Aggregation of Metallo-β-oxoporphyrins

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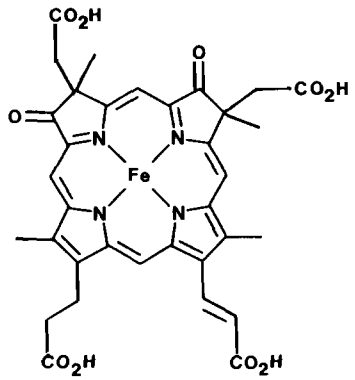
The heme d_1 prosthetic group isolated from the bacterial nitrite reductase/cytochrome oxidases of *Pseudomonas aeruginosa, Parracoccus denitrificans*, and *Thiobacillus denitrificans* has an iron β-dioxoporphyrin structure, **1** [1]. Compounds of this type have long been known to result from the hydrogen peroxide oxidation of octaalkylporphyrins in acidic media [2, 3], but until recently [4] the properties of this biologically relevant macrocycle class received little attention. Oxoporphyrins were first viewed



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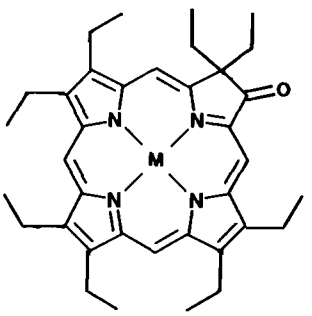
as analogues of hydroporphyrins, due to the similarity of interruptions in the π-systems and the superficial resemblance of the UV–Vis spectra of the two classes of macrocycles [5, 6]. It is now clear that the chemical behavior of these two classes of macrocycles are quite different [4, 7].

One obvious difference between hydroporphyrins and oxoporphyrins is the presence of β-carbonyl groups in the latter. Contrary to the expected reactivity of these sites, oxoporphyrins are remarkably inert towards the reagents that typically react with carbonyl groups [4]. On the other hand, the carbonyl group appears to function as a Lewis base despite severe steric congestion. The lanthanide shift reagent Eu(fod) preferentially binds to these sites in the free-base compounds [7, 8]. In addition, the selectivity of meso deuteration of free-base oxoporphyrin compounds is consistent with electronic effects that would result from protonation of the carbonyl oxygen atom [7].

The acidity of most metal ions in a porphyrin coordination environment and the basicity of the oxoporphyrin β-carbonyl group suggest that metallo-oxoporphyrin complexes may be prone to self-association. In this paper we present evidence of aggregation in Zn and Mg octaethyloxoporphyrin complexes. Owing to the mode of association, the physical properties and chemical reactivity of many metallo-β-oxoporphyrins are likely to exhibit marked dependences upon solution concentration.

Experimental

3,3,7,8,12,13,17,18-octaethyl-2-porphinone, H_2-(**2**), was obtained by literature methods [4, 9, 10].



M(2)
(M = H_2, Zn, Mg)

Zn(**2**) and Mg(**2**) were prepared by reaction of H_2(**2**) with zinc acetate in dimethylformamide or with methylmagnesium bromide, respectively. Characterization of these complexes is described elsewhere [4]. Chloroform-d_1, methanol-d_4, pyridine-d_5, and Resolve-Al Eu(fod) were obtained from Aldrich Chemical Co. Acetone-d_6 and acetonitrile-d_3 were obtained from Cambridge Isotope Labs. Chloroform-d_1 was treated before use by passage down a (Merck) grade I basic alumina column. The initial runnings were discarded. Other deuterated solvents were taken from ampules opened immediately prior to use and were used as received. Methylene chloride was dried by distillation from calcium hydride. Solutions of complexes were prepared under a dry argon atmosphere.

NMR spectra were determined on either a Brucker WH-90 or a Varian XL-300 spectrometer. Chemical shifts are reported relative to TMS. IR spectra were recorded on a Perkin Elmer 683 spectrophotometer equipped with a data station. Low temperature spectra were obtained in a Beckman RIIC VLT 2 variable temperature cell. Reported temperatures are thermocouple readings. True sample temperatures are expected to be within 2 °C of these values.

