CHEMISTRY LETTERS, pp. 1809-1812, 1992.

Probing Behavior of 1-Pyrenylalanine for Interaction of Two α-Helices Anchored on a Bipyridyl Group

Hisakazu MIHARA, Norikazu NISHINO,* and Tsutomu FUJIMOTO
Department of Applied Chemistry, Faculty of Engineering, Kyushu Institute of Technology, Tobata, Kitakyushu 804

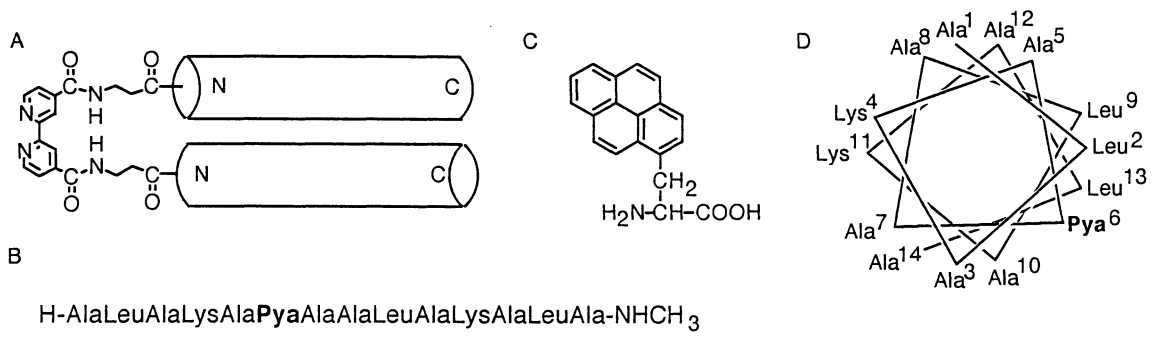
Two strands of an amphiphilic α-helical 14-peptide were anchored in parallel on a bipyridyl group to construct a helix-helix structure. The interaction of two α-helices in various circumstances was probed by behavior of 1-pyrenylalanine residues in circular dichroism and fluorescence spectra.

A number of trials have been reported on the *de novo* design of model peptides which mimic the protein structure and function.[1] The construction of three-dimensional structures was achieved by the use of various tricks in assembling peptide segments. Especially, attentions are focused on the design of α-helix bundle structures.[2] The secondary structure such as α-helix in the constructed three-dimensional structures, however, is evaluated only by the circular dichroism (CD) measurement at the amide region. Recently, we reported the convenient use of 1-pyrenylalanine (Pya)[3,4] as a probe for the detection of the interaction between α-helix segments in a four α-helix bundle polypeptide.[5] The pyrenyl group showed great advantages based on the strong excimer formation and sharp induced CD at different absorption bands from the amide region. This probe is thus expected to give useful information for the intersegmental interaction in super-secondary structures.

In order to elucidate the behavior of the pyrene probe in the detection of the helix-helix interaction, we attempted to construct a parallel helix-helix super-secondary structure (Fig. 1A) employing a bipyridyl group as an anchor of two strands of an amphiphilic α-helical[6] 14-peptide (Fig. 1B). The bipyridyl group is rigid, but a good candidate as a loop model with appropriate hydrophilicity. Furthermore this group can be derived to the functional group by complexation as ruthenium trisbipyridine[7] in the future use. The fluorescent amino acid, Pya, was introduced near the center of the model 14-peptide (Fig. 1B and C).

The model 14-peptide[8] was designed to take an amphiphilic α-helical conformation (Fig. 1D) and synthesized by fragment couplings on a *p*-nitrobenzophenone oxime resin.[9] Two 14-peptides were condensed with β-Ala moieties on 2,2'-bipyridyl-4,4'-dicarboxylic acid by using benzotriazole-1-yl-oxy-tris(dimethyl-amino)phosphonium hexafluorophosphate (BOP).[10] The obtained bipyridyl hybrid peptide was deprotected with trimethylsilyl trifluoromethane sulfonate[11] and the crude peptide was purified by C4 reversed-phase HPLC to give the homogeneous product.[12] Details of the synthesis will be reported elsewhere.

