Coordination of Amino Acids by the Fe^{3+} Porphyrin Microperoxidase-8: Possible Role for the Invariant Phe in the Peroxidase Enzymes

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Equilibrium constants have been determined for the substitution of coordinated H_2O in the Fe^{3+} MP-8 by various amino acids in 20% aqueous MeOH at 25 °C; the presence of an aromatic side chain significantly increases log *K* from, *e.g.* Gly 3.5 to Phe 4.8 and Trp 5.6 and causes a shift in the wavelength of the Soret band from 403 to 406 nm, which is ascribed to donor–acceptor interaction between the porphyrin and aromatic/heterocyclic rings and suggests a role for the invariant distal Phe in the peroxidases.

The typical peroxidases (which catalyse the rapid reduction of H_2O_2 to H_2O by a variety of reagents)[1] and the O_2 binding myoglobins (Mbs) are monomeric haemoproteins with the same axially coordinated 'proximal' His and often the identical porphyrin ring and substituents; they provide the classic case of control of activity by the protein. Although the coordinated His has been implicated in the peroxidase mechanism,[2] the recent finding that His can be replaced (using site-directed mutagenesis) by an *O*-bonded Glu or Gln with little change in activity[3] allows one to focus on the role of the 'distal' groups surrounding the active coordination site of the Fe in controlling activity. An X-ray structure determination[4] of cytochrome c peroxidase (CCP) established that the active site is surrounded by Arg, His and Trp; this Trp-51 lies adjacent to the porphyrin ring and can be replaced by Phe with no significant change in enzymatic activity.[5] Comparison with the amino acid (AA) sequences of plant peroxidases suggests that Arg, His and Phe are invariant distal residues in the peroxidases,[6] while the groups surrounding the distal site in Mbs include invariant His, Val *etc.*, but no Arg or Phe.[7] Attention is therefore directed to the role of Arg and Phe in promoting peroxidase activity. An important, but so far unidentified, role for Phe is further suggested by the presence of an analogous distal Phe in the catalases, but discussion is here complicated by the replacement of His by Tyr as axial ligand.[8]

Differences in peroxidase activity between the Mbs and the enzymes include the far greater rate of reaction of H_2O_2 with the Fe^{3+} in the enzymes,[9] and the greater stability towards irreversible change of the highly oxidised intermediates of the enzymes called Compound I (Fe^{4+} + oxidised porphyrin ring[10] or, in the case of CCP,[11] of an oxidised Trp other than Trp-51) than of the Mbs [Fe^{4+} + unidentified protein radical(s), no oxidised porphyrin yet observed].[12] We have already demonstrated a role for the guanidinium ion (representing the Arg side chain) in the peroxidase activity of the Fe porphyrin microperoxidase-8 (MP-8), which possesses an octapeptide side chain including one axially coordinated His and serves as a suitable protein-free cofactor, and have discussed the basis of the increased rate of reaction of H_2O_2 with the enzymes.[13] We have now used the coordination of AAs *via* their NH_2 group to anchor relevant side chains near the porphyrin ring and to examine the electronic effects (changes in UV–VIS spectra) and energetics (changes in binding constant) of porphyrin–benzene (and indole) interactions as models for Phe (and Trp)-haemoprotein interactions.

Equilibrium constants for the substitution of coordinated H_2O on the Fe^{3+} in MP-8 by a series of AAs with non-coordinating side chains (Sigma, used without further purification; also second sample of Ala from BDH) were determined using *ca.* 5 μmol dm^{-3} MP-8 in 20% v/v aqueous MeOH (to suppress dimerisation),[14] phosphate buffer pH 8.75 and I = 0.08 mol dm^{-3} at 25 °C by spectrophotometric titration in 1 cm cells. The changes in spectra were established rapidly and good isosbestic points observed. At least three separate determinations were carried out for each AA and a second source of Ala was used to confirm an unexpectedly low value. The observed equilibrium constants all corresponded to the binding of one AA and were corrected for the pK of the AA[15] as shown in Table 1 (assumed to be unaffected by the presence of 20% MeOH)[16] and for the pK = 8.90 for conversion to the hydroxo complex[14] to give values of K = [Fe–NH_2R]/[Fe–OH_2][NH_2R] where NH_2R is the amine form of the AA. The values of log K and the wavelengths of the main (Soret) band of the product are given in Table 1, together with results for NH_3[17] and spectroscopic data for certain simple amines.

The data show very similar wavelengths for the complexes with NH_3, alkylamines (including cyclohexylamine) and simple AAs (all 403–404 nm) but a significant shift to longer wavelength (405.5–406 nm) where π-electrons are present (benzylamine, Phe and Trp), which we ascribe to π–π interactions of the donor–acceptor (D–A) type between the porphyrin and benzene/indole rings. Similar values of log K (3.3–4.0) were obtained for NH_3 and the simple AAs except for Ala (2.9), which may reflect the predominance of adverse steric over favourable inductive effects of introducing the Me group into Gly (*cf.* ref. 18), but significant increases with Phe (4.8) and Trp (5.6), which can reasonably be ascribed to the effects of the D–A interaction. A smaller increase in log K for Trp, but little or no increase for Phe, has been reported for a Zn porphyrin (*cf.* Leu 2.3, Phe 2.5, Trp 3.7).[19] D–A interactions resulting in significant binding constants are well known in the adducts of Fe porphyrins with heterocyclic reagents such as caffeine[20] and the *N*-methylpyridinium cation,[21] but their formation usually involves additional changes in structure (*e.g.* dissociation of dimeric haematin to give monomeric adducts) and hence in spectra. Our results provide direct (UV–VIS) evidence for D–A interaction in Fe porphyrins under the simplest conditions (*i.e.* where the state of aggregation and the nature of the axial ligands are held constant) and with the simple benzene ring (in Phe and benzylamine); they suggest that the invariant Phe may moderate the activity of the Fe porphyrin through D–A interactions.

One result of introducing D–A interactions with Phe is, in effect, to increase the polarisability of the porphyrin system.

Table 1 Equilibrium constants and positions of the Soret bands for the amino acid complexes

Ligand	pK	log K^a	λ/nm
Gly	9.6	3.46 (0.09)	403
Ala	9.7	2.89 (0.07)	403
Val	9.6	3.73 (0.08)	403
Leu	9.6	3.99 (0.03)	403.5
Phe	9.1	4.76 (0.12)	406
Trp	9.4	5.64 (0.09)	406
NH_3[b]	9.1	3.34 (0.03)	403.5
$EtNH_2$[b]			403.5
$BuNH_2$[b]			403.5
$C_6H_{11}NH_2$[b]			404
$PhCH_2NH_2$[b]			405.5

[a] Standard deviation in parentheses. [b] From ref. 17.

This could serve, firstly, to stabilise the highly oxidising Fe^{4+} and porphyrin radical cations (this provides a natural explanation for the enhanced stability of the oxidised porphyrin ring in the enzymes compared to the Mbs; the further stabilisation of oxidised Trp over porphyrin in CCP obviously requires a different explanation) and, secondly, to facilitate any nearby movement of charge in the protein environment of low dielectric constant (*e.g.* the swinging of the flexible charged side chain of Arg,[4] which appears to play a key role in the peroxidase mechanism[13]) through an enhanced condenser effect. D–A interactions would also alter the properties of the Fe^{3+} ion and might help marginally to anchor the key pentapeptide loop (Arg 48 to His 52 in CCP) near the active site. These possibilities will be discussed in more detail elsewhere.

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