Annealing of Two-α-Helix Structure by Metal Ion Binding Regulated with Trifluoroethanol

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(Received May 13, 1998; CL-980369)

A designed two-α-helix peptide His-2α bound effectively a transition metal ion, such as Cu^{2+} and Zn^{2+} in buffer containing 10-30% trifluoroethanol, with the α-helix structure being annealed by the metal ion binding.

Metal ions play an important role in nature as active sites of metalloproteins. Additionally, metal ions are known to be important factors in defining the three-dimensional (3D) structure of proteins, such as DNA-binding zing-finger motifs. Along with this aspect, design and synthesis of artificial metalloproteins and metal-binding peptides have been of great interest in the field of *de novo* protein design.[1-5] Especially, considerable effort has been devoted to construction of 3D structure induced and/or stabilized by the metal binding. Recently, we reported the design and synthesis of a two-α-helix peptide His-2α, which bound a heme only when the α-helix structure was annealed by the addition of 10-20% trifluoroethanol (TFE).[6] In this communication, we attempted to examine ability of the peptide for the metal binding in order to construct an artificial metallopeptide. The peptide bound a transition metal ion, such as Zn^{2+} and Cu^{2+}, in 10-30% TFE, selectively to Co^{2+} and Ni^{2+}. Furthermore, the metal binding increased the α-helicity of the peptide.

The 14-peptide segment in the peptide[6] was designed to take an amphiphilic α-helix structure in a manner similar to that of a portion of coiled-coil proteins (Figure 1).[5-7] The two segments were dimerized *via* the disulfide linkage of Cys^{16} residues. As ligands of metal ion, His residues were introduced at the sixth positions instead of Leu to bind a metal ion between the two α-helices. In the designed structure, it would be expected that the His residues can interact with transition metal ions to form a bidentate complex and concurrently fix the peptide backbone in an α-helix structure. The peptide was synthesized by the solid-phase method using the Fmoc strategy and purified with HPLC to high purity (>98%).[6]

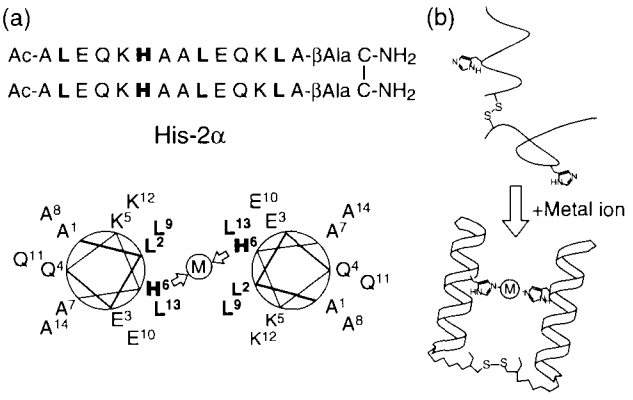

Figure 1. Structure of the metal-binding peptide, His-2α. (a) Amino acid sequence of His-2α and helix wheel drawing of the two 14-peptides in a coiled-coil form (b) Schematic illustration of two-α-helix peptide structure induced by a metal binding.

The conformation of His-2α (20 μM) in buffer (pH 7.4) was almost random due to the introduction of His residues (Figure 2).[6] In the buffer, the addition of transition metal ions, such as Zn^{2+} and Cu^{2+}, did not change the CD spectrum of the peptide, suggesting that the peptide did not bind the metal ion effectively under the conditions. Because the conformation of His-2α is predominantly random in the buffer, His residues may not be oriented at proper positions for the effective metal binding. In order to assist the metal binding of the peptide, various amounts of TFE, which is known to be an α-helix stabilizing solvent[5], were added. With increasing percentage volume of TFE, the α-helicity[8] of His-2α gradually increased until it reached about 70% at 30% TFE (Figure 2a). Interestingly, in the presence of $ZnCl_2$ (1.0 equiv.), a further increase in α-helicity was observed at 10-30% TFE (Figure 2a, closed circles). A similar result has been observed previously for the binding of Fe(III)-mesoporphyrin (heme) by the same peptide,[6] indicating that the α-helix improvement was attained only by the metal coordination. However, the heme binding was more effective to increase the α-helix structure, probably due to the presence of hydrophobic interactions between the porphyrin ring and amphiphilic helices. A proper percentage volume of TFE (10-30%) assisted the metal binding and the metal binding induced further α-helix improvement of the peptide. The largest increase of α-helicity by the addition of Zn^{2+} ion was obtained at around 15% TFE (Figure 2b). On the other hand, the addition of transition metal ions at a higher TFE content (>40%), did not change the CD spectrum of peptide, suggesting that the peptide did not bind the metal ion. In the solution containing a high percentage volume of TFE (>40%), His residues may not be fixed at appropriate positions for the metal binding, because the two-α-helix packing is perturbed so that each α-helix segment is free to move, due to loss of the hydrophobic interactions. The addition of an excess

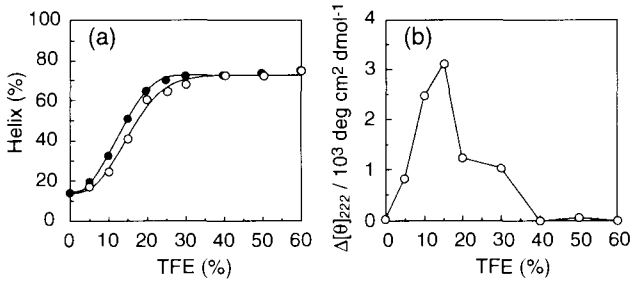

Figure 2. (a) Effect of TFE content on the α-helicity in the absence (○) and presence of Zn^{2+} (1.0 equiv.) (●) in 20 mM Tris HCl buffer (pH 7.4). [His-2α] = 20 μM. (b) Increase in ellipticity at 222 nm by the addition of $ZnCl_2$ as a function of TFE content.

