Fluorescence Energy Transfer to Tailed Acridine Oranges from Pyrene Excimer Formed in Four α-Helix Bundle 53-Peptide

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Fluorescence energy transfer from pyrene excimer in four α-helix bundle polypeptide to 10-*n*-alkyl acridine oranges was alkyl chain-length-dependent. The hydrophobic space in the bundle structure could accommodate as long alkyl chain as decyl by 1:1 stoichiometry.

One of the major goals of protein *de novo* design is to elucidate the enzymatic functions of polypeptide including the substrate binding and catalytic activities. However, the protein models designed so far are regarded as molten globules,[1] which represent the metastable state of proteins under unusual conditions. The artificial proteins designed *de novo* barely mimic the three dimensional conformation such as four α-helix bundle structure at present, but obtain no specific folding like native enzymes.

We also have designed and synthesized four α-helix polypeptides containing a pair of 1-pyrenylalanines to characterize the conformational behaviors in various environments.[2-4] These polypeptides could form hydrophobic space in a four α-helix bundle structure in aqueous solution. The hydrophobic space in so-called molten globule is expected to accommodate hydrophobic moieties such as alkyl groups of appropriate substrates. Therefore, it is of interest to evaluate the hydrophobicity of the inside of the bundle for the usefulness as substrate binding site, even though it is still in molten globule state.

On the other hand, acridine orange 10-*n*-dodecyl bromide ($C_{12}AO$) is known as a fluorescent probe for hydrophobic micro-environment.[5] It has been used to characterize the α-helix bundle structure by the increase in fluorescence intensity.[6-8] In the previous study, we observed the fluorescence energy transfer from pyrene excimer in the bundle structure to $C_{12}AO$.[3] This observation led us to an idea that four α-helix bundle structure might be able to mimic the enzyme-like substrate intake.

In order to investigate the relationship between the efficiency of the fluorescence energy transfer and the length of alkyl chain tethering acridine orange (AO) dye, we additionally prepared methyl, *n*-butyl, *n*-hexyl, *n*-octyl, and *n*-decyl AO (C_nAO : n=number of methylene groups) iodides (Figure 1).[9] $C_{12}AO$ was purchased from Dojindo Laboratories. These dyes are examined for the difference in the efficiency of the fluorescence energy transfer from pyrene excimer generated in the hydrophobic space in the 53-peptide (Figure 1).

The addition of C_nAO in the 53-peptide solution gave new emission at 530 nm from AO with disappearance of excimer emission at 470 nm upon excitation at 345 nm, at which AO has no absorption band (Figure 2). These AO dyes in buffer without 53-peptide did not show emission at 530 nm when excited at 345 nm. Moreover, C_nAO with the 53-peptide showed very weak emission in methanol. In methanol condition, 53-peptide took α-helix conformation but did not show excimer emission due to the melting of bundle structure.[3] Obviously, such fluorescence energy transfer was induced by pyrene excimer formation. It is also noteworthy that the increase at AO emission was accompanied with the apparent decrease at pyrene monomer emission (400 nm) as seen in Figure 2. Therefore, to compare the efficiencies in fluorescence energy transfer, the ratio of the intensity at 530 nm (I_{AO}) to that at 400 nm (pyrene monomer emission, I_M) was plotted against alkyl chain length (Figure 3). The result indicated that the energy transfer efficiency depends on the alkyl chain length. The AO dyes with shorter alkyl chains than octyl group appeared very poor in energy transfer. The longer tails remarkably enhanced the energy transfer. The inner space of the four α-helix bundle structure is assumed hydrophobic enough to incorporate alkyl chains longer than *n*-octyl group. The aromatic group of AO dyes may remain outside of the hydrophobic space .

To confirm the mode of the binding between C_nAO and 53-peptide, various equivalents of each AO derivative were added to the peptide solution. As shown in Figure 4, the I_{AO}/I_M increased differently with the addition of these dyes. Dyes with long alkyl chain such as decyl and dodecyl groups showed

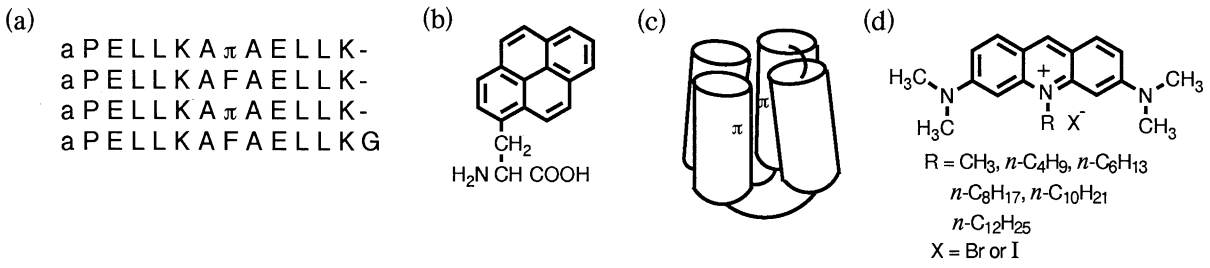

Figure 1. Structures of 53-peptide and chromophores. (a) Amino acid sequence of the 53-peptide. One letter symbols of amino acids are A, Ala; a, D-Ala; E, Glu; F, Phe; G, Gly; K, Lys; L, Leu; P, Pro; π, L-1-pyrenylalanine. (b) Structure of L-1-pyrenylalanine. (c) Four α-helix bundle structure of the 53-peptide. (d) Structure of 10-alkyl acridine oranges (C_nAO).

