Molecular assembly of two-α-helix peptide induced by haem binding

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A designed two-α-helix peptide H2α-17 bound effectively Fe^{III}–mesoporphyrin (haem), and the haem binding simultaneously induced the molecular assembly of the peptide from a monomeric to a tetrameric form.

The self-assembly of polypeptides and functional chromophores, such as chlorophyll and haem, has a significant role in nature, the assembled species displaying highly efficient functions. For example, in the bacterial light-harvesting complex LH2, nine identical units, each consisting of two kinds of α-helical polypeptides and three bacteriochlorophyll *a* (Bchl*a*) units, are combined into a ring-shaped assembly.[1] In the LH2 protein, the orientation and assembly of a large number of Bchl*a* units are regulated by the polypeptide three-dimensional structure in membranes and effective energy-transfer is accomplished. So far, in the field of *de novo* protein design, considerable effort has been devoted to construction of polypeptide three-dimensional structure and conjugation of porphyrin molecules *via* chelation[2–4] or covalent linkage[5–8] with peptides. However, to develop a larger supramolecular system, such as LH2, it will be necessary to construct and regulate self-assembling systems composed of multiple polypeptide units and porphyrin molecules. In line with this aim, we have designed and synthesized a two-α-helix peptide which binds Fe^{III}–mesoporphyrin (haem). Furthermore, we found that the haem-binding simultaneously induced the self-association of the peptide, and that the haem groups were highly oriented in the self-assembled peptides.

17-peptide segment was designed to take an amphiphilic α-helix structure, which was stabilized by two sets of E–K salt bridges (Fig. 1). The two segments were dimerized *via* the disulfide linkage of the Cys^{21} residues. As axial ligands of haem, His was introduced at the ninth position to deploy a haem parallel to the helix. Four Leu residues per helix were arranged around the His to construct a hydrophobic haem-binding site. Even though the hydrophobicity of the sequence may be low, it is expected that the haem binding increases the overall hydrophobicity of the peptide and induces molecular association through hydrophobic interactions. The peptide was synthesized *via* solid-phase methodology using the Fmoc strategy and purified with HPLC to high purity (> 98%). The peptide gave a molecular ion peak at *m*/*z* 4012.2 [(M + H)$^+$] (calc. 4011.7) *via* matrix assisted laser desorption ionization time-of-flight mass spectrometry.

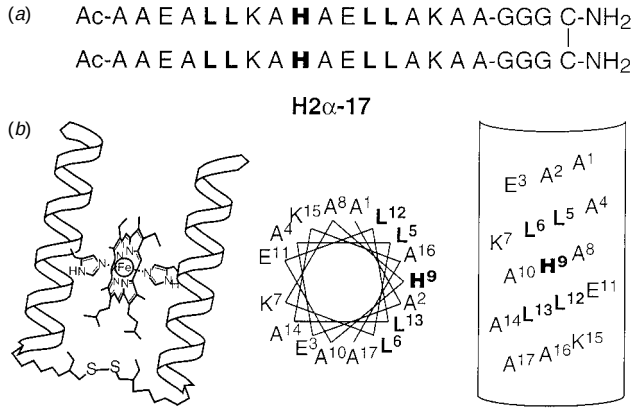

Fig. 1 Structure of the designed peptide, H2α-17. (*a*) Amino acid sequence of H2α-17; (*b*) illustration of the two-α-helix peptide structure bound to the haem, and helix wheel and net drawings of the 17-peptide.

Circular dichroism (CD) studies revealed that the peptide H2α-17 showed a typical α-helical pattern in buffer (pH 7.4) [Fig. 2(*a*)]. From the ellipticity at 222 nm ($[\theta]_{222}$ = −17 100 deg cm^2 dmol^{-1}), the α-helicity was estimated as 54%.[9] Since the monomeric peptide (H1α-17) showed a lower α-helix content ($[\theta]_{222}$ = −6800 deg cm^2 dmol^{-1}, 20%), the two α-helix segments in H2α-17 gathered together by orienting the hydrophobic side-chains inside, resulting in stabilization of the three-dimensional structure. Interestingly, the α-helicity of H2α-17 was increased by the addition of haem ($[\theta]_{222}$ = −26 800 deg cm^2 dmol^{-1}, 85%) [Fig. 2(*a*)]. The addition of an excess amount of cyanide ion (2.5×10^{-2} mol dm^{-3}, 2.5×10^3 equiv.) inhibited the coordination of H2α-17, resulting in a decrease in the α-helicity to the level observed 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 *ca.* 6.0, the pH effect is attributed to the protonation of the His side chains such that they cannot act as a ligand. Therefore, we concluded that the increase in α-helicity of H2α-17 took place *via* the haem-binding by ligation with His residues. The thermal stability of the peptide in the presence or absence of haem was also examined by CD measurements. H2α-17 showed a midpoint of thermal transition (T_m) at 35 °C [Fig. 2(*b*)]. Similar to the α-helicity, haem binding increased remarkably the T_m value of the peptide to 62 °C. This result indicates that the haem binding increases the stability of the 2α-helix structure.

