Preparation and Characterisation of Iron(IV) Porphyrin in Aqueous Solution at Room Temperature: a Proposed Model for Peroxidase Compound II

Vinay P. Shedbalkar, Sandeep Modi, and Samaresh Mitra*

Chemical Physics Group, Tata Institute of Fundamental Research, Colaba, Bombay 400 005, India

By use of porphyrin C as ligand, the Fe^{IV} state has been stabilised in aqueous solution at room temperature for the first time, and characterised by optical spectroscopy, ^1H n.m.r., e.s.r., and magnetic moment measurements.

The enzyme peroxidase catalyses the oxidation of various substrates by hydrogen peroxide or substituted peroxides.[1—3] The catalytic cycle of horse radish peroxidase involves two key intermediates, compounds I and II, which are 2 and 1 oxidising equivalents, respectively, above the native iron(III) enzyme.[2,3] The prosthetic group in compounds I and II contains an iron(IV) porphyrin as active centre. In modelling the active site of the peroxidases, stabilisation of the iron(IV) state is crucial.

Several attempts have been made to stabilise iron(IV) in model synthetic and natural iron porphyrins.[4—14] Iron(IV) porphyrins have been stabilised in organic solvents at temperatures around −40 to −70 °C, and have been electrochemically generated[12] in solution in organic solvents and produced as transient species in the oxidation of iron(III) deuteroporphyrin.[9] However, to our knowledge no definitive evidence of the formation of iron(IV) porphyrins in aqueous solution at room temperature has yet been reported.

By using the ligand porphyrin C [see structure (**1**)] we have been able to stabilize iron(IV) porphyrin in aqueous solution at room temperature. Porphyrin C was synthesised from haematoporphyrin bishydrochloride (Sigma Chemicals) and cysteine hydrochloride (Sigma Chemicals), and purified by a reported procedure.[15] The absorption spectrum ($\lambda_{max.}$ 618, 564, 528, 495, and 370 nm) matched the reported values.[16] The Fe^{III} haem C was obtained by metallation of the porphyrin C by a standard procedure.[17] In the synthesis of the ligand and the metal complex, exposure to light and oxygen had to be strictly avoided. The visible absorption spectrum of an aqueous 10^{-5} M iron(III) haem C solution in 0.1 M phosphate buffer at 22 °C is shown in Figure 1. The spectrum is typical of high-spin Fe^{III} porphyrins with a characteristic band at 628 nm. The magnetic moment of this complex in solution; determined by Evan's method, was 5.7 ± 0.2 Bohr magnetons at 25 °C.

Addition of 1.2 equiv. of H_2O_2 (in the same buffer) to the aqueous solution of Fe^{III} haem C at room temperature produced a new species, the absorption spectrum of which (Figure 1) is typical of Fe^{IV} porphyrin ($\lambda_{max.}$ 572, 545, and 418 nm). The spectrum is similar to that reported for horse radish peroxidase and the Fe^{IV} species obtained by oxidation of metmyoglobin (see Figure 1). On addition of $K_4Fe(CN)_6$ to the Fe^{IV} haem C solution, the Fe^{III} haem C spectrum is regenerated. The oxidation–reduction cycle $Fe^{III} \rightleftharpoons Fe^{IV}$ haem C was similar to that reported for the analogous species.[2,18] The magnetic moment of the oxidised haem C between 290 and 320 K was 2.9 ± 0.2 Bohr magnetons, consistent with an $S = 1$ ground state of the Fe^{IV} haem. The

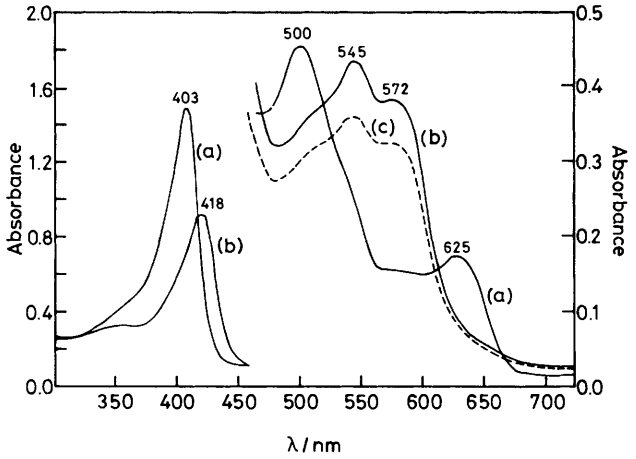
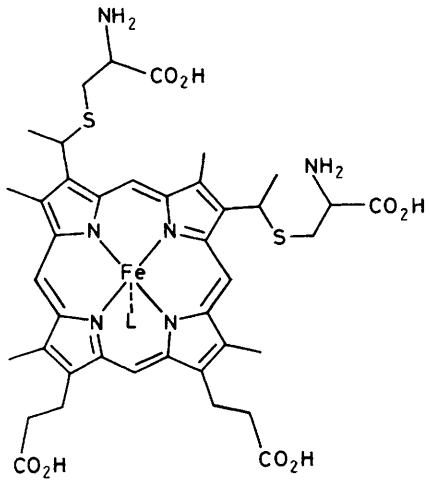


Figure 1. Electronic absorption spectra of (a) Fe^{III} haem C (**1**); (b) Fe^{IV} haem C, obtained by oxidation of (a) by H_2O_2; and (c) Fe^{IV} metmyoglobin, obtained by oxidation of Fe^{III} metmyoglobin by H_2O_2; recorded in aqueous solution at pH 7 and *ca.* 22 °C.