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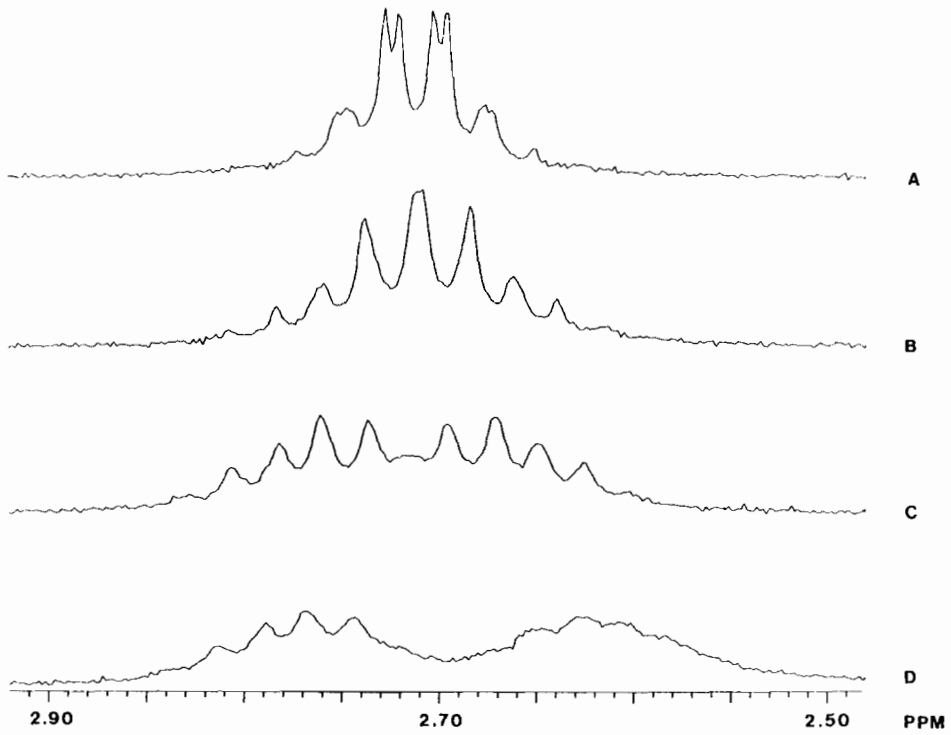

Fig. 1. Variable temperature 300 MHz ^{1}H NMR spectra of the gem-diethyl methylene protons of 5 mM Zn(2) in $CDCl_3$. (a) 25 °C, (b) 0 °C, (c) −25 °C, (d) −50 °C.

Results and Discussion

The ^{1}H NMR spectrum of Zn(2) (5 mM $CDCl_3$ solution) at room temperature consisted of very broad, unresolvable resonances when recorded on a 90 MHz spectrometer. This observation agreed with a previous report of the spectrum recorded at 100 MHz [11]. Upon cooling to −50 °C, the meso proton peaks began to resolve. The entire spectrum of Zn(2) was resonably well resolved at room temperature when recorded at 300 MHz. One striking feature of the spectrum was the multiplet pattern of the methylene protons of the gem-diethyl groups. As the temperature was decreased from 25 °C to −50 °C (at 300 MHz), this multiplet shifted upfield, broadened, and eventually split into two multiplets, Fig. 1. At each temperature, irradiation of the gem-diethyl methyl protons collapsed the methylene proton multiplets to an AB quartet. Shift differences between the A and B protons increased to 0.16 ppm as the temperature was decreased to −50 °C. All other lines in the spectrum shifted upfield. Upfield shifts were greatest for protons near the carbonyl group.

