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Four α-Helix Bundle Structure Built with Alternating D- and L-Segments

Yuji Tanaka, Koichi Nagamoto, Hiroshi Sasaki, Tsutomu Fujimoto, and Norikazu Nishino*

Department of Applied Chemistry, Faculty of Engineering, Kyushu Institute of Technology, Tobata, Kitakyushu 804, Japan

Masahito Oka

Research Institute for Advanced Science and Technology, University of Osaka Prefecture, Gakuen-cho, Sakai, Osaka 593, Japan

Abstract: *The 57-peptide for four α-helix bundle structure with alternating D-, L-, D-, and L-segments was synthesized, characterized, and compared with that of all L-segment-57-peptide by the aid of a 1-pyrenylalanine probe.*

The recent successes in construction of artificial proteins established some principles in *de novo* design[1], such as stabilization of the α-helix conformation and application of the amphiphilic α-helix motif[2] to the α-helix bundle structure. However, the artificial proteins are regarded as molten globules, and to acquire native-protein-like firm packing of several segments, development of a more sophisticated design is needed. Among such several efforts including coordination with metal ions[3], salt bridge formation[4], and incorporation of porphyrin derivative[5], we envisioned that compact packing could be achieved by the employment of a D-amino acid segment in combination with the usual L-amino acid segment. The preliminary conformational energy optimization was carried out by using the energy function of ECEPP[6] on a model peptide with alternating D- and L-segments.[7] The lowest-energy conformation of four α-helix bundle structure with alternating D- and L-segments (Fig. 1A) appeared more compact than the all L-segments peptide (Fig. 1B). Particularly, there is great difference between them in the twisting mode of each segment. Therefore, we attempted the synthesis and characterization of such totally unusual protein model.

For the convenient evaluation of four α-helix bundle structure of polypeptides, we have demonstrated the usefulness of 1-pyrenylalanine (Pya) as circular dichroism (CD) and fluorescent probe.[8] Thus, for the actual synthesis, the 57-peptides (57-DLDL and 57-LLLL) were designed as shown in Fig. 2. The former 53-peptides were improved at the turning part by adding Gly residue to make -D-Ala-Pro-Gly- sequence. Thus, the 11-peptide segments to be assembled into 57-peptides were connected with D-Ala-Pro-Gly or Ala-D-Pro-Gly as β-turn moieties (Fig. 2). The D- and L-pyrenylalanines (π and Π, respectively) are introduced at the first and third segments to evaluate the formation of four α-helix bundle structure by CD and fluorescence measurements.

The synthesis of 57-peptides was carried out by solid-phase-synthesis using Kaiser's oxime resin and segment condensation in solution.[8b,9] The intermediates of the protected peptides were purified with the Sephadex LH-60 column (DMF). The protecting groups of 57-peptides were removed with anhydrous HF and the free peptides were purified by Sephadex G-50 (40% acetic acid). TOF-MS (Shimadzu MALDI II)

A. Ac-a15-G3-A15-G3-a15-G3-A15-NHCH3

B. Ac-A15-G3-A15-G3-A15-G3-A15-NHCH3

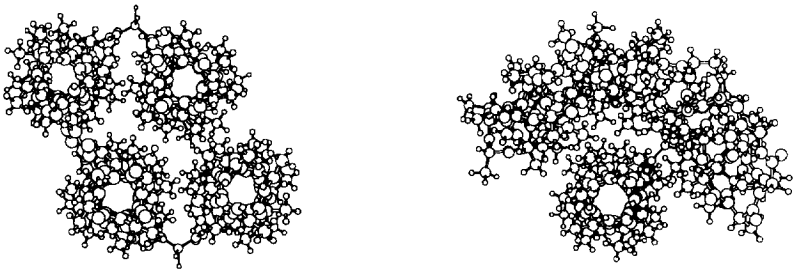
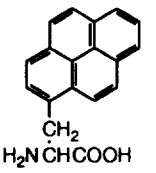


Fig. 1. Lowest-energy conformations of four α-helix bundle polypeptides (top view) with alternating D- and L-segments (A) and with all L-segments (B). The a and A denote D- and L-alanine residues, respectively.

A. ApGellkaπaellk-
aPGELLKAFAELLK-
ApGellkaπaellk-
aPGELLKAFAELLKG

B. aPGELLKAΠAELLK-
aPGELLKAFAELLK-
aPGELLKAΠAELLK-
aPGELLKAFAELLKG

C.



D.

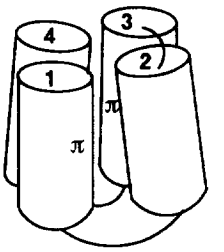


Fig. 2. Design of single-chained four α-helix bundle polypeptides. A, B. Amino acid sequences of 57-DLDL and 57-LLLL, respectively. Capital and small letters denote L- and D-amino acid residues, respectively, except glycine. C. D- or L-1-pyrenylalanine (π, Π) for CD and fluorescent probe. D. Illustration of four α-helix bundle conformation of 57-peptides. Cylinder and loop represent α-helix and β-turn, respectively.

gave a broad peak around 6250 (M+H=6251) probably due to too many Leu residues in the peptides.

The 57-DLDL in aqueous solution showed no CD at the far UV region, because the right-handed and left-handed α-helical segments may cancel each other's CD. The 57-LLLL showed typical α-helical CD pattern. Both 57-peptides showed induced CD derived from the amino acid configuration at the pyrene absorption region (Fig. 3A). Especially, split CD patterns were observed at 350 nm (^{1}La band region) in H_2O/TFE (96/4, v/v). These split CD disappeared on the addition of MeOH. Generally, an amphiphilic folding of 4α-helix bundle structure is effected by the solvent environment. With increase of MeOH content, the intensities of split CD ($\Delta[\theta]=[\theta]_{355}-[\theta]_{345}$) of 57-peptides increased to the maximum $\Delta[\theta]$ (20% and 60% for 57-DLDL and 57-LLLL, respectively) (Fig. 3B), then diminished finally. The ellipticities of 57-LLLL at the amide region showed no changes on the addition of MeOH ($[\theta]_{222}$ = -26,000 deg·cm^2·dmol^{-1}) as observed previously for 53-peptide.

Fluorescence spectra of both 57-peptides showed strong excimer emission at 470 nm in H_2O (Fig. 4A). These results indicate that two pyrene rings in polypeptides are in close proximity by forming four α-helix bundle structure in aqueous condition. Excimer emissions disappeared on the addition of MeOH. The ratio of fluorescence intensities at 470 nm (I_E) to that at 400 nm (I_M) were plotted against MeOH contents (Fig. 4B). For 57-DLDL, the ratio gradually decreased with increasing MeOH content over 30%, while 57-LLLL showed increase in I_E/I_M up to 50% MeOH, then decrease with higher MeOH content. Obviously, the

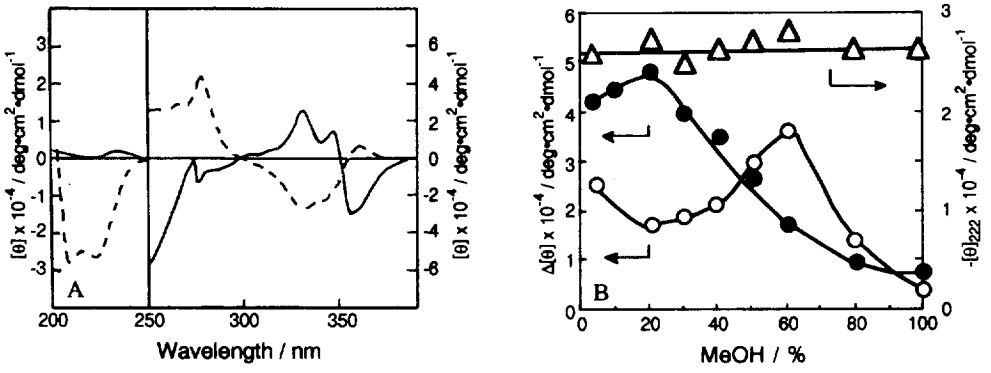


Fig. 3. CD spectra of 57-peptides. A. 57-DLDL (——) and 57-LLLL (---)in H_2O/TFE (96/4, v/v). B. Effect of MeOH to the ellipticities at ^1La band region of 57-DLDL (●), 57-LLLL (○) and at 222 nm of 57-LLLL (△). [Peptide] = 3.0 x 10^{-5} M.

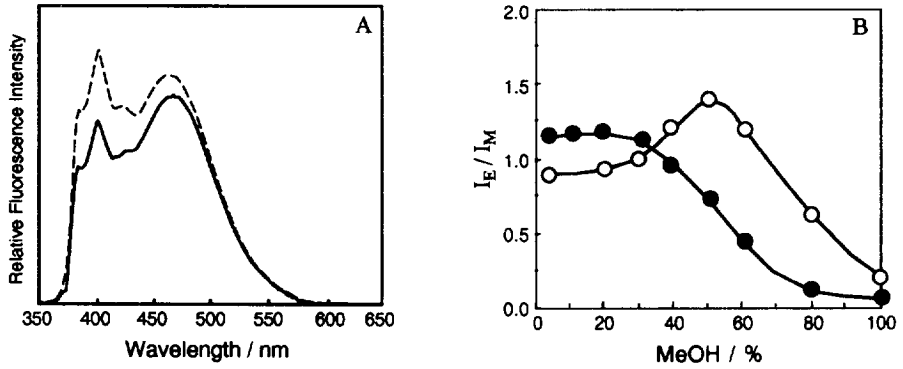


Fig. 4. Fluorescence spectra of 57-peptides. A. 57-DLDL (——) and 57-LLLL (---) in H_2O/TFE (96/4, v/v). B. Changes on the I_E/I_M of 57-DLDL (●) and 57-LLLL (○) by the addition of MeOH. I_E/I_M denotes the ratio of fluorescence intensities at 470 nm to 400 nm. [Peptide] = 3.0 x 10^{-5} M. Excited at 345 nm.

bundle structure of 57-DLDL can be melted by MeOH more easily than that of 57-LLLL.[8a] The hydrophobic packing of α-helices in 57-DLDL seems to be loosened with less MeOH content than that of 57-LLLL probably due to the compact packing without twisting as shown in Fig. 1A.

Denaturation experiment with guanidine hydrochloride (Gu·HCl) was also performed to investigate the difference in the stabilities of 57-peptides (Fig. 5). The process was followed by fluorescence spectra. The 57-DLDL kept the I_E/I_M at 3.3 up to 3 M of Gu·HCl, then the ratio gradually decreased on further addition of Gu·HCl. The concentration of the transition midpoint of 57-DLDL was 5.5 M. On the other hand, the I_E/I_M of 57-LLLL made a slow descent on the addition of Gu·HCl suggesting that unfolding of 57-LLLL was less cooperative than that of 57-DLDL. Since protein folding generally depends on temperature, the thermal stability of the four α-helix bundle conformation was examined by the CD measurements. The CD spectra at the ^1La band region were compared between 57-DLDL and 57-LLLL at varying temperatures. The Δ[θ] of 57-LLLL disappeared at 80°C while that of 57-DLDL still remained. These results again demonstrate that the

folding of the bundle structure of 57-DLDL is more stable than that of 57-LLLL. The Gu·HCl and heat destabilize the hydrogen bonding in the α-helices. In 57-LLLL, the hydrophobic interaction among the twisting α-helices (Fig. 1B) may provide some tension to each helical rod and make it sensitive to the denaturant and heat. Therefore, it is likely that the packing of α-helices in 57-DLDL is more resistant against the hydrogen bond breakers than the twisting packing in 57-LLLL.

In conclusion, a new four α-helix bundle polypeptide built with alternating D- and L-segments was for the first time synthesized and characterized by a pair of 1-pyrenylalanines. The helix-helix interaction in the bundle structure was distinctly different between 57-DLDL and 57-LLLL as predicted by energy calculation. In the design of artificial functional proteins, the D-segments will be useful parts in the arrangement of the functional groups.

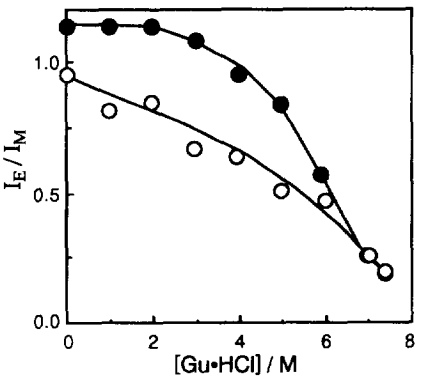


Fig. 5. Guanidine denaturation curves of 57-DLDL (●) and 57-LLLL (○). [Peptide] = 1.0×10^{-5} M. Excited at 345 nm.

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