(**1**)

magnetic moments of horse radish peroxidase compound II and other Fe^{IV} haems are similar.[12,13]

The 500 MHz ^1H n.m.r. spectrum of the oxidised species in aqueous solution (pH 7) at room temperature (Figure 2) is almost identical with that reported for compound II.[19] As noted previously,[19] the ^1H n.m.r. behaviour of compound II and Fe^{IV} haems is diagnostic of the oxidation state of the iron. An *X*-band e.s.r. study of the oxidised species in aqueous solution at room temperature and at 4.2 K (frozen solution) did not show any signal either of any free radical (if the species were low-spin Fe^{III} porphyrinyl) or of the metal atom. All these results establish conclusively that when the Fe^{III} haem C is oxidised by H_2O_2 in aqueous solution at room temperature, only the Fe^{IV} species is present in solution.

The Fe^{IV} state was found to be stable in solution for at least 2—3 h; complete return to the Fe^{III} state took 5—6 h. A 5% loss in the intensity of the Fe^{III} species absorption was observed during the oxidation–reduction process. The rate of formation of the Fe^{IV} haem C from its Fe^{III} analogue was determined spectrophotometrically by keeping the porphyrin concentration constant and varying the H_2O_2 concentration. The value obtained was 5×10^2 dm^3 mol^{-1} s^{-1}; the corresponding value for myoglobin is 1×10^3.[18] During the first 0.6 min, 80% conversion from Fe^{III} haem C to Fe^{IV} haem C was observed; *cf.* 90% conversion in myoglobin.[18]

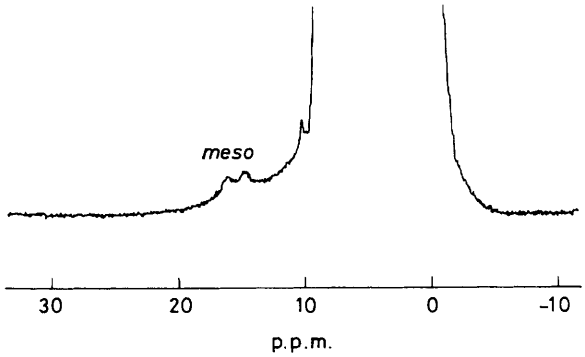


Figure 2. ^1H N.m.r. (500 MHz) spectrum of Fe^{IV} haem C in aqueous solution at room temperature.

These observations establish that the Fe^{IV} state can be stabilized in the synthetic haem C in aqueous solution at room temperature by oxidation of the corresponding Fe^{III} analogue. Iron(IV) haem C appears to be, therefore, an attractive model for horse radish peroxidase compound II.

We thank the 500 MHz FT-NMR National Facility for the ^1H n.m.r. work reported here.

Received, 28th March 1988; Com. 8/01229F

References

1 H. B. Dunford, 'Advances in Inorganic Biochemistry,' vol. 4, ed. G. L. Eichhorn and L. G. Marzilli, Elsevier, New York, 1982, p. 41 *et seq.*
2 W. D. Hewson and L. P. Hager, 'The Porphyrins,' ed. D. Dolphin, Academic Press, New York, 1979, p. 295 *et seq.*
3 B. C. Saunders, 'Inorganic Biochemistry,' ed. G. L. Eichhorn, Elsevier, New York, 1973, p. 988 *et seq.*
4 R. H. Felton, G. S. Owen, D. Dolphin, and J. Fajer, *J. Am. Chem. Soc.*, 1971, **93**, 6332.
5 D. H. Chin, A. L. Balch, and G. N. La Mar, *J. Am. Chem. Soc.*, 1980, **102**, 1446.
6 D. H. Chin, G. N. La Mar, and A. L. Balch, *J. Am. Chem. Soc.*, 1980, **102**, 4344.
7 D. H. Chin, G. N. La Mar, and A. L. Balch, *J. Am. Chem. Soc.*, 1980, **102**, 5945.
8 H. C. Kelly, K. J. Pariji, I. Wilson, D. M. Davis, P. Jones, and L. J. Roettger, *Inorg. Chem.*, 1981, **20**, 1086.
9 A. L. Balch, Y. W. Chan, R. J. Cheng, G. N. La Mar, L. L. Grazynski, and M. W. Renner, *J. Am. Chem. Soc.*, 1984, **106**, 7779.
10 A. L. Balch, L. L. Grazynski, and M. W. Renner, *J. Am. Chem. Soc.*, 1985, **107**, 2983.
11 J. T. Groves and J. A. Gilbert, *Inorg. Chem.*, 1986, **25**, 123.
12 A. L. Balch and M. W. Renner, *J. Am. Chem. Soc.*, 1986, **108**, 2603.
13 J. T. Groves, R. Quinn, T. J. McMurry, M. Nakamura, G. Lang, and B. Baso, *J. Am. Chem. Soc.*, 1985, **107**, 354, and references therein.
14 K. Shin and H. M. Goff, *J. Am. Chem. Soc.*, 1987, **109**, 3140.
15 P. A. Scourides, G. Morstyn, and M. Ngu, *J. Chem. Soc., Chem. Commun.*, 1986, 1817.
16 R. K. DiNello and C. K. Chang, 'The Porphyrins,' ed. D. Dolphin, Academic Press, 1978, vol. 1, p. 289 *et seq.*
17 J. E. Falk, 'Porphyrins and Metalloporphyrins,' Elsevier, New York, 1964, p. 131 *et seq.*
18 N. K. King and M. E. Winfield, *J. Biol. Chem.*, 1963, **238**, 1520.
19 G. N. La Mar, J. S. de Ropp, L. Latos-Grazynski, A. L. Balch, R. B. Johnson, K. M. Smith, D. W. Parish, and R. J. Cheng, *J. Am. Chem. Soc.*, 1983, **105**, 782.