The behaviour of Zn(2) contrasted sharply with that of H_2(2), Ni(2), and free-base dioxooctaethylporphyrin compounds. The NMR spectra of these compounds were well resolved at all field strengths, temperatures, and concentrations [4, 9, 11]. Furthermore, the diastereotopic methylene protons of the gem-diethyl groups were nearly isochronous ($\Delta\delta <$ 0.03 ppm) and gave rise to a 'simple quartet' rather than a multiplet pattern like that observed for Zn(2) [4]. (The simple quartet resolved to a narrow 'doublet' of a quartet when near maximal resolution was achieved.)

The broadness of the spectrum of Zn(2) and its dependence upon field strength and temperature were suggestive of an exchange process, given the diamagnetism of both Zn and H_2(2). One possibility was an equilibrium between monomeric Zn(2) and a labile oligomeric complex formed by coordination of the basic carbonyl oxygen atom of one molecule to the acidic zinc atom of the second. Upon coordination, the ring current of one macrocycle in the oligomeric complex would induce changes in the chemical shifts of protons in the second [12]. This mode of association is chemically reasonable inasmuch as zinc porphyrin complexes have a strong tendency to exhibit square-pyramidal coordination [13]. Furthermore, aggregation involving interactions between the magnesium atom of one molecule and the ring V carbonyl group of a second has been demonstrated in dimers of chlorophyll *a* in CCl_4 solution [12]. Not surprisingly, the ^{1}H NMR spectrum of Mg(2) was extremely broad at all field

strengths. Peaks were shifted in excess of 2 ppm upfield from the corresponding positions in $H_2(2)$, suggesting that the extent of oligomer formation is significantly greater in the case of Mg than for Zn.

Other evidence provided support for the proposed mode of aggregation. As would be expected, the appearance of the spectrum of Zn(2) depended markedly upon the concentration of the complex. When the concentration of Zn(2) was increased, the upfield shifts of all peaks and the shift difference between the A and B protons of the gem-diethyl methylene proton multiplet increased. In contrast, the spectrum of a 0.5 mM solution showed no evidence of aggregation: the gem-diethyl methylene proton peak appeared as a 'simple quartet' and all peaks were downfield of their positions in spectra of more concentrated samples. The conversion to monomeric Zn(2) at lower concentration cannot be attributed to the decrease in the mole ratio of Zn(2) to nucleophilic solvent impurities such as water (see below). The spectrum of Mg(2) remained broad at all accessible concentrations.

The frequencies of the carbonyl band in the IR spectra of Zn(2) and Mg(2) supported the involvement of the β-carbonyl in aggregation. This band was generally found between 1711 and 1714 cm^{-1} in KBr or solution spectra of free-base, Ni, and Cu oxoporphyrins [4]. Two carbonyl bands were found in the IR spectrum of Zn(2) (KBr), a small shoulder at 1709 cm^{-1} and a main band at 1686 cm^{-1}. The carbonyl band of Mg(2) was found at 1671 cm^{-1} (KBr). The shift of the band to lower frequency was consistent with coordination of the carbonyl to a metal ion. It was reminiscent of and similar in magnitude to the shifts observed for the ring V carbonyl group in chlorophyll dimers [12]. The frequency shifts of the carbonyl bands of Zn(2) and Mg(2) were not as large in solution spectra. At 25 °C, a 5 mM CH_2Cl_2 solution of Zn(2) had a sharp, symmetrically shaped carbonyl band at 1708 cm^{-1}. Upon cooling to −65 °C, the maximum of the band shifted to 1700 cm^{-1}, the band broadened substantially, and became skewed towards lower frequencies. These changes are compatible with the expected increase in the amount of oligomeric forms at lower temperature. Mg(2) had a broad, skewed band at 1695 cm^{-1} in solution at room temperature, consistent with the greater extent of oligomer formation observed by NMR.