To further characterize the haem-binding with the peptide, UV–VIS titration of the haem with H2α-17 was carried out in buffer. With increasing peptide concentration, an increase of the Soret band at 405 nm and a decrease of the band at 355 nm of haem were observed (Fig. 3). That is, the UV–VIS spectrum of haem was converted from that of the high spin to the low spin form with an isosbestic point at 390 nm.[8] The UV–VIS spectrum of the haem in the presence of peptide resembles those

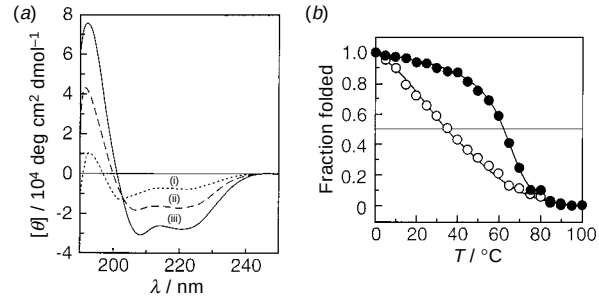

Fig. 2 (*a*) CD spectra of (i) H1α-17 and H2α-17 in the (ii) absence and (iii) presence of haem (1.0 equiv.) in 2.0×10^{-2} mol dm^{-3} Tris·HCl buffer (pH 7.4) at 25 °C. [H2α-17] = 1.0×10^{-5} mol dm^{-3} and [H1α-17] = 2.0×10^{-5} mol dm^{-3}. (*b*) Temperature denaturation profiles of H2α-17 in the (○) absence and (●) presence of haem (1.0 equiv.) in the buffer.

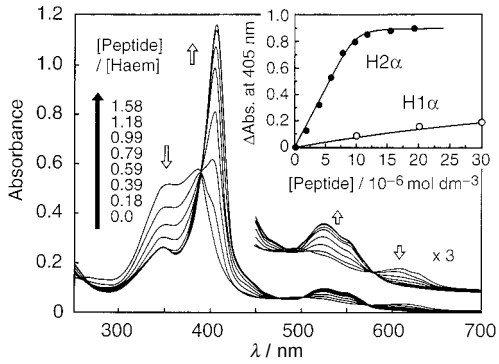

Fig. 3 UV–VIS spectra of haem with increasing H2α-17 concentration in the buffer (pH 7.4) at 25 °C. [haem] = 1.0×10^{-5} mol dm^{-3}. Inset: plots of absorbance at the Soret band of haem as a function of concentration.

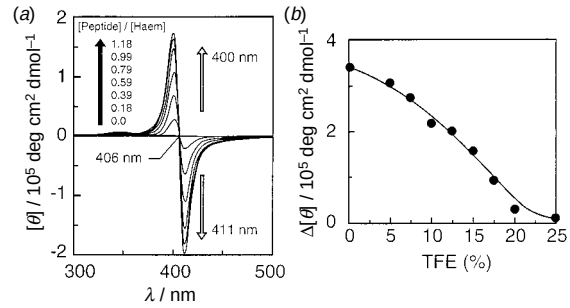

Fig. 5 (*a*) CD spectra of haem with increasing H2α-17 concentration in the buffer (pH 7.4) at 25 °C. [haem] = 1.0×10^{-5} mol dm^{-3}. (*b*) Effect of TFE content on the Soret CD intensity of haem bound to the peptide. $\Delta[\theta] = [\theta]_{max} - [\theta]_{min}$.

of natural cytochromes with six-coordinate iron. The binding constant (K_a) determined from the absorbance change at the Soret band using a single site binding equation,[4] was 1.1×10^7 mol^{-1} dm^3 (Fig. 3, inset). On the other hand, addition of H1α-17 caused little increase of the Soret band, suggesting that the monomeric peptide could not bind the haem effectively in this concentration range. This result implies that the 2α-helix structure and the consequent formation of the hydrophobic pocket are essential for the haem-binding.[4]