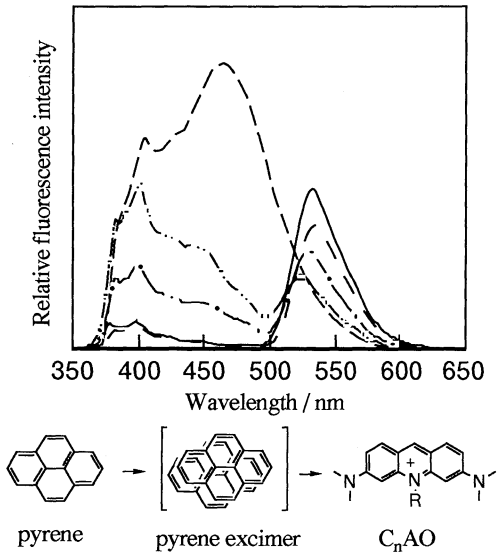

Figure 2. Fluorescence spectra of the 53-peptide in 2.0 x 10^{-2} mol·dm^{-3} Tris·HCl buffer (pH 7.4) (- - -), with C_1AO (-•••-), with C_6AO (-•-), with $C_{10}AO$ (— —), and with $C_{12}AO$ (———). [Peptide]=[Dye]=3.0 x 10^{-5} mol·dm^{-3}. Excited at 345 nm, 25 °C. The illustration is to explain the fluorescence energy transfer.

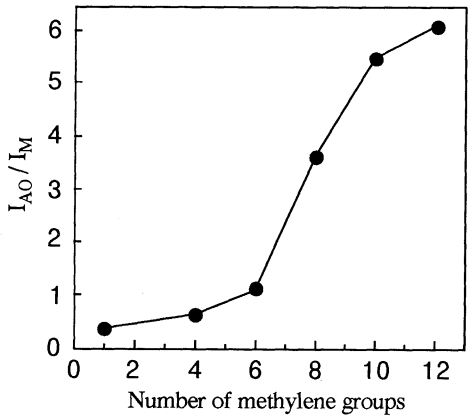

Figure 3. Dependence of I_{AO}/I_M on alkyl-chain-length of C_nAO. [Peptide]=[Dye]=3.0 x 10^{-5} mol·dm^{-3}. Excited at 345 nm, 25 °C. I_{AO}/I_M denotes the ratio of fluorescence intensities at 530 nm to 400 nm.

saturation at about one equivalent to the 53-peptide. These results suggest that the dye with long tail would penetrate into the inside of the four α-helix bundle structure and form 1:1 complex with the 53-peptide. The association constants between the 53-peptide and $C_{10}AO$ and $C_{12}AO$ were estimated to be 5.5 x 10^4 and 1.5 x 10^5 mol^{-1}·dm^3, respectively, by calculation according to the literature.[7] The shorter alkyl chains could not afford

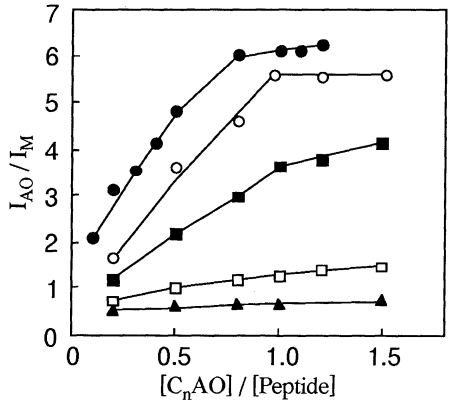

Figure 4. Changes of I_{AO}/I_M with increasing amounts of C_4AO (▲), C_6AO (□), C_8AO (■), $C_{10}AO$ (○), and $C_{12}AO$ (●) to the 53-peptide. [Peptide]=3.0 x 10^{-5} mol·dm^{-3}. Excited at 345 nm, 25 °C. I_{AO}/I_M denotes the ratio of fluorescence intensities at 530 nm to 400 nm.

significant hydrophobic incorporation into the inner space of the bundle structure even with more than one equivalent. Octyl group gave intermediate efficiency to the dye in fluorescence energy transfer.

In conclusion, the energy transfer from pyrene excimer to various AO dyes depended on the chain length. Greater hydrophobicity afforded by longer chain than octyl group was required for efficient energy transfer. One equivalent of tailed AO was enough to obtain the maximum efficiency. This hydrophobic space in polypeptide will be useful to form complexes with other functional small molecules by mimicking enzyme-like substrate incorporation.

References and Notes

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9 Preparation according to ref. 5 with exception of the use of alkyl iodides. FAB-MS gave peaks of m/z, which correspond to $[M]^+$ for all CnAOs. Each CnAO showed absorption spectrum similar to each other with λ_{max} (H_2O) at 472 nm and ε of 32400 dm^3·mol^{-1}·cm^{-1}.