Super-Secondary Structure with Amphiphilic β-Strands Probed by Pyrenylalanine

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(Received July 24, 1995)

A peptide composed of two amphiphilic β-strands was designed and synthesized. The circular dichroism and fluorescence spectra of L-1-pyrenylalanine introduced in each segment probed that a super-secondary structure with two β-strands was formed with a left-handed twist and transformed to α-helices by the addition of trifluoroethanol.

The β-structure is an important secondary structure of proteins. Some models for β-structure rich proteins such as all β-protein betabellin, and mixed α/β proteins octarellin and albebetin have been designed and prepared by using chemical and biological methods.[1] Their conformational properties are usually investigated by circular dichroism (CD) measurements. The NMR and crystallographic characterizations, however, are not accomplished satisfactorily due to the various obstructions like solubility. The spectroscopic methods, such as CD, IR, and NMR, give some information on the presence and content of β-sheet structure but not regarding the mode of interaction between β-strands and the tertiary structure. Therefore, a convenient analytical principle proving the interaction between β-strands is required in the further *de novo* design of β-structural polypeptides

Recently, Mihara *et al.* reported the employment of a pair of L-1-pyrenylalanine (Pya) residues as CD and fluorescent probes for the detection of super-secondary structure composed of two α-helices.[2] When the peptide segments adopted a 2α-helix conformation, strong splits and pyrene-excimer emission were observed in the CD and fluorescence spectra, respectively. In particular, it was clear from the CD pattern that two α-helix segments were arranged in a right-handed sense as seen in leucine-zipper-like coiled-coil peptides. In the present study, we applied the Pya probe to characterize a super-secondary structure consisting of two amphiphilic β-strands. Two amphiphilic β-strands were connected to bipyridine dicarboxylate, the non-peptide moiety (Figure 1).[2] The amino acid sequence was designed to have alternating hydrophobic and hydrophilic amino acid residues. Such sequences have been found to form amphiphilic β-structures.[1,3] In addition to Leu and Lys as hydrophobic and hydrophilic amino acids, Ala residues were employed at the hydrophilic face. The fluorescent and hydrophobic amino acid, Pya, was introduced at the 5th position from *N*-terminus of the 12-peptide instead of the Leu residue. The 12-peptide was combined to the anchoring group with a spacer of β-Ala to make a parallel two-β-strand peptide (**24B**). A single chain peptide **12S** was also synthesized to compare the conformational properties with **24B** for the effect of dimerization.

The 12-peptide segment was synthesized by the segment condensation between protected Boc-6-peptide-OH and H-6-peptide-$NHCH_3$.[4] A pair of 12-peptides were condensed with β-Ala moieties on 2,2'-bipyridyl-4,4'-dicarboxylic acid by using benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexa-fluorophosphate (BOP).[2] The obtained bipyridyl peptide hybrid was deprotected with trimethylsilyl trifluoromethanesulfonate[5] and the crude product was purified by C4 reversed-phase HPLC to give the peptide **24B** in pure form.[4]

CD spectra of the peptide hybrid **24B** were measured in 2.0 x 10^{-2} mol dm^{-3} TrisHCl buffer (pH 7.4) containing various

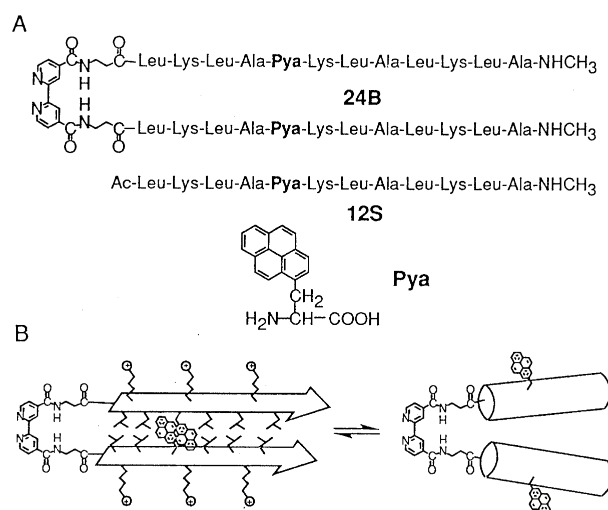

Figure 1. (A) Structure of the designed polypeptide with 2β-strands (**24B**) and the monomer peptide (**12S**). The structure of 1-pyrenylalanine (Pya) is also shown. (B) Illustration of the amphiphilic folding of 2β-strands and the transition between β-strands and α-helices.

