1.999(6) Å, Fe–N(NO) = 1.652(5) Å, and Fe–O = 2.001(5) Å. For [Fe(OEP)(NO)]ClO_4 (Fig. 1), Fe–N_p = 1.994(1) Å, Fe–N(NO) = 1.644(3) Å; the iron(III) atom is displaced 0.29 Å from the mean plane of the porphinato core. The structural parameters of both complexes are appropriate for low-spin ferric porphyrinates. [Fe(OEP)(NO)]ClO_4 forms a remarkable π–π dimer in the solid state (Fig. 2). The two planar cores are parallel with an interplanar separation of only 3.36 Å.

Q27

Corrinoid Catalysis of Thiol Oxidation

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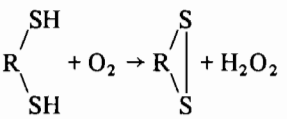
Under appropriate conditions, thiols can react with corrinoids to form relatively stable complexes [1–7], or reduced corrinoids [1, 2, 8–11]. If alkyl halides are present during the reduction of corrinoids by thiols, alkyl corrinoids are produced [4, 9, 12, 13]. In the presence of oxidizing substrates, corrinoids will efficiently catalyze the oxidation of thiols to their corresponding disulfides [14–18]. In view of the important role that enzyme sulfhydryl groups play in corrinoid–coenzyme-dependent catalysis [19, 20], we have further characterized aerobic thiol oxidation catalyzed by a selected group of biologically important corrinoids and have determined the stoichiometry of the reaction.

Hydrogen peroxide and superoxide have been identified as reaction products during the aerobic catalysis of 2-mercaptoethanol [ME, 21] by the corrinoids listed in Table I. The reactions were conducted in a polarographic cell equipped with a Clark O_2 electrode [22]. The corrinoids used to initiate the reactions were prepared as described previously [23, 24] and their purity was determined by hplc [25]. H_2O_2 was detected by adding catalase to the reaction system. This resulted in an abrupt increase in O_2 concentration which is consistent with the catalytic activity of the enzyme. As anticipated, the pseudo-first order rate constant (k_1) of O_2 consumption in the presence of catalase decreased 50 per cent. Using a similar approach, superoxide dismutase was used in an attempt to detect the presence of O_2^- as a reaction product. This was unsuccessful except for one corrinoid, namely Aq-Cbl (Table I). Thus, during the catalysis of ME oxidation by Aq-Cbl, O_2^- is the primary reaction product. In the presence of dismutase, k_1 for the Aq-Cbl-catalyzed reaction decreased approximately 50 per cent which is consistent with the catalytic activity of this enzyme. H_2O_2 was also detected during Aq-Cbl-catalyzed oxidation of ME and may have been produced by the spontaneous dismutation of O_2^-.

Disulfide bond formation was monitored spectrophotometrically [26] during the oxidation of DTE. Pseudo-first-order rate constants for O_2 disappearance and for DTE_{ox} appearance were in good agreement (Table II). These results, which differ from previously published studies [16, 17, 27], suggest that the reactions and their stoichiometries for aerobic oxidation of mono- and dithiols by corrinoids are:

$$2\ RSH + O_2 \rightarrow RSSR + H_2O_2 \qquad (1)$$



(2)

TABLE I. Pseudo-First-Order Rate Constants, pH Optima, Products and Photosensitivity for Catalysis of ME Oxidation by Corrinoids.[a]

Group	Catalyst	k_1, sec^{-1}	pH Optimum	Reduced Product	k_1, sec^{-1} (after photolysis)[b]
I	Ado-Cbl	0.003	Broad	H_2O_2	0.102 (20 sec)
	Me-Cbl	0.003	7.0[c]	H_2O_2	0.184 (20 sec)
II	CN-Cbl	0.080	8.0[c]	H_2O_2	0.080 (60 sec)
	Aq-Cbl	0.180	8.4	HO_2	0.180 (20 sec)
	Ado-Cbi	0.23		H_2O_2	177.0 (60 sec)
	Me-Cbi	0.56	11–13	H_2O_2	122.0 (90 sec)
III	CN-Cbi	191	8.5–9.5	H_2O_2	190.0 (30 sec)
	$(Aq)_2$-Cbi	211	8.8	H_2O_2	210.0 (60 sec)

[a]ME = 2.5 × 10^{-2} *M*; 25 ± 0.2 °C. [b]At the midway point of O_2 consumption, the polarographic cell was irradiated with light from a tungsten-filament high intensity lamp for the time indicated. [c]Measurement was made at the indicated pH only.