The CD spectra of the hybrid peptide **1** in H_2O were shown in Fig. 2.[13] It gave a typical α-helical CD profile ($[\theta]_{222}$=-22000 deg·cm^2·dmol^{-1}) which corresponds to 75% α-helicity.[14] The 14-peptide itself did not take α-helical structure in H_2O but random one ($[\theta]_{222}$=-5200 deg·cm^2·dmol^{-1}) as same as the previously reported parent 14-peptide.[8] These facts indicate that the peptide **1** is stabilized in α-helical conformation in H_2O by hydrophobic interaction between the peptide segments. Furthermore, the peptide **1** in H_2O showed CD



H-AlaLeuAlaLysAla**Pya**AlaAlaLeuAlaLysAlaLeuAla-NHCH$_3$

Fig. 1. (A) Structure of the bipyridyl-hybrid peptide **1**. Cylinders represent α-helix segments. (B) Amino acid sequence of the amphiphilic model 14-peptide. (C) Structure of Pya as a probe. (D) An amphiphilic α-helical characteristic of the 14-peptide represented by the α-helical wheel drawing.

spectrum with extraordinary strong splits at both ^{1}Bb and ^{1}La bands of pyrene group (molar ellipticity (deg·cm^2·dmol^{-1}): 7.0 x 10^5 at 280 nm and 1.3 x 10^5 at 250 nm) (Fig. 2). Both splits consisted of a pair of positive (280 and 350 nm) and negative peaks (277 and 343 nm, respectively). This CD pattern indicates that two pyrene rings are sterically fixed in close proximity between the two α-helices and arranged in right-handed sense according to the exciton chirality principle.[15] Ellipticity of these splits decreased by the addition of MeOH, while the α-helicity was not changed by varying MeOH contents (Fig. 3A). The role of MeOH is to loosen the hydrophobic interaction between two α-helices. These results suggest that in H_2O, 6 Leu and 2 Pya in two segments are gathered up and deployed on the α-helical rods in the same manner as a part in coiled-coil peptides, namely as Leu-zipper mode,[16] because the 14-peptide sequence fulfills the terms for this mode (Fig. 4). Since the helix-helix interaction is in right-handed sense in coiled-coil peptides, two pyrene rings should be arranged in the same sense. This arrangement is consistent with the splitting pattern in CD of pyrene rings (Fig. 2). It should be noted that the bipyridyl hybrid with two α-helices in antiparallel anchoring did not show such a splitted CD (data not shown).

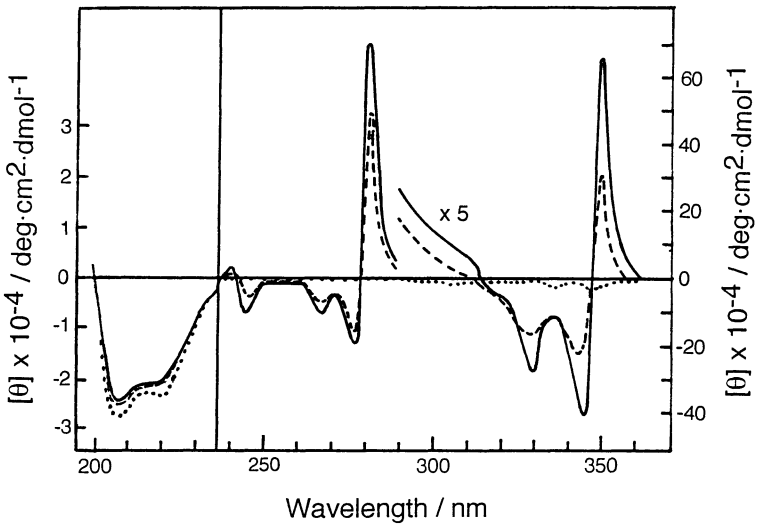


Fig. 2. CD spectrum of the bipyridyl-hybrid peptide in H_2O (——), 25% MeOH (– – –), and MeOH (••••). [Peptide] = 3.0 x 10^{-5} mol·dm^{-3}.

In fluorescence spectrum the aqueous solution of **1** gave faint pyrene-excimer emission. On the contrary, the addition of MeOH to certain contents (40%) generated the excimer emission, where the CD split almost disappeared. Ellipticity at 350 nm and the ratio of excimer to monomer emission of pyrene of **1** were plotted as a function of MeOH content (Fig. 3B). Obviously, the increase in the excimer formation was followed by the decrease in the CD split (especially at 20-40% MeOH content). These findings on the pyrene CD and

fluorescence spectra could present the presumed conclusion as follows. In H_2O, two α-helical segments were kept tightly in contact with each other stabilized by the Leu-zipper like interaction (Fig. 4). The tight packing of two α-helices probably forced pyrene rings to fix in different orientation, which allowed the sharp splits in CD and simultaneously prohibited the stacking of pyrene rings for excimer formation. On the other hand, the addition of MeOH loosened the helix-helix packing, then two pyrene rings acquired the opportunity to form the excimer. In these circumstances, possibly considered is the presence of molten-globule-like metastable conformer,[17] in which the specific organization of side chains is lost. Further addition of MeOH (>60%) decreased the excimer emission as a result of the complete melt of the helix-helix packing.

