Remarkable helix stabilization *via* edge-to-face tryptophan–porphyrin interactions in a peptide-sandwiched mesoheme

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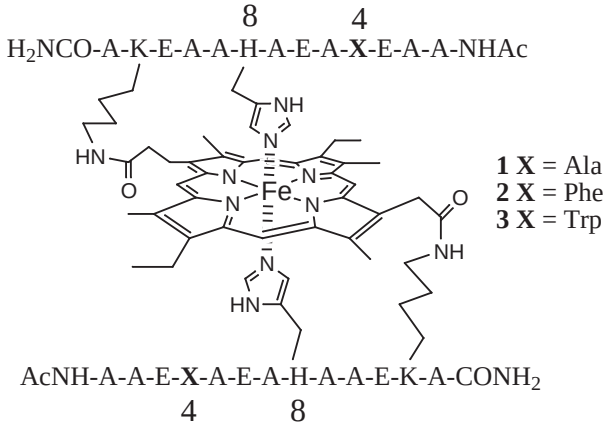
Tryptophan residues in a peptide-sandwiched mesoheme provide helix stabilization *via* edge-to-face interactions between the Trp indole side-chains and the porphyrin ring.

Binding of heme by apomyoglobin and cytochrome b_5 is driven to a large extent by interactions between apolar amino acid side-chains and the porphyrin ring. This was convincingly demonstrated by an experiment in which the proximal histidine (His) ligand of myoglobin (Mb) was mutated to glycine without greatly diminishing the apoprotein's affinity for heme.[1] Furthermore, removal of heme from Mb[2] and from cytochrome b_5[3] leads to reduced protein helix content and decreased protein stability as interactions between the heme and amino acid side-chains in the heme binding pocket are lost.

The F-helix of *Aplysia limacina* Mb provides the proximal ligand (His-95) to the heme iron (Fig. 1).[4] A phenylalanine residue (Phe-91), which is also within the F-helix, is located at position $i - 4$ relative to His-95 (in mammalian Mbs, this position is usually occupied by leucine). The phenyl ring of Phe-91 is nearly perpendicular to the heme plane, and the hydrogen atoms in the 3- and 4-positions of the phenyl side-chain make van der Waals contact with the heme.[4] Similar interactions between heme and Phe residues are observed in cytochrome b_5.[5] An edge-to-face orientation between two aromatic groups is energetically favorable.[6] Stabilization arises from electrostatic interactions between hydrogen atoms on one ring, which bear a partial positive charge, and the π-electrons of the second ring.[7]

We have developed a class of hemoprotein models that we call peptide-sandwiched mesohemes (*e.g.* **1**).[8–11] His-to-iron



coordination in **1** induces the covalently attached peptides to adopt conformations with *ca.* 50% helix content, in aqueous solution at 8 °C.[10] Helix content can be increased to > 90% by addition of propan-1-ol (PrOH). From molecular modelling studies we have predicted that in **1** the angle between the peptide helix axis and the porphyrin plane will be about 30°.[12] When His ligands to heme iron in natural hemoproteins reside within helices, this angle is usually closer to 0°. The different

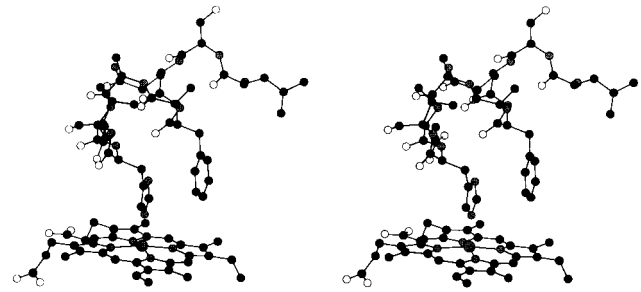


Fig. 1 Heme and F-helix of *Aplysia Limocina* Mb.[4] Atom colors: carbon (black); nitrogen (grey); oxygen (white).

orientations result from alternate combinations of His side-chain torsional angles χ_1 and χ_2. In **1** we predict that $\chi_1/\chi_2 \approx 180°/-90°$,[9,12] whereas in most hemoproteins utilizing His residues that reside in α-helices (including *Aplysia limacina* Mb), these values are closer to $-60°/90°$.[8]

In **1**, Ala-4 is at position $i - 4$ relative to the His ligand (His-8). Using the structure of **1** predicted from molecular modeling studies,[12] we replaced Ala-4 by Phe and by tryptophan (Trp) to investigate whether edge-to-face interactions between either aromatic amino acid side-chain and the heme was possible. From these studies we found that the indole side-chain of Trp can make such interactions if its side-chain torsional angles χ_1 and χ_2 are limited to *ca.* 180 and 90°, respectively. An energy minimized structure predicted for **3** is shown in Fig. 2. In contrast, the side-chain of Phe appears to be too small to permit contact with the heme in **2**.

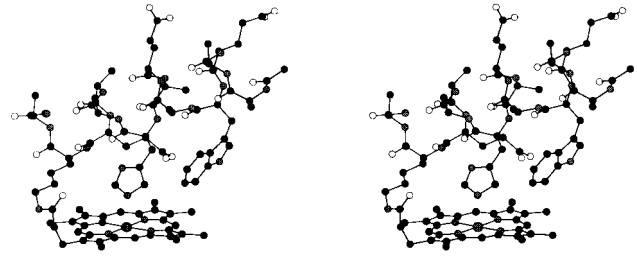


Fig. 2 Structure of **3** predicted by molecular modelling. Only one peptide is shown. Atom colors as in Fig. 1.

We have prepared **2** and **3** using our previously reported synthetic method.[8,9] CD spectra of **1** and **3** in neutral aqueous solution at 8 °C are shown in Fig. 3. The spectrum of **2** (not shown) is nearly identical to that of **1**. Using the mean residue ellipticity at 220 nm (θ_{220}) as a measure of peptide helix content,[8] we estimate that changing Ala-4 to Trp increases peptide helix content from *ca.* 50 to *ca.* 90% (maximum θ_{220} for **1**–**3** is calculated to be $-27\,700$ deg cm^2 dmol^{-1}). In contrast, changing Ala-4 to Phe leaves the peptide conformation unaltered. Both results are consistent with predictions from molecular modelling.

Aromatic amino acid side-chains,[13] as well as heme itself,[8,14] can contribute to CD spectra in the wavelength range normally

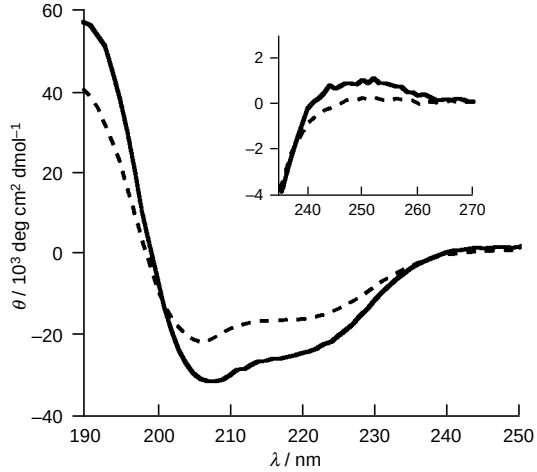


Fig. 3 CD spectra of **1** (- - -) and **3** (——) in 2 mM pH 7 potassium phosphate buffer at 8 °C

employed to determine peptide helix content. In fact, we observe a positive contribution in the CD spectrum of **3** near 250 nm that is absent in the spectra of **1** and **2** (Fig. 3, inset). This positive ellipticity probably arises *via* exciton coupling of the Trp side-chain with the peptide backbone amides and/or with the heme. Such contributions can lead to errors in peptide helix content determined by CD.[13] The amount of organic solvent required to achieve the maximum value of θ_{220} provides additional information about relative helix content in pure aqueous solution when comparing similar systems such as **1**–**3**. Fig. 4 shows θ_{220} plotted *vs.* volume percent of PrOH for **1** and **3** (the plot for **2** is identical to that for **1**, and thus is not shown). Whereas **1** and **2** require more than 30% (v/v) PrOH to reach maximal helix content, **3** reaches maximal helix content with only about 5% PrOH. This is consistent with **3** having much higher helix content than **1** and **2** in aqueous solution.

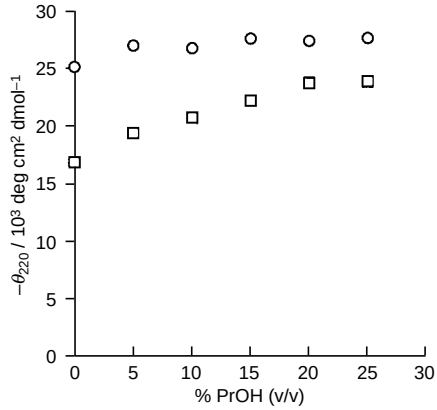


Fig. 4 θ_{220} as a function of PrOH concentration for **1** (□) and **3** (○). The plot for **2** is identical to that for **1**.

Aromatic amino acids are considered to be helix breakers[15] because their side-chain torsional angle χ_1 is restricted to the g^+ (300°) and t (180°) regions when they are forced to reside within a helix.[16] However, when the amino acid is within the first (*N*-terminal) turn of a helix, $\chi_1 = g^-$ (60°) is also accessible.[16] Because Phe-4 is within the first helical turn of **2**, it is not surprising that **1** and **2** exhibit similar conformational properties. In order for Trp to make edge-to-face contact with the heme in **3**, however, we predict that its side-chain must be restricted to $\chi_1 \approx 180°$ and $\chi_2 \approx 90°$. Our CD data indicate that the indole–heme interactions are sufficiently stabilizing to overcome the large entropy loss associated with such conformational restrictions. This is in keeping with the importance of such interactions in maintaining the structural integrity of natural hemoproteins.[2,3]

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Notes and References

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