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Dansyl and Indolyl Groups as a Probing Pair for Intersegmental Arrangement in Four α-Helix Bundle Structure of a Polypeptide

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Dansylaminoalanine and tryptophan were separately incorporated into the α-helical segments of the four α-helix bundle structure of a 53-peptide. The perfect transfer of fluorescence energy of the indolyl group to the dansyl moiety was observed in aqueous solution by the emission at 480 nm upon excitation at 280 nm. The addition of MeOH unfolded the bundle structure and resulted in the interruption of the efficient energy transfer.

A number of polypeptides with α-helix bundle structure have been designed.[1] Such bundle structure is considered as a model of functional artificial proteins. In so-called *de novo* design of polypeptides, the amphiphilic α-helical motif[2] is commonly employed to build up the stable secondary structure of a main chain. However, the arrangement of side chains on the α-helical segments is merely expected and at most estimated by computer-graphic drawing.[1a,d] The multi-dimensional NMR measurement has not given the clear conclusion on the side chain arrangement in the designed polypeptides yet.[3] Furthermore, even the incorporation of some functional groups as side chains of artificial amino acids has not been attempted so far.

The artificial protein by *de novo* design will acquire some function only when particular amino acids are successfully incorporated under three-dimensional arrangement. Though the mode of arrangement between segments (intersegmental arrangement) should be clarified by X-ray crystallography and NMR techniques, a more convenient procedure is awaited to estimate the conformation by spectrophotometric measurements. As an example, in the search for a fluorescent probe for the intersegmental arrangement in the 4α-bundle structure of a 53-peptide, we proposed the utilization of excimer formation of pyrene rings on different α-helical segments.[4] However, four pyrene rings seemed too bulky in the narrow hydrophobic core in the bundle structure, when it was compared with other aromatic amino acids such as Phe and Trp. In the present study, therefore, we attempted the incorporation of dansylaminoalanine (Dsa) and Trp,[5] as a paired probe for the bundle structure by detection of energy migration from the indolyl group to the dansyl moiety.[6]

The 53-peptide was designed as shown in Fig. 1. Four 13-peptide segments were connected in series starting from Gly. The D-Ala-Pro sequence and remaining 11-peptide in each segment were designed to undertake the tasks to form type II' β-turn and the amphiphilic α-helix with about 3 spiral spins, respectively. In the center of the 11-peptide sequence of the first and third segments were placed Dsa and Trp, respectively (X and Y in Fig. 1). Those α-helical segments may be organized in parallel orientation in the 4α-bundle

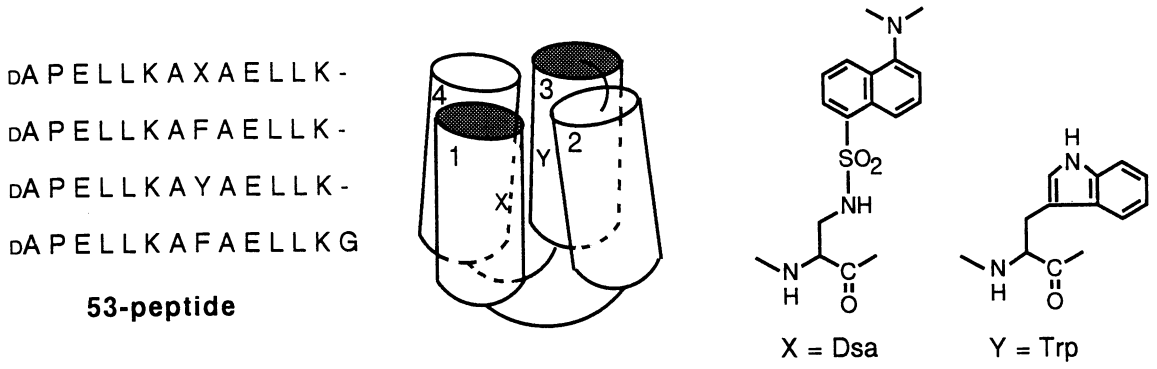

Fig. 1. Design of the 4α-bundle 53-peptide containing dansylaminoalanine (Dsa, X) and Trp (Y) in the first and third segments, respectively. A, Ala; DA, D-Ala; E, Glu; K, Lys; L, Leu; P, Pro.

structure (Fig. 1). Thus, two fluorophores may also be arranged in close proximity in hydrophobic inside of the bundle structure.

