Haem binding and catalytic activity of two-α-helix peptide annealed by trifluoroethanol

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A designed two-α-helix peptide His-2α binds effectively Fe^{III}–mesoporphyrin (haem) and enhances *N*-demethylase activity of the haem, when the 2α-helix structure is annealed by the addition of 10–20% trifluoroethanol.

Iron porphyrins occur widely in nature as cofactors of haemproteins and display diverse functions.[1] The specific function of the haemprotein is determined by the microenvironments around the haem.[2] Thus, if we can establish the haem environments by designing polypeptide 3D structures, it will be possible to control the haem functions. So far, considerable effort has been devoted to the conjugation of porphyrin molecules by chelation[3] or covalent linkage[4–6] with designed peptide 3D structures. To develop a mini haemprotein, we here report the design and synthesis of a 2α-helix peptide His-2α, which could bind Fe–mesoporphyrin (haem) in a regulated manner. Furthermore, catalytic activity of the haem that resembled that of peroxidase was significantly increased by the binding with the peptide.

A 14-peptide segment in the peptide was designed to take an amphiphilic α-helix structure in a manner similar to that of a portion of coiled-coil proteins (Fig. 1).[7,8] The two segments were dimerized by disulfide linkage of the Cys^{16} residues. As axial ligands of haem, His residues were introduced at the sixth position instead of Leu to deploy a haem parallel to the helix. The peptide was synthesized by the solid-phase method using the Fmoc strategy and purified with HPLC to high purity (> 98%). The peptide gave a molecular ion peak at *m*/*z* 3529.6 [(M + H)$^+$] (calc. 3529.1) on matrix assisted laser desorption ionization times-of-flight mass spectrometry. The monomer peptide (His-1α) was also synthesized and gave an ion peak at *m*/*z* 1836.9 [(M + H)$^+$] (calc. 1837.2).

The conformation of His-2α (2.0×10^{-5} mol dm^{-3}) in buffer (pH 7.4) was almost random due to the introduction of His residues (Fig. 2).† With increasing percentage volume of trifluoroethanol (TFE), which is known to be an α-helix stabilizing solvent,[8] the α-helicity[9] of His-2α gradually increased until it reached about 70% at 30% TFE [Fig. 2(*b*)]. Interestingly, in the presence of the haem, a further increase in α-helicity was observed at 10–20% TFE [Fig. 2(*b*), closed circles]. The largest increase of α-helicity by the addition of haem was obtained at around 15% TFE (1.4-fold). When His-2α was titrated with the haem in 15% TFE, the CD spectra of His-2α changed gradually to that of a typical α-helix structure with an isodichroic point at 204 nm (Fig. 3). The binding constant (K_a) in 15% TFE, which was estimated from molecular ellipticities at 222 nm using a single site binding equation,[10] was 2.8×10^5 dm^3 mol^{-1}. The addition of 1-methylimidazole (200 equiv.) inhibited the coordination of His-2α with the haem, resulting in the decrease of the α-helicity to the level without the haem. Additionally, there was no significant change in the CD spectra by the addition of haem at acidic pH (2.0–6.0). Because the pK_a of imidazole is about 6.0, the pH effect is attributed to the protonation of His side chains such that they cannot act as ligands. Therefore, we concluded that the increase in α-helicity

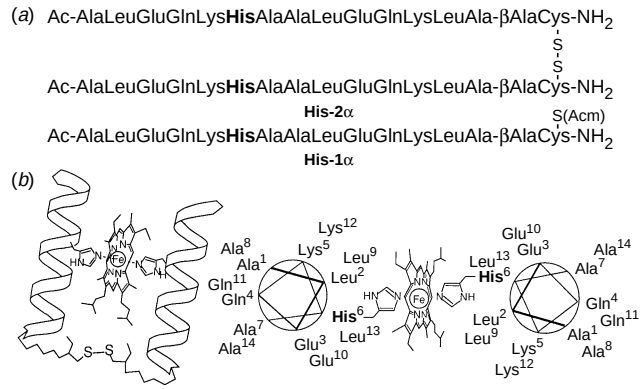

Fig. 1 Structure of the designed peptides, His-2α and His-1α. (*a*) Amino acid sequences of His-2α and His-1α. (*b*) Illustration of two-α-helix peptide structure bound to the haem and helix wheel drawing of the two 14-peptides in coiled-coil form. Acm = acetamidomethyl.

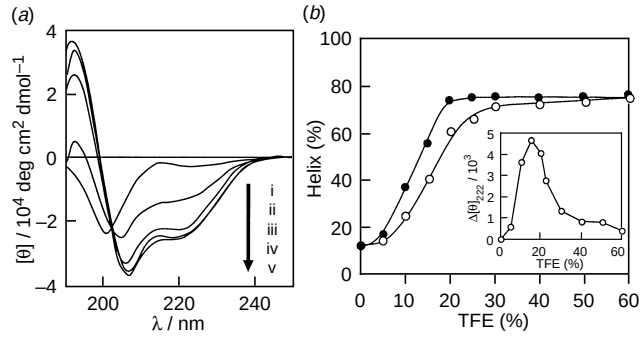

Fig. 2 (*a*) CD spectra of His-2α with increasing percentage volume of TFE in 2.0×10^{-2} mol dm^{-3} Tris HCl buffer (pH 7.4) at 25 °C. i, 0%; ii, 15%; iii, 30%; iv, 60%; v, 97%. [His-2α] = 2.0×10^{-5} mol dm^{-3}. (*b*) Effect of TFE content on the α-helicity (○) in the absence and (●) the presence of haem (2.0×10^{-5} mol dm^{-3}). Inset: increase in ellipticity at 222 nm by the addition of haem as a function of TFE content.

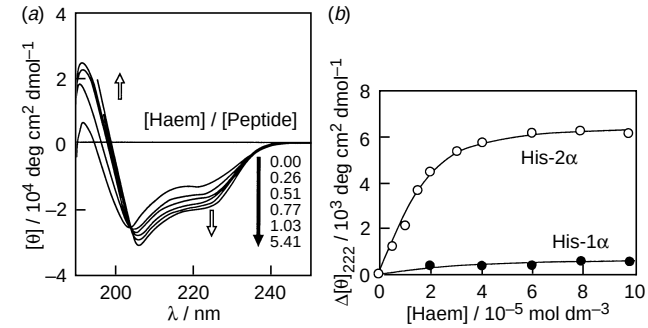

Fig. 3 (*a*) CD spectra of His-2α with increasing haem concentration in the buffer containing 15% TFE at 25 °C. [His-2α] = 2.0×10^{-5} mol dm^{-3}. (*b*) Improvement of α-helix for (○) His-2α (2.0×10^{-5} mol dm^{-3}) and (●) His-1α (4.0×10^{-5} mol dm^{-3}) by the addition of the haem.

of His-2α took place *via* the haem binding by ligation with His residues. In contrast, α-helicity of His-1α was little affected by the addition of haem at any percentage volume of TFE (0–90%), indicating that His-1α could not bind the haem in this concentration.

Titration of the haem with His-2α was also carried out in the buffer containing various amounts of TFE. At 15% TFE, the Soret band at 401 nm increased and the band at around 355 nm decreased upon the addition of His-2α [Fig. 4(*a*)]. That is, an UV–VIS spectrum of the haem was converted from the high spin to the low spin form with an isosbestic point at 390 nm. The decrease in absorbance at 355 nm indicated that the peptide acted to break up haem aggregates.[6] The binding constant for the haem with His-2α was strongly dependent on the TFE content [Fig. 4(*b*)]. His-2α showed the highest binding constant at 15% TFE (K_a = 5.8 × 10^5 dm^3 mol^{-1}). In contrast, His-2α could not bind the haem effectively at < 10% and > 30% TFE (K_a < 3.0 × 10^4 dm^3 mol^{-1}). These TFE titrations revealed that the 2α-helix structure annealed by TFE and the consequent formation of a hydrophobic pocket are important for the haem binding. That is, at the lower TFE content (< 10%), the hydrophobic pocket is not formed, because the conformation of His-2α is predominantly a random-coil. On the other hand, at the higher TFE content (> 30%), the 2α-helix structure is destroyed so that each α-helix segment is free to move. Because there is no hydrophobic pocket at either lower or higher TFE content, the peptide cannot bind the haem effectively. The monomer peptide His-1α and 1-methylimidazole needed a concentration of an order of *ca.* 10^{-3} mol dm^{-3} for the haem binding and did not show such a TFE dependence. These results also confirm that the formation of the 2α-helix structure is essential for the haem binding.

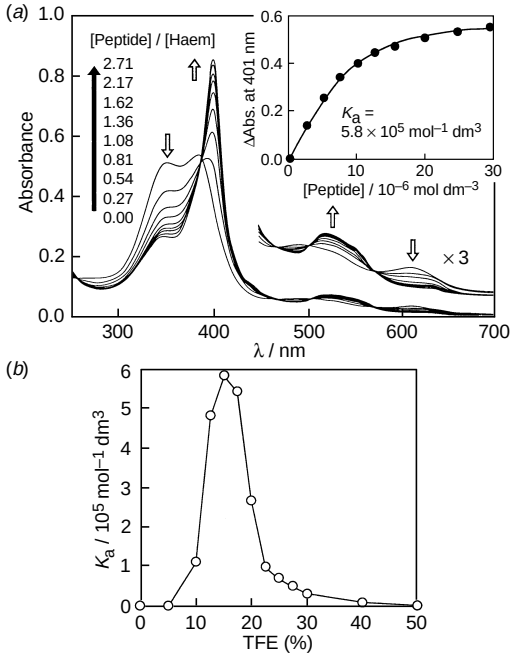

Fig. 4 (*a*) UV–VIS spectra of the haem with increasing His-2α concentration in the buffer containing 15% TFE at 25 °C. [haem] = 1.0 × 10^{-5} mol dm^{-3}. (*b*) Effect of TFE content on the binding constant for His-2α with the haem.

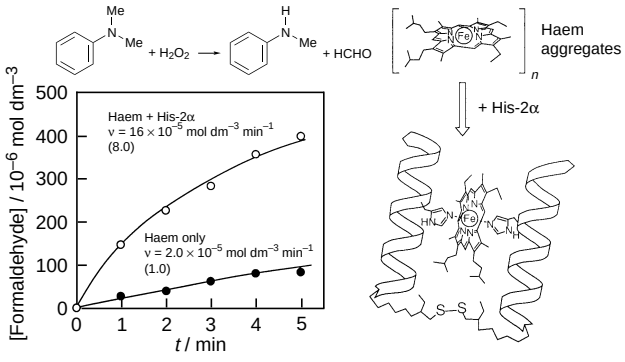

Fig. 5 Time course of formaldehyde formation catalysed by the haem (○) in the presence and (●) absence of His-2α. The reaction was initiated by addition of hydrogen peroxide (4.9 × 10^{-3} mol dm^{-3}) to mixtures of *N*,*N*-dimethylaniline (5.0 × 10^{-3} mol dm^{-3}), haem (4.8 × 10^{-6} mol dm^{-3}) and His-2α (1.0 × 10^{-5} mol dm^{-3}) in 0.1 mol dm^{-3} Tris HCl buffer (pH 7.4) containing 15% TFE at 30 °C. About 80% of the haem was bound to the peptide under the conditions according to the binding constant.

Next, demethylation catalysis of the haem bound to His-2α was demonstrated using *N*,*N*-dimethylaniline as a substrate.[11] As shown in Fig. 5, the initial rate *v* of the reaction in the presence of His-2α (*v* = 16 × 10^{-5} mol dm^{-3} min^{-1}) was accelerated by a factor of 8.0, relative to that in the absence of the peptide (*v* = 2.0 × 10^{-5} mol dm^{-3} min^{-1}). The acceleration of the reaction was dependent on the TFE content and showed a maximum value at 15% TFE. The *N*-demethylase activity was comparable to that of bilayer-bound cytochrome *c* as reported by Hamachi *et al.*[11] The initial rate was dependent on the concentration of H_2O_2, but not on that of *N*,*N*-dimethylaniline. Therefore, the rate-determining step is the reaction between the haem and H_2O_2. The peptide seems to enhance the activity by isolating the haem in the peptide structure from the haem aggregates in solution.

In conclusion, the haem binding of the His-peptide was controlled by the peptide conformation with TFE. The catalytic activity was enhanced by the formation of the haem–peptide complex, which might be another kind of catalytic molten globule.[12] The strategy using designed peptides conjugated with functional groups, such as haem, is expected to be applied to the elucidation of the roles of polypeptide 3D structure on the diverse functions of natural proteins, and the obtained information will be useful for the design artificial proteins.

Footnotes

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† Leu-2α, a non-His peptide, had 55% helicity in the buffer.

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Received in Cambridge, UK, 17th March 1997; Com. 7/01852E