contents of trifluoroethanol (TFE) (Figure 2). The peptide hybrid **24B** in 5% TFE showed a typical β-structural CD profile ($[\theta]_{215}$ = -19000 deg dm^2 mol^{-1}) at the amide absorption region, indicating an almost complete β-structure conformation,[6] whereas a typical α-helical one in 60% TFE ($[\theta]_{222}$ = −29000 deg dm^2 mol^{-1} α-helicity 90%). With increasing TFE content from 5% to 100%, a single minimum at 215 nm characteristic of the β-structure gradually disappeared. Instead of the incident, double minima at 208 and 222 nm characteristic of the α-helix appeared at 30% TFE and the α-helicity increased up to 90% at 60% TFE (Figure 2B). On the other hand, in the region of pyrene absorption, the peptide hybrid **24B** in 5% TFE showed a CD spectrum with two splits at 343 (^1L_a band) and 278 nm (^1B_b band). These splits consisted of positive peaks at lower wavelengths (272 and 339 nm) and negative peaks at longer wavelengths (283 and 349 nm), respectively. This CD pattern strongly suggests that the two pyrene rings are sterically fixed in close proximity between the two β-strands and arranged in a left-handed sense according to the exciton chirality principle.[7] No split pattern was observed for **24B** in 60% TFE, indicating that the two pyrene rings on the α-helical segments were separated far apart from each other and free to move preventing the exciton-exciton interaction. The split CD was decreased with increasing TFE content parallel to the deformation of the β-structure (Figure 2B). The CD patterns of single peptide **12S** in 5% and 60% TFE were similar to those of **24B** at both amide and pyrene absorption regions. However, the ellipticities of two splits for **24B** ($\Delta[\theta]_{278}$ = 30000 and $\Delta[\theta]_{343}$ = 28000 deg dm^2 mol^{-1}) in 5% TFE at the pyrene absorption region were greater than those for **12S** ($\Delta[\theta]_{278}$ = 27000 and $\Delta[\theta]_{343}$ = 20000 deg dm^2 mol^{-1}).

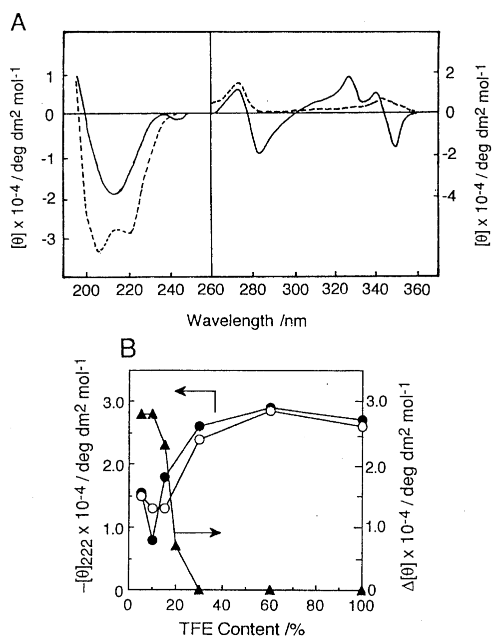

Figure 2. (A) CD spectra of the peptide **24B** in the buffer solution containing 5% TFE (——) and 60% TFE (– – –); [Peptide] = 1.0 x 10^{-5} mol dm^{-3}; 25 °C. Ellipticity at the amide region represents mean residue one (24 residues) and at the pyrene adsorption region is per peptide. (B) Dependences of CD spectra on the TFE content. $[\theta]_{222}$ denotes the ellipticities at 222 nm representing α-helix conformation of **24B** (●) and **12S** (○). $\Delta[\theta]$ denotes the difference of ellipticities of **24B** between the positive peak (339 nm) and the negative peak (349 nm) of the split CD at 343 nm (▲).

This comparison suggests that two pyrene groups in the 2β-strands bridged by the bipyridine moiety were better fixed in the super-secondary structure than those in the monomer.