Dsa was prepared from dansyl chloride and *t*-butyloxycarbonyl-L-2,3-diaminopropionic acid [Dsa: mp 100-102 °C; $[\alpha]_D^{25}$ +5.7° (*c*1, MeOH); FAB-MS m/z 437 (M^+)]. The synthesis of the 53-peptide was carried out by the similar manner as described in the previous report.[4,7] The 13-peptide and the 40-peptide containing Dsa and Trp, respectively, were also prepared as reference compounds to the 53-peptide in the fluorescent behaviors.

The 53-peptide showed a typical α-helix pattern in CD spectrum at 3.0 x 10^{-5} M (1 M = 1 mol dm^{-3}) in Tris·HCl buffer (20 mM, pH 7.4) at 25 °C. From the ellipticity at 222 nm ($[\theta]_{222}$ = -19000 deg cm^2 $dmol^{-1}$), the α-helicity was estimated as 60%,[8] which suggests that about 32 residues in 53 or 8 in 13 are involved in the α-helix conformation. The α-helical segments may gather together with hydrophobic side chains inside of the bundle, resulting in stabilization of the three-dimensional structure. In MeOH, the α-helicity of 53-peptide was increased to 82%. This fact should be attributed to the stabilization of the main chain in the α-helix structure but not to the mutual organization of α-helices into any particular arrangement.

Figure 2 shows the fluorescence spectra of the 53-peptide in aqueous and MeOH solutions at 25 °C. The excitation at 280 nm afforded no emission at 350 nm but at 480 nm in aqueous solution. The fluorescence energy of Trp was perfectly transferred to the dansyl group (>98% efficiency).[6a] This fact evidences that two fluorophores exist in very close proximity in the 4α-bundle structure as expected in the illustration in Fig. 1. In contrast, the addition of MeOH gradually increased the fluorescence intensity of Trp at 347 nm with the decrease of the dansyl emissions shown in Fig. 3A. The emission of the Trp fluorescence can be responsible for the separation of the dansyl group from Trp as illustrated in Fig. 3B.

It should be noted that the maximum wavelength of the dansyl emission shifted from 480 nm to 510 nm by the addition of MeOH (from 0 to 100%). The red shift of the fluorescence band also demonstrates that the dansyl group is exposed to the hydrophilic circumstances. Thus, it is considered that MeOH unfolded the bundle conformation by sneaking into the hydrophobic inside. This melting of the bundle structure results in the exposure of dansyl group to more polar solvent than the hydrophobic circumstances formed with Leu and Phe residues. When an equimolar amount of the 13-peptide containing Dsa was added to the 40-peptide consisting of three α-helix segments probably in the bundle structure (58% α-helicity), both Trp and dansyl

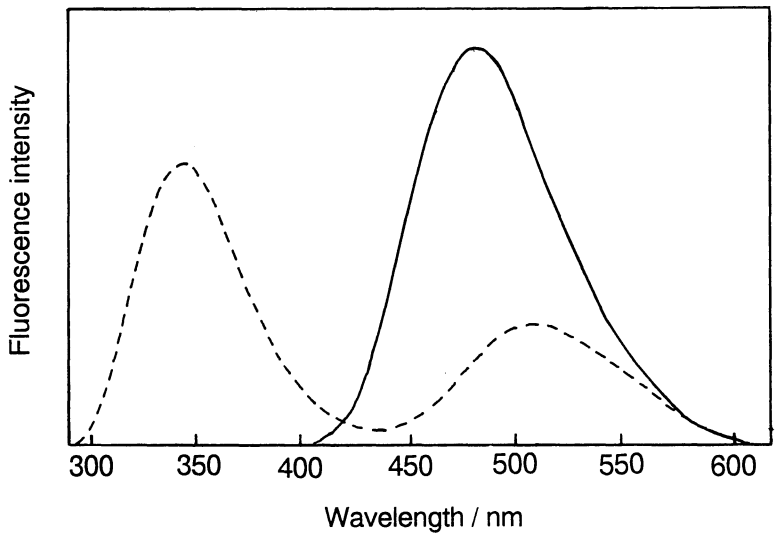

Fig. 2. Fluorescence spectra of the 53-peptide in 20 mM Tris·HCl, pH 7.4 (——) and MeOH (----). Concentration, 3.0 x 10^{-5} M. Excited at 280 nm. 25 °C.

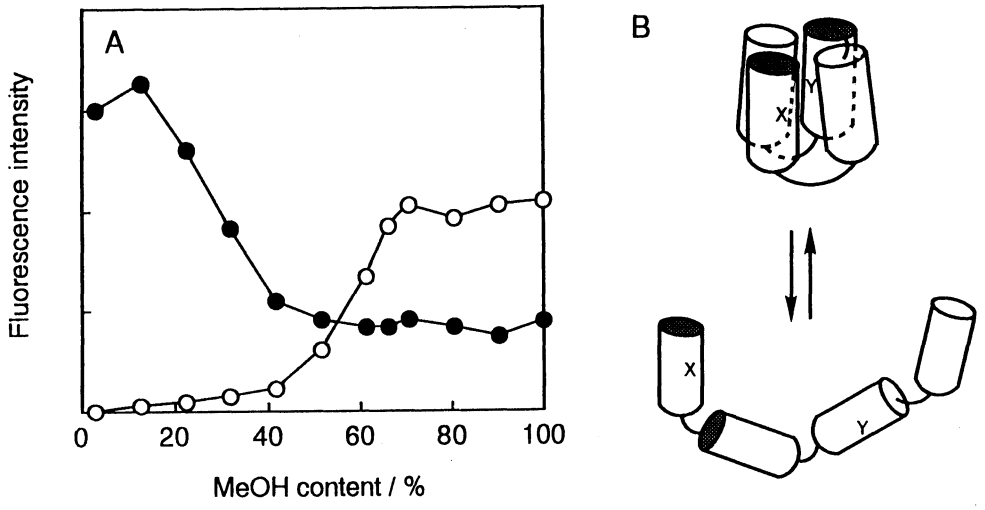

Fig. 3. (A) The effect of MeOH content on the fluorescence energy transfer from Trp to the dansyl group. The fluorescence intensity, Trp at 347 nm (○) and the dansyl group at 480 nm (●). Concentration, 3.0 x 10^{-5} M. Excited at 280 nm. 25 °C. (B) Illustrations for the 4α-helix bundle structure of the 53-peptide in aqueous solution and for the undefined conformation in MeOH.

fluorescences were emitted. The increasing equivalent of the 13-peptide to the 40-peptide gradually diminished the Trp fluorescence and increased the dansyl emission by excitation at 280 nm. This fact suggests that these unconnected fragments can form a complex with rather weak binding capacity.

The effect of guanidine hydrochloride (Gu·HCl) was further investigated to estimate the stability of the secondary structure of the 53-peptide. As shown in Fig. 4, the peptide lost its α-helicity by the addition of the denaturant at higher than 3 M concentration (Fig. 4A). The fluorescence at 480 nm also decreased along with the addition of Gu·HCl with shifting the emission maximum to a longer wavelength (510 nm). The destruction of the secondary structure again interrupted the energy migration from Trp as shown in Fig. 4B.

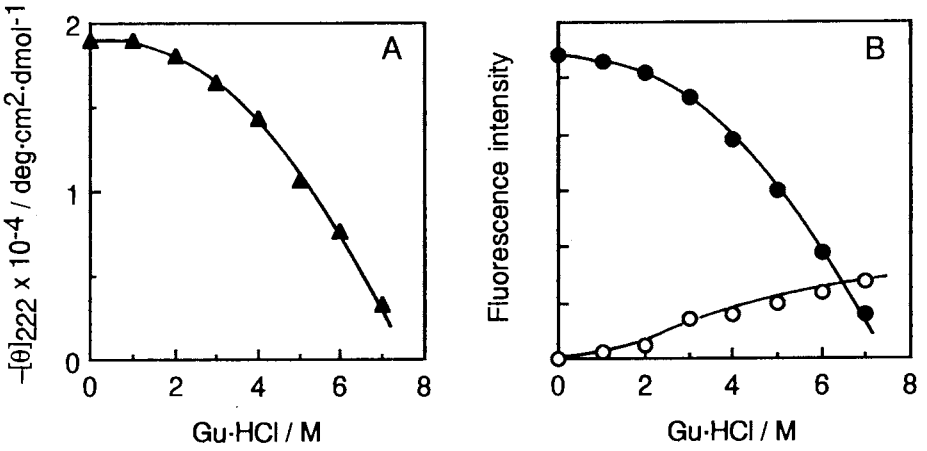

Fig. 4. The effect of guanidine hydrochloride concentration on the conformation (A) and the fluorescent incident (B) of the 53-peptide. The ellipticity, at 222 nm. The fluorescence intensity, Trp at 347 nm (○) and the dansyl group at 480 nm (●). Concentration, 3.0 x 10^{-5} M. Excited at 280 nm. 25 °C.

In conclusion, the fluorescence energy transfer system of indolyl and dansyl groups was for the first time applied to detect the intersegmental arrangement in the 4α-bundle structure. The fluorescent incident was well correlated with the folding properties of the 53-peptide in various conditions. This system may be employed in further design of artificial enzymes, in which particular functional groups must be arranged in close relation.

References

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