Regulation of Catalytic Activity of Peptide–Heme Conjugate by Conformational Change with Trifluoroethanol

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A peptide–heme conjugate in which two α-helix segments were covalently coupled at the heme propionates was designed, and the relationship between the structure and the peroxidase-like catalytic activity was examined. The activity and coordination state of heme were regulated by the conformational change of peptide induced upon the addition of trifluoroethanol.

Iron porphyrins (heme) occur widely in nature as cofactors of hemoproteins and display diverse functions.[1] The specific function of the hemoprotein is determined by the environment around the heme which is provided by protein three-dimensional (3D) structure. So far, considerable effort has been devoted to the conjugation of porphyrin molecules with designed peptide 3D structures.[2–11] However, yet little is known about the factors that regulate the heme function in the designed peptides. It is necessary to obtain more detailed information in order to establish an artificial hemoprotein that meets minimal requirements for function. Along with these aspects, we here report the design and synthesis of a peptide–heme conjugate Heme-2α, in which designed His-containing α-helix peptides were covalently linked at the heme propionate groups.

A 17-peptide segment in the conjugate was designed to take an amphiphilic α-helix structure (Figure 1). As axial ligands of heme, His was introduced at the middle of helix. Four Leu residues per helix were arranged around the His to construct a hydrophobic heme-binding pocket. The peptide segments were conjugated at the ends of heme propionate groups via flexible linkers to avoid straining and distortion of heme-binding 2α-helix structure. The peptides were synthesized by means of both solid-phase and solution methods using the Fmoc protocol according to previously reported procedures.[12] The Heme-2α was purified with HPLC to give a high purity (>98% on analytical HPLC) and identified by MALDI-TOFMS, m/z, 4923.6 [$(M + H)^+$] (calc. = 4922.5).

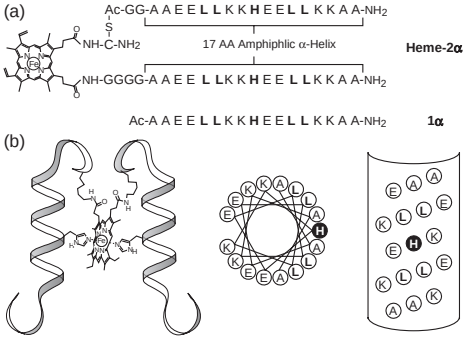

Figure 1. (a) Amino acid sequence of Heme-2α. (b) Illustration of two-α-helix peptide structure bound to the heme, and helix wheel and net drawings.

To characterize the coordination state of the heme iron in the peptide–heme conjugate, we measured UV–vis spectra of the Heme-2α in a buffer (pH 7.4). Heme-2α exhibited the sharp Soret band at 412 nm and α/β band at 537 nm. Upon the addition of sodium dithionite, the Soret band red-shifted to 427 nm and sharp α and β bands appeared at 561 and 531 nm, respectively. These UV–vis spectra of ferric- and ferrous-Heme-2α resemble those of natural cytochromes with 6-coordinate iron. On the other hand, free heme molecule showed a broad Soret band at 393 nm and the addition of the 1α peptide caused little change in the spectrum, suggesting that the monomeric peptide could not interact with the heme effectively in this concentration range. This result implies that the covalent conjugation of peptide segments to heme is one of valid methods to achieve the bis-His coordination state in the designed peptide–heme conjugate.

Circular dichroism (CD) spectrum of the peptide Heme-2α in the buffer showed a typical α-helical pattern (Figure 2a). From the ellipticity at 222 nm ($[\theta]_{222} = -19800\,\mathrm{deg\cdot cm^2\cdot dmol^{-1}}$), the α-helicity was estimated as 63%.[13] Since the monomeric peptide showed a lower α-helix content ($[\theta]_{222} = -4800\,\mathrm{deg\cdot cm^2\cdot dmol^{-1}}$, 15%), the interaction between the heme and α-helix segments in Heme-2α seemed to contribute to the stabilization of the 3D structure. It is unlikely that the helix improvement of Heme-2α was the result of intermolecular aggregation, since the peptide did not show any concentration dependence of α-helicity at 1–20 μM. Meanwhile, the α-helicity of Heme-2α was pH sensitive and was decreased to ca. 40% at acidic pH (2.0–5.0). Since 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 a ligand. Because the peptide 1α did not show such a pH dependence, we concluded that the improvement of α-helicity in Heme-2α took place via the ligation with His residues.

Because the folding of amphiphilic peptide is known to be regulated by the addition of organic solvents,[6,10] the spectra were

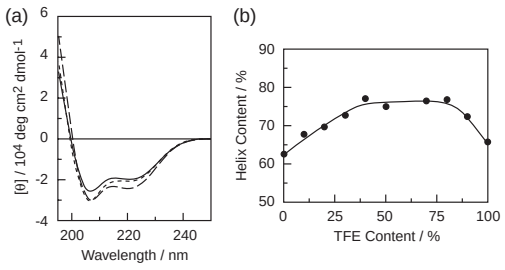

Figure 2. (a) CD spectra of Heme-2α in 20 mM Tris HCl/150 mM NaCl buffer (pH 7.4) (—), in 50% TFE (– –), and TFE (- - -). (b) Dependence of α-helicity of Heme-2α on TFE content. [Heme-2α] = 10 μM, 25 °C.