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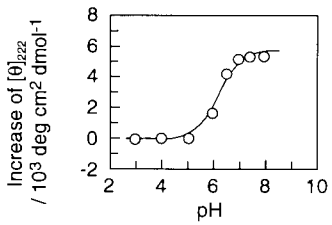

Figure 3. Effect of solution pH on the α-helix improvement by the addition of Zn^{2+} in 15% TFE at 25 °C. Data are fitted by an equation for pH titration. [His-2α] = 20 μM. [$ZnCl_2$] = 100 μM.

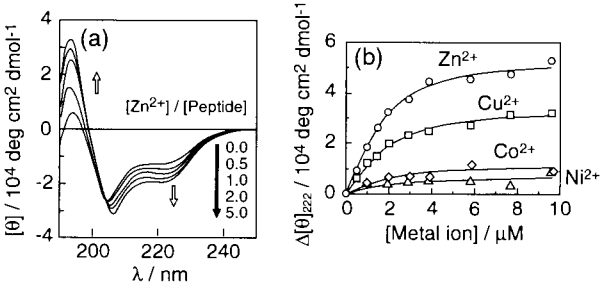

Figure 4. (a) CD spectra of His-2α with increasing Zn^{2+} concentration in the buffer containing 15% TFE at 25 °C. [His-2α] = 20 μM. (b) Improvement of α-helix for His-2α (20 μM) by the addition of Zn^{2+} (○), Cu^{2+} (□), Co^{2+} (◇) and Ni^{2+} (△) ions.

amount of KCN (100 equiv.), as a masking reagent, prevented the coordination of His-2α to Zn^{2+}, resulting in a decrease of the α-helicity to the level observed without $ZnCl_2$. Additionally, the α-helix enhancement by the addition of $ZnCl_2$ was highly dependent on the solution pH (Figure 3). There was no significant difference in the CD spectra between the presence and absence of Zn^{2+} at acidic pH (2.0-5.0). The midpoint of the α-helix improvement was observed at pH 6.2. Because the pK_a of imidazole is *ca.* 6.0, the pH effect is attributed to the protonation of His side chains such that they cannot act as a ligand. In contrast to His-2α, α-helix contents of the monomer peptide, His-1α and non-His peptide, Leu-2α, were little affected by the addition of Zn^{2+} at any percentage of TFE (0-90%). Therefore, we concluded that the increase in α-helicity of His-2α took place *via* the Zn^{2+} binding by ligation with two His residues in the 2α-helix structure.

When His-2α was titrated with $ZnCl_2$ 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 (Figure 4). The binding constant (K_a) in 15% TFE, which was estimated from molecular ellipticities at 222 nm using a single site binding equation,[6] was 1.7 x 10^5 M^{-1}. Titration of His-2α by other divalent transition metal ions was also carried out in the buffer containing 15% TFE, in order to examine a metal selectivity of the peptide according to the size and/or preferred coordination numbers. The addition of $CuCl_2$ also increased the α-helicity of the peptide with a binding constant similar to that of Zn^{2+} (K_a=1.4 x 10^5 M^{-1}). However, the α-helix improvement effect of Cu^{2+} was smaller than that of Zn^{2+}. The smaller radius of Zn^{2+} (about 0.74 Å) *vs* that of Cu^{2+} (about 0.96 Å) might be favorable for the binding by the peptide and consequent α-helix induction. In contrast to the results obtained with $ZnCl_2$ and $CuCl_2$, addition of $NiCl_2$ or $CoCl_2$ caused little increase of the α-helicity of the peptide. In general, Ni^{2+} and Co^{2+} are known to prefer an octahedral coordination due to ligand field stabilization. On the other hand, Cu^{2+} and Zn^{2+} tend to take a tetrahedral geometry rather than other geometries. Because a computer modeling study showed that Glu, Gln, or Lys residue in the peptide was too apart from the metal-binding site to act as additional ligands, nonligated metal coordination site are likely occupied by water molecules. Thus, the peptide must accommodate four water molecules in the hydrophobic metal-binding site, when the peptide binds a metal with a six-coordinate form. It can be considered that the permeation of the water molecules into the hydrophobic region is a disadvantage for the effective metal binding and consequent α-helix improvement of the peptide. The difference in the size, electronic configuration in the 3d orbitals, and preferred coordination numbers among the metal ions are possibly responsible for their different effects on the α-helix improvement.

In conclusion, the His-peptide bound Zn^{2+} and Cu^{2+} in 10-30% TFE/buffer solution, and the metal binding selectively enhanced the α-helix structure. The selectivity in α-helix enhancement by the special ion, such as Zn^{2+} or Cu^{2+}, will be applicable to metal ion sensors.[3] In addition, since metal ions have catalytic properties, a metal-binding peptide could be used for the template of a synthetic enzyme. Although detailed examination of metal binding and further increment of metal ion selectivity will be needed, the strategy using designed peptides conjugated with functional groups, such as transition metal ion or heme, is expected to be applied to the elucidation of a role of polypeptide 3D structure on diverse functions of natural proteins, and the obtained information will be useful for designing artificial proteins.

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