Thus, the Pya in different peptide chains was demonstrated as a useful probe to evaluate the intersegmental interaction in a designed polypeptide. It behaved specifically depending on various modes of interaction of the two α-helices. The development of this probing method in combination with the hybridization approach would be a step advance to design artificial proteins.

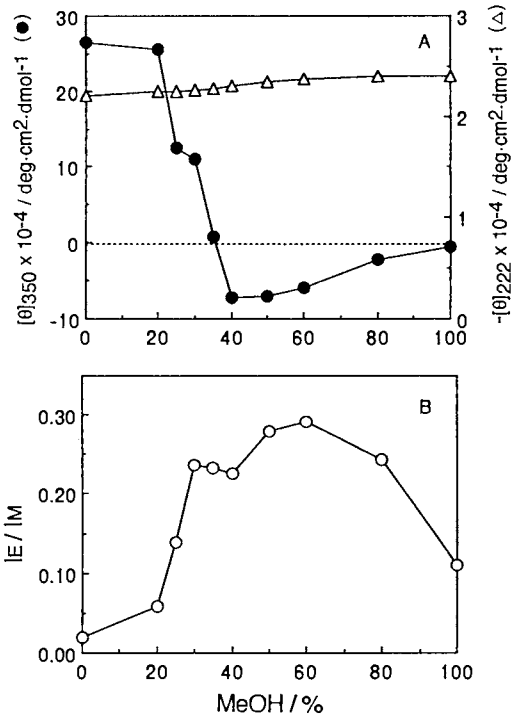


Fig. 3. Plots of the CD split and α-helicity (A), and the pyrene-excimer formation (B) as a function of MeOH content. (A) $[\theta]_{350}$ and $[\theta]_{222}$ are molar and mean residue ellipticity of the peptide at 350 nm and 222 nm, respectively. Over 40% MeOH content, a negative peak without split was observed at 345 nm. [Peptide] = 3.0 x 10^{-5} mol·dm^{-3}. (B) I_M and I_E mean fluorescence intensities at 395 nm and 480 nm, respectively, excited at 342 nm. [Peptide] = 1.0 x 10^{-6} mol·dm^{-3}.

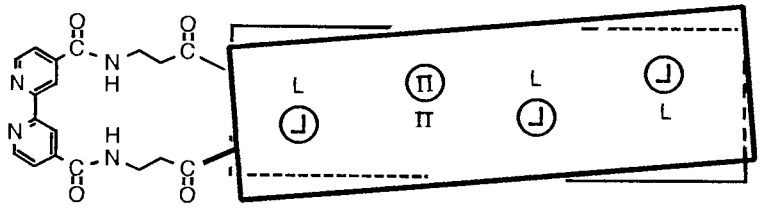


Fig. 4. Illustration of the Leu-zipper like interaction of two helices. Π denotes the Pya residue. The circled Π and reversed sign of L correspond Pya and Leu residues on the opposite side of the front helix, and other residues are on the back helix. Two helical rods may be arranged with twisting angle of about 18 degree.[16a] Two helices of peptides in coiled-coil or Leu-zipper structure wrap around each other to form a left-handed supercoil. The arrangement of these helices is in right-handed sense. Also, in these peptides, hydrophobic residues such as Leu are placed at a and d residues in a heptad repeat $(abcdefg)_n$.[16b]

We thank Professors K. Ohkubo and H. Ishida, Kumamoto University, for generous gift of 2,2'-bipyridyl-4,4'-dicarboxylic acid. This work was funded in part by a Grant-in-Aid for Scientific Research on Priority Area from the Ministry of Education, Science and Culture, Japan.

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12) The retention time of **1** was 21 min on a YMC C4 column (1.0 cm x 25 cm) with a linear gradient of 37-100% CH_3CN/0.1% CF_3COOH over 30 min; flow rate, 3.0 ml/min; detection, 340 nm. Absorption spectrum in MeOH, ε (λmax, nm): 34800 (342), 23300 (326), 12700 (312), 10000 (299), 43300 (276), 74600 (242), and 61700 (233). Molecular weight was estimated as 4000 by gel-filtration on a Sephadex G-50 column (2.0 cm x 85 cm).

13) The CD profile in H_2O was the same as that in 2.0 x 10^{-2} mol·dm^{-3} Tris·HCl buffer, pH 7.4.

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(Received June 15, 1992)