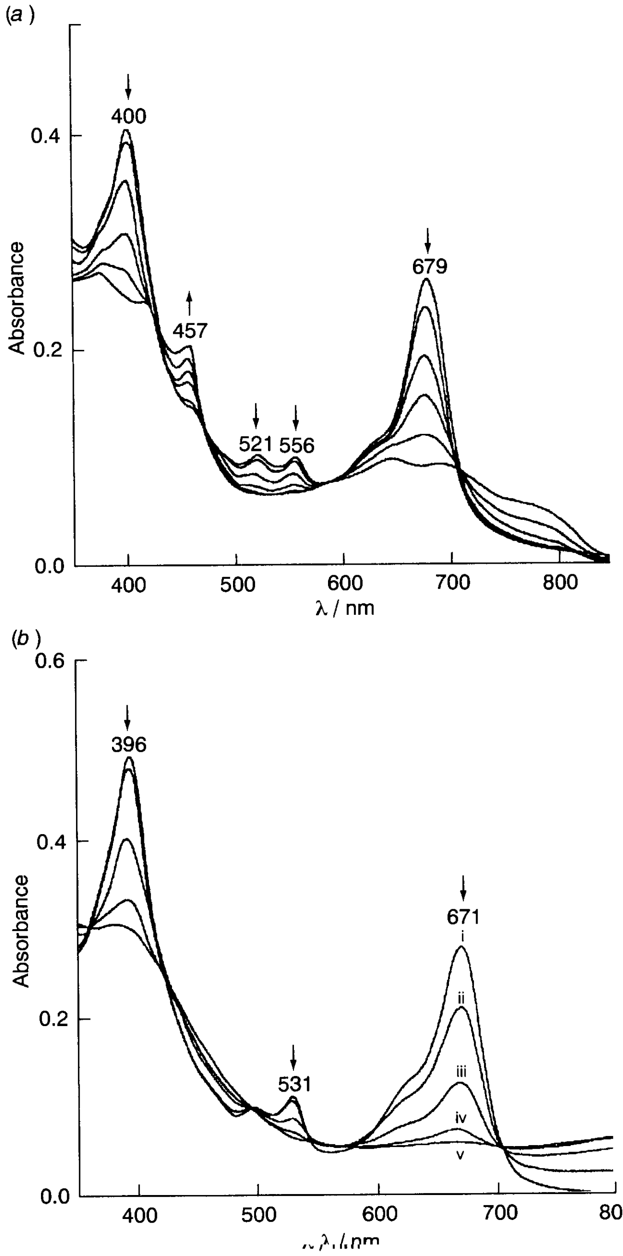
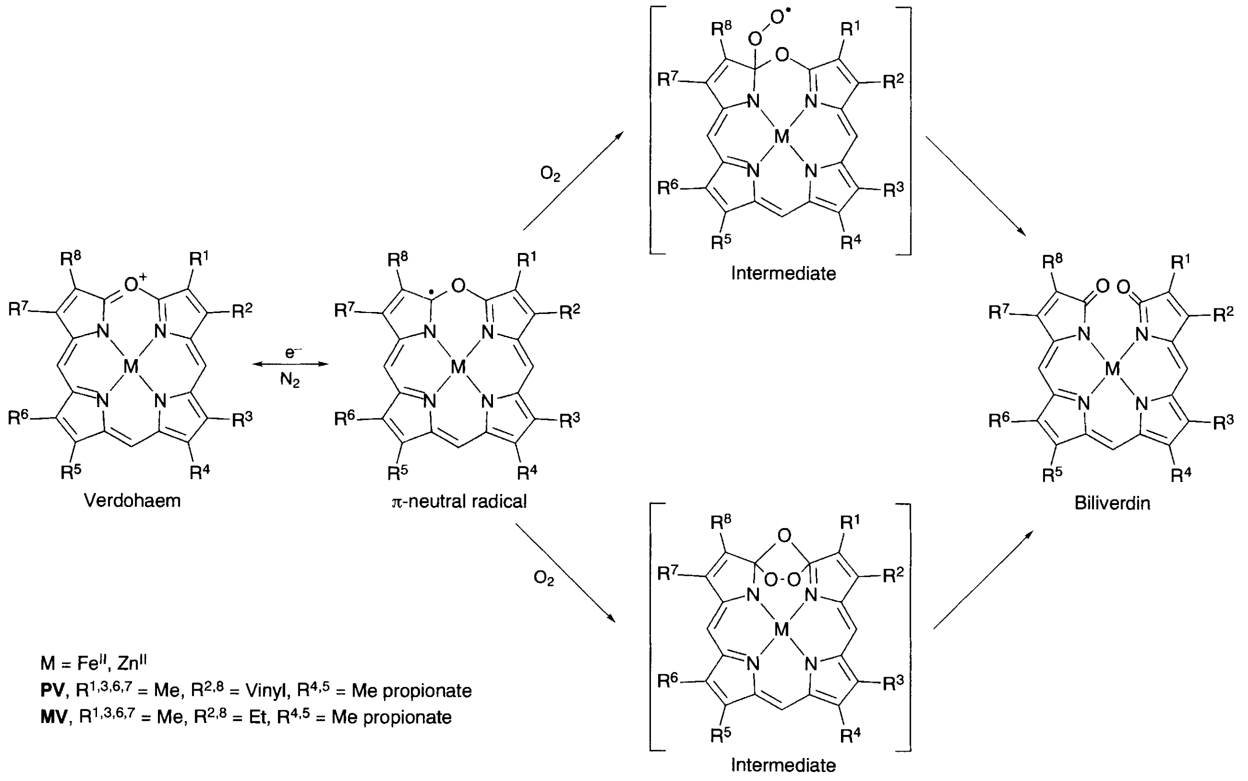