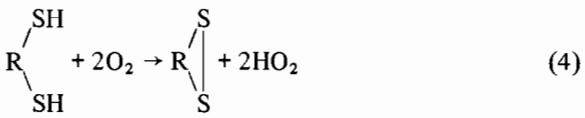
TABLE II. Pseudo-First-Order Rate Constants for the Disappearance of O_2 and the Formation of Oxidized DTE.[a]

Catalyst	O_2 Disappearance[b] k_1, sec^{-1}	DTE_{ox} Formation[c] k_1, sec^{-1}	Ratio O_2/DTE_{ox}
Aq-Cbl	0.57	0.63	0.91
Ado-Cbi	0.49	0.46	1.07
$(Aq)_2$-Cbi	567	597	0.95

[a]Initial substrate DTE = 1.25×10^{-2} *M*; pH = 8.0; 25 ± 0.2 °C. [b]The rate of O_2 disappearance was determined polarographically. [c]The rate of oxidized DTE formation was determined spectrophotometrically.

The results of the dismutase study and the data in Table II suggest that, in the case of Aq-Cbl, the reactions are:

$$2RSH + 2O_2 \rightarrow RSSR + 2HO_2 \quad (3)$$



$$+ 2O_2 \rightarrow + 2HO_2 \quad (4)$$

Autocatalytic behavior was not observed for reactions catalyzed by the alkylcobalamins (Ado-Cbl and Me-Cbl) and the alkylcobinamides (Ado-Cbi and Me-Cbi) suggesting that the carbon–cobalt bond remains intact during the catalytic cycle. Similar observations have been made by Pellizer *et al.* [28] for the catalysis of cysteine oxidation by the model compound $[MeCo(tn)H_2O]^+$.

The corrinoids in Table I have been grouped according to their catalytic activity. The alkylcobalamins (Ado-Cbl and Me-Cbl; Group I) are poor catalysts. The second group of corrinoids includes CN-Cbl, Aq-Cbl, Ado-Cbi and Me-Cbi. The most active catalysts (CN-Cbi and $(Aq)_2$-Cbi; Group III) are cobinamides which lack covalently attached ligands. Thus, the catalytic activity of corrinoids with respect to thiol oxidation appears to be determined by the number (1 *vs.* 2) and accessibility (coordinate *vs.* covalent) of axial-ligand positions.

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21 Abbreviations: ME, 2-mercaptoethanol; DTE, dithioerythritol; Aq-Cbl, aquocobalamin; Ado-Cbl, adenosylcobalamin; Me-Cbl, methylcobalamin; CN-Cbl, cyanocobalamin; Ado-Cbi, adenosylcobinamide; Me-Cbi, methylcobinamide; CN-Cbi, cyanocobinamide; $(Aq)_2$-Cbi, diaquocobinamide.
22 The rate of O_2 disappearance during corrinoid catalysis of ME and DTE oxidation was measured polarographically using a Gibson Oxygraph (Model KM) equipped with a Clark O_2 electrode. The electrode cell was maintained at 25 ± 0.2 °C and all measurements were made in a darkroom under dim red illumination. After establishing background oxidation rates (usually small on EDTA containing buffers), the catalytic reaction was initiated by injection of corrinoid. The rate of O_2 disappearance was usually linear over at least 85 per cent of the concentration range. Catalase (Type CTR, Worthington Biochemical Corp.) and/or superoxide dismutase (bovine, Sigma Chemical Co.) were injected into the cell at the midway point of O_2 consumption.
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Q28

Proton Magnetic Resonance Spectra of the Heme Undecapeptide (Microperoxidase), its Reaction with Hydrogen Peroxide and its Cyanide Ligated Form

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Until recently, model systems of heme protein spectroscopy and function were primarily naked iron porphyrins of varying synthetic complexity [1–5]. The detailed use of heme–peptide complexes [6–10] such as the heme undecapeptide (microperoxidase, MP-11) and the heme octapeptide (OP) from the enzymic degradation of cytochrome *c* has lagged. The structure of these complexes includes the heme which is covalently bound to an 8–11 amino acid segment from the native protein *via* cys-14 and cys-17 in the enzyme's primary sequence [11–13]. NMR spectroscopy of OP has revealed interesting comparisons to cytochrome *c* [6]. This preliminary report represents an interest in the microperoxidase which originates with its peroxidase activity (hence its common name) and comparisons which might be made with cytochrome *c* peroxidase [14–17] and horseradish peroxidase [18, 19].

Experimental

Nuclear magnetic resonance (NMR) spectra of the heme undecapeptide were obtained at proton frequencies of 360 and 470 MHz. These experiments were carried out on Nicolet Magnetics spectrometers at the Purdue University Biochemical Magnetic Resonance Laboratory. Samples were purchased from Sigma and passed through a gel filtration column (Sephadex G-50) which was equilibrated with 1.0 *M* aqueous ammonia. This procedure takes advantage of MP-11 aggregation in nonligating bases [20]. The MP-11 band was collected, neutralized with 0.1 *M* HCl, extensively dialyzed against water and lyophilized. Isoelectric focusing using an LKB multiphor and pH 3–12 PAG plates produced a major and minor band as previously described [9]. NMR samples were prepared in 99.8% ^2H_2O (Merck). MP-11-CN was formed using KCN (Aldrich) and all pH s were adjusted to 7.0 ± 0.5 as reported in the figure captions.

Results and Discussion

The proton NMR spectra presented here reveal many resolved resonances outside the diamagnetic region of 0–10 ppm. These large shifts are caused by the paramagnetism which is characteristic of high, intermediate and low spin forms of ferric hemes. Current understanding of the theory and interpretation of such shifts for hemes, porphyrins and heme proteins has been presented elsewhere [4, 17, 21].

Figure 1A reveals the low and high field proton hyperfine shift region of the ferric heme undecapeptide. In 1A the low field spectrum shows a group of broad resonances, A–D, which are located in a position typical of heme methyl resonances in high spin ferric heme proteins (100–60 ppm) [4]. Simultaneously, a group of resonances (E–I) occur in a portion of the spectrum which is typical of low spin ferric heme proteins and heme models [4, 17, 21]. This spectrum, taken in aqueous solution without buffer, indicates that aggregation, which is common to water soluble natural hemins and porphyrins [22], including OP [6, 7] and MP-11 [8], may be characterized by a high spin ⇌ low spin equilibrium. The high spin form presumably reflects aqueo-MP-11, by analogy with native (aquo) cytochrome *c* peroxidase where ^2H_2O is the sixth ligand, in this case. The low spin resonances are most likely due to the aggregated species.

As reported earlier, MP-11 has peroxidase activity [23] and its NMR spectroscopic behavior upon reaction with H_2O_2 is of interest in comparison to that observed for the peroxidase enzymes [14, 15, 18, 19]. Figure 1B indicates that addition of a 25% mole excess of hydrogen peroxide to the aqueous MP-11 solution results in complete loss of the hyperfine resonances downfield (A–K) and nearly a complete bleaching of the upfield resonances (L–T). These spectral changes mimic those of cytochrome *c*

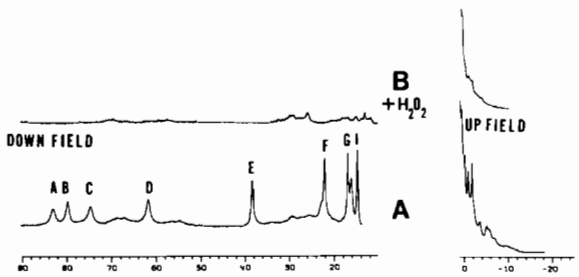

Fig. 1. (A) Proton NMR spectrum (360 MHz) of the hyperfine shift region of 8 × 10^{-4} *M* ferric heme undecapeptide (MP-11) in 99.8% ^2H_2O, pH′ 7.2. Peaks A–D are characteristic of the heme methyl groups of a high spin ferric heme protein. The pH′ is the observed meter reading. Peaks E–I are characteristic of a low spin ferric heme protein. (B) Spectrum which results from the addition of a 25% mole excess of hydrogen peroxide to the solution in (A). This bleached spectrum is characteristic of the cytochrome *c* peroxidase oxidized intermediates.