To examine the molecular assembly of the peptide in aqueous solution, the peptide samples were passed through a size-exclusion column. In the absence of haem, the peptide showed a sharp single peak [Fig. 4(*a*)]. It is reasonable to conclude that the peptide is in the monomeric form in the buffer, because sedimentation equilibrium studies demonstrated that the peptide existed as the monomeric form [MW_{obs} = 3600 (MW_{calc} = 4010)] under the same conditions. Furthermore, the peptide did not show any concentration dependence of the α-helicity at (0.1–2.0) $\times$ 10^{-5} mol dm^{-3}. When the peptide solution containing the haem (1.0 equiv.) was chromatographed, the haem was co-eluted with the peptide in a sharp peak at a higher molecular weight, suggesting that the haem was tightly bound to the peptide and that the haem-binding gave rise to the formation of a higher self-association state of the peptide [Fig. 4(*b*),(*c*)]. The sedimentation equilibrium studies revealed that the peptide was in a tetrameric form (MW_{obs} = 19 300, 4.2-mer) after haem-binding [Fig. 4(*d*)]. As further evidence supporting the self-association of peptide–haem conjugates, the haem bound to the peptide showed a strong induced CD peak in the Soret region, which was split into a negative peak at a longer wavelength and a positive peak at a shorter wavelength (Fig. 5). The split Cotton effect, *i.e.* exciton-coupling, indicates that the haem groups are highly oriented with respect to each other in close positions in the self-assembled peptides. With increasing percentage volume of trifluoroethanol (TFE), the ellipticity gradually decreased [Fig. 5(*b*)]. Dissociation of the haem from the peptide cannot explain the reduced Soret CD, because the UV–VIS study indicated that the peptide bound the haem with the six-coordinate form below 25% TFE (K_a = *ca.* x 10^7 mol^{-1} dm^3). Thus, the decrease of the Soret CD seems to be attributed to a change of association state of the peptide–haem conjugate from a tetrameric to a monomeric form. This TFE titration study implies that the hydrophobic interaction is important for the self-association of the peptide–haem conjugates. That is, the haem-binding increases the overall hydrophobicity of the amphiphilic 2α-helix peptide and induces the molecular assembly of the peptide–haem conjugates.

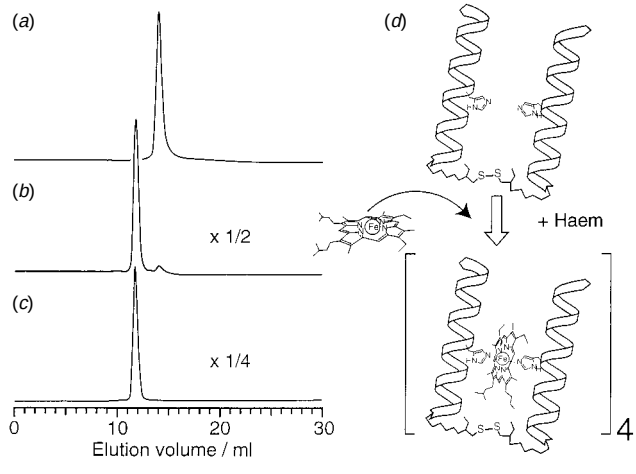

Fig. 4 Size-exclusion chromatograms of H2α-17 (*a*)–(*c*) and the schematic representation of self-assembly of two-α-helix peptide induced by the haem binding (*d*). (*a*) H2α-17 (1.0×10^{-5} mol dm^{-3}) in the absence of haem, detection at 220 nm. (*b*) H2α-17 (1.0×10^{-5} mol dm^{-3}) in the presence of haem 1.0 equiv.), detection at 220 nm. (*c*) H2α-17 (1.0×10^{-5} mol dm^{-3}) in the presence of haem (1.0 equiv.), detection at 405 nm. Column, Superdex 75 HR 10/30 (10 $\times$ 300 mm), 0.1 mol dm^{-3} NaCl/5.0 $\times$ 10^{-2} mol dm^{-3} Tris·HCl buffer (pH 7.4) at 25 °C; flow rate, 0.5 ml min^{-1}.

In conclusion, the association and orientation of functional chromophores can be accomplished using an artificially designed polypeptide. These findings could lead to studies applicable to the regulation of haem functions by polypeptide three-dimensional structures and to the development of peptidyl devices with haem functions.

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Notes and References

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