Aggregation of the type proposed should not occur in solvents capable of coordinating to Zn or Mg. Addition of 1% pyridine-d_5 (by volume) to a benzene-d_6 solution of Mg(2) led to dramatic changes in the ^1H NMR spectrum. The broad peaks characteristic of Mg(2) shifted over 2 ppm downfield and resolved to peaks with normal linewidths. The chemical shifts of the new peaks due to $Mg(2)(C_5D_5N)_2$ were generally similar to those observed for $H_2(2)$. Changes in the spectrum of Zn(2) in $CDCl_3$ upon addition of pyridine-d_5 were less pronounced. The most noteworthy change was a 0.22 ppm upfield shift of the methyls of the gem-diethyl group from their position in aggregated solutions of Zn(2) (*i.e.* net upfield shift of 0.35 ppm from the position in dilute, monomeric Zn(2)). Thus, the methyl group would appear to be located in the shielding region of the ring current of an axially coordinated pyridine. The remainder of the peaks in the spectrum of Zn(2) shifted slightly downfield upon addition of pyridine. Although the shift difference between the A and B protons of the gem-diethyl methylene groups decreased substantially, the multiplet did not collapse to a 'simple quartet'. This is not inconsistent with the expected suppression of aggregation, however. Unlike Mg porphyrins which can become six-coordinate, Zn porphyrins are strictly five-coordinate in the presence of excess ligand [14]. Thus, the mirror plane containing the oxoporphyrin macrocycle cannot exist in an axially ligated Zn(2). The chemical equivalence of the gem-diethyl methylene protons is consequently destroyed. Not surprisingly, multiplets were observed for these protons in all coordinating solvents including acetone-d_6, acetonitrile-d_3, and methanol-d_4.

Our results imply that oxoporphyrin complexes of metals capable of coordinating to and preferring more than four ligands will aggregate in solutions above a critical concentration. While the effects of aggregation for Zn(2) are small, larger effects will occur for metals with greater binding constants. Certain high-spin ferric porphyrin and hydroporphyrin complexes have a strong tendency to bind a sixth ligand [15]. This could result in conversion to a low-spin complex, autoreduction, and/or labilization of axial ligands among other effects. Thus, any investigation of iron oxoporphyrin complexes as model systems for the heme-d_1 prosthetic group must examine aggregation behavior to insure that the measured properties pertain to monomeric complexes.

Acknowledgements

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References

1 C. K. Chang, R. Timkovich and W. Wu, *Biochemistry, 25*, 8447 (1986).
2 R. Bonnett, D. Dolphin, A. W. Johnson, D. Oldfield and G. F. Stephenson, *Proc. Chem. Soc.*, 371 (1964).

3 H. H. Inhoffen and W. Nolte, *Tetrahedron Lett.*, 2185 (1967).
4 A. M. Stolzenberg, P. A. Glazer and B. M. Foxman, *Inorg. Chem., 25*, 983 (1986).
5 H. Scheer and H. H. Inhoffen, in D. Dolphin (ed.), 'The Porphyrins', Vol. 2, Academic Press, New York, 1978, pp. 45–90.
6 J. H. Furhop, in K. M. Smith (ed.), 'Porphyrins and Metalloporphyrins', Elsevier, Amsterdam, 1975, p. 639.
7 A. M. Stolzenberg and M. A. Laliberte, *J. Org. Chem., 52*, 1022 (1987).
8 R. Timkovich, M. S. Cork and P. V. Taylor, *J. Biol. Chem., 259*, 15089 (1984).
9 H. H. Inhoffen and W. Nolte, *Liebigs Ann. Chem., 725*, 167 (1969).
10 C. K. Chang, *Biochemistry, 19*, 1971 (1980).
11 H. H. Inhoffen and A. Gossauer, *Liebigs Ann. Chem., 723*, 135 (1969).
12 J. J. Katz, L. L. Shipman, T. M. Cotton and T. R. Janson, in D. Dolphin (ed.), 'The Porphyrins', Vol. 5, Academic Press, New York, 1978, p. 401.
13 J. W. Buchler, in D. Dolphin (ed.), 'The Porphyrins', Vol. 1, Academic Press, New York, 1978, p. 425.
14 R. J. Abraham, P. Leighton and J. K. M. Sanders, *J. Am. Chem. Soc., 107*, 3472 (1985).
15 A. M. Stolzenberg, S. H. Strauss and R. H. Holm, *J. Am. Chem. Soc., 103*, 4763 (1981).