Fig. 2 Electrochemical optical absorption and ESR spectra observed for (*a*) [Zn^{II}**PV**]$^+$ and (*b*) [Fe^{II}**PV**(Py)$_2$]$^+$ complexes under oxygen at 25 °C. (*a*) Applied potential for reduction of [Zn^{II}**PV**]$^+$; i, before reduction, ii, −650; iii, −690; iv, −730; v, −770 and vi, −800 mV *vs.* Ag/Ag$^+$. (*b*) Applied potential for reduction of [Fe^{II}**PV**(Py)$_2$]$^+$; i, before reduction; ii, −720; iii, −780; iv, −840; v, −870 and vi, −900 mV *vs.* Ag/Ag$^+$.



M = Fe^{II}, Zn^{II}
PV, $R^{1,3,6,7}$ = Me, $R^{2,8}$ = Vinyl, $R^{4,5}$ = Me propionate
MV, $R^{1,3,6,7}$ = Me, $R^{2,8}$ = Et, $R^{4,5}$ = Me propionate

Scheme 1 Possible reaction mechanisms for the reductive decomposition of Zn^{II}- and Fe^{II}-protoverdohaem to biliverdin

activation of molecular oxygen relating to the verdohaem cleavage reaction.

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Footnotes

† OEP octaethylporphyrin; PV$^+$ protoverdohaem dimethylester; TBAP tetra-*n*-butylammonium perchlorate.

‡ ^{1}H NMR parameters of verdohaems were as follows; [Zn^{II}**PV**]$^+$ (in $CDCl_3$): δ 9.28, 9.05, 9.00 (3H, s *meso*), 3.00 (6H, s, CH_3), 3.60, 3.10 (6H, s, CH_3), 3.90 (4H, t, py-CH_2), 3.02 (4H, t, py-CH_2), 3.68 (6H, s, OCH_3), 7.60, 7.38 (2H, d, py-CH), 6.70, 6.00 (2H, d, =CH_2), 6.20, 6.10 (2H, d, =CH); [Fe^{II}**PV**$(Py)_2$]$^+$ ([$^2H_5$]-pyridine): δ 9.00, 8.65, 9.05 (3H, s, meso), 3.48 (6H, s, CH_3), 3.45, 3.41 (6H, s, CH_3), 3.90 (4H, t, py-CH_2), 3.02 (4H, t, –CH_2–), 3.50 (6H, s, OCH_3), 7.41, 7.21 (2H, d, py-CH), 6.56, 5.93 (2H, d, =CH_2), 6.02, 5.88 (2H, d, =CH), 3.22 (4H, α-pyridine), 5.80 (4H, β-pyridine), 6.50 (2H, γ-pyridine).

§ 0 mV *vs.* (Ag/0.1 mol dm^{-3} Ag$^+$) corresponds closely to 390 mV *vs.* SCE in the present reaction system.

¶ The *g*-values of observed ESR spectra were estimated by using lithium salt of tetracyanoquinodimethane (Li-TCNQ; g = 2.0025) as an external standard.

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