In the fluorescence measurements of **24B** in the same buffer solution containing 5% TFE, a significant pyrene excimer emission was observed (Figure 3). However, the excimer emission almost disappeared in TFE, in which the peptide attained the α-helical structure. The single peptide **12S** in the buffer solution also showed the excimer emission, but its intensity was rather low (Figure 3 inset). The ratio of the intensity of the excimer emission at 460 nm (I_E) to the monomer emission at 400 nm (I_M) for **24B** and **12S** was decreased by the addition of TFE. The I_E/I_M value of **24B** gradually decreased with increasing TFE content, whereas that of **12S** disappeared under a lower TFE content (ca. 20% TFE). The excimer emission directly demonstrated that the β-strands in **24B** were close to each other in aqueous solution (Figure 1B). The pyrene-pyrene interaction was gradually canceled by the addition of TFE, because the hydrophobic aggregation of the two segments untied by the effects of TFE. In this condition, the peptide segments formed the α-helix structure, because the amino acids contained in the sequence, Ala, Leu and Lys, prefer to take an α-helix structure. On the other hand, the monomer peptide **12S** seemed to be aggregated intermolecularly in aqueous solution as evident from the weak excimer formation. However, the aggregation was not tight and canceled with a much lower content of TFE.

The TFE-dependent deformation of the excimer and the disappearance of the CD exciton interaction suggested that the hydrophobic interaction of the peptide segments was responsible for the stable β-structural formation of the peptide **24B**. This suggestion was supported by the CD measurements in the presence of guanidine hydrochloride (GuHCl). In the aqueous solution of **24B** containing GuHCl (0–7.3 mol dm^{-3}), no

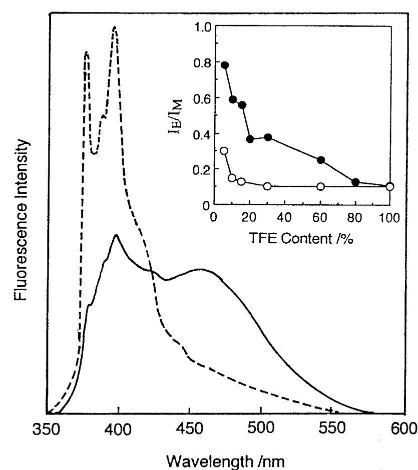

Figure 3. Fluorescence spectra of the peptide **24B** in the buffer solution containing 5% TFE (——) and TFE (– – –); [Peptide] = 1.0 x 10^{-5} mol dm^{-3}; λex 345 nm; 25 °C. Dependences of the ratio I_E/I_M of **24B** (●) and **12S** (○) on TFE content are shown in the inset.

significant difference in CD at 215 nm was observed, suggesting that the intersegmental hydrogen bonds were not significant for the stabilization of the β-structure. Therefore, it is considered that the hydrophobic side chains in the two segments face to each other by forming the β-structure in aqueous solution (Figure 1B). Similar β-structure formation stabilized by hydrophobic interaction, so called the β-zipper was found as a proposed folding nucleation site in Greek key β-barrel proteins.[8] Moreover, the orientation of the β-strands with a left-handed sense suggested by the CD measurements is comparable to the segment orientation of β-sheets in natural proteins. The method of utilizing a pair of pyrenes was useful to elucidate the intersegmental interaction in polypeptides with not only α-helices[2] but also β-strands. The β⇄α transition was also figured by the spectral observations under various conditions. Though the β⇄α transition by solvents and pH was observed with designed single-chain amphiphilic peptides by Mutter *et al.*,[9] the conformational conversion in a three-dimensional structure with the β-zipper character was, for the first time, elucidated in this study. Furthermore, the conformational transition of the designed polypeptides provides useful information for designing "molecular machines" with artificial proteins.

References and Notes

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4 All the protected peptides (6- and 12-peptides, N^{α}, *t*-butyloxycarbonyl (Boc); Lys, 2-chlorobenzyloxycarbonyl) gave satisfactory results on the elemental analyses (within error ranges of +/- 0.3%). The purified peptides gave good results on amino acid analyses including β-Ala (+/- 5%). λmax(TFE)/nm 342 (ε/dm^3 mol^{-1} cm^{-1} 38200), 324 (28000), 311(21000), 274(47300), 264(36500), 241(83200), 232(72900) for **12S**; λmax(TFE)/nm 342(76400), 324(56500), 311(37100), 274(99900), 264(67800), 241(177800), 232(150000) for **24B**.
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