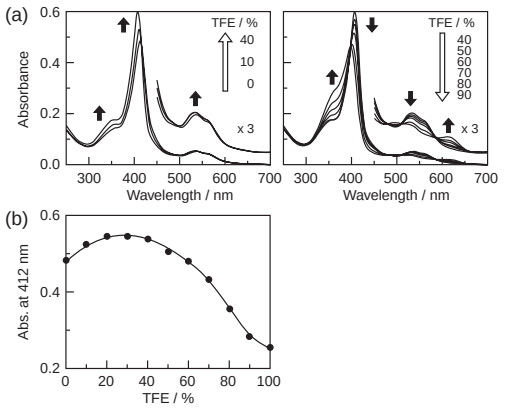

Figure 3. (a) UV–vis spectra of Heme-2α in a buffer (pH 7.4) containing the various content of TFE at 25 °C. [Heme-2α] = 5 μM. (b) Absorbance change of Heme-2α at 412 nm depending on the TFE content.

measured at various TFE content. In the initial range (<40%), the α-helicity increased until it reached 75% by the helix stabilizing effect of TFE (Figure 2b).[6] In the next TFE concentration range (40–80%), the Heme-2α kept its α-helix content. With further addition of TFE (>80%), the helicity gradually decreased. The UV–vis spectrum of Heme-2α also changed by the TFE addition (Figure 3). At the beginning of titration (<40%), the Soret band at 412 nm increased with a small blue-shift of the peak top to 407 nm, suggesting the heme microenvironment seemed to be optimized for the 6-coordination by the improvement of 2α-helix structure. Upon further addition of TFE (>40%), the Soret band steeply decreased and the spectrum changed to that characteristic for the high-spin heme. In 40–80% TFE, although the helicity of the peptide remained at 75%, the interaction between the porphyrin plane and hydrophobic faces of α-helices might be weakened, leading to the gradual loss of His-coordination in the conjugate. In higher TFE contents (>80%), since the His residues were almost completely dissociated, the α-helicity was decreased by the addition of TFE. These results confirmed that the hydrophobic interaction was essential for the formation of 6-coordinate heme-iron in Heme-2α. In other words, the heme coordination state could be regulated by the conformational change induced with TFE addition.[6]

Finally, peroxidase-like catalytic reaction by Heme-2α was demonstrated using *o*-methoxyphenol (*o*-MP) as a substrate and H_2O_2 as an oxidant.[6,11] In the buffer, the initial rate, v, of the reaction by Heme-2α ($v = 1.5\,\mu M\,min^{-1}$) was four times as large as that catalyzed by the free heme molecule ($v = 0.38\,\mu M\,min^{-1}$). Interestingly, the acceleration of the reaction was dependent on the TFE content and showed a maximum value at 50% TFE (Heme-2α, $v = 7.3\,\mu M\,min^{-1}$; heme, $v = 0.93\,\mu M\,min^{-1}$; relative activity, 8) (Figure 4a). The reaction consists of two steps; the formation of active intermediate of heme and the subsequent oxidation of *o*-MP. In the design of Heme-2α, the heme was fixed in the structure through bis-His coordination, as seen in the natural *b*-type cytochromes. For the complex having such a structure, the replacement of one of two His ligands with H_2O_2 is required for the active species formation. It is considered that the heme reacts less readily with H_2O_2 as the peptide segments interact with the heme more tightly. The addition of the proper amount of TFE seemed to loosen the interaction between the peptide and heme, resulting in the easier coordination of H_2O_2 at the one side of the axial sites of heme and the subsequent reaction at the heme center.[6] However, in the presence of excess amounts of TFE, it is anticipated that the hydrophobic pocket and His-ligations are destroyed so that the reactivity of heme is reduced. The rate-determining step is the reaction between the heme and H_2O_2 to give an active intermediate because the initial rate was dependent on the concentration of H_2O_2 in a saturated manner (Figure 4b). In 50% TFE, double-reciprocal plots against H_2O_2 gave a linear relationship both in the cases of Heme-2α and free heme. The parameters obtained by the Michaelis–Menten scheme were as follows: Heme-2α, $k_{cat.} = 70\,min^{-1}$, $K_m = 9.0$ mM; heme, $k_{cat} = 27\,min^{-1}$, $K_m = 28.9$ mM. The enhanced reactivity is attributed not only to the catalytic efficiency of the heme conjugated to peptides, but also to the affinity against H_2O_2. The peptide seems to enhance the heme reactivity by the effect of His-ligation and the formation of hydrophobic reaction site.

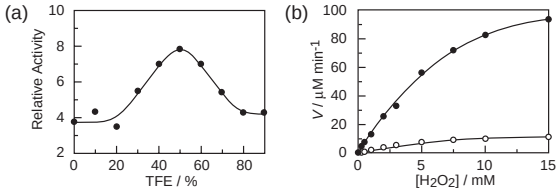

Figure 4. (a) Effect of TFE content on the acceleration of the *o*-MP oxidation catalyzed by Heme-2α in a buffer (pH 7.4) at 25 °C. [Heme-2α] = 2 μM, [*o*-MP] = 5 mM, and [H_2O_2] = 0.5 mM. (b) Dependence of *o*-MP oxidation catalyzed by Heme-2α (●) and heme (○) on H_2O_2 in 50% TFE/buffer (pH 7.4) at 25 °C. [Heme-2α] and [heme] = 2 μM. [*o*-MP] = 5 mM.

In summary, we designed and synthesized a novel 2α-helix peptide bearing the heme. The strategy using designed peptides conjugated with functional groups, such as heme, is expected to be applied to elucidation of roles of polypeptide 3D structure on the diverse functions of natural proteins, and the information obtained here will be useful in designing artificial proteins.

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