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Amphiphilic Tailor-made Proteins as Novel Chiral Hosts

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We have designed and synthesized the proteins made up of several amphiphilic helices and the mobile linkage parts. They were proved to serve as host molecules providing hydrophobic inclusion sites. Induced circular dichroism spectra of the protein-acridine orange derivative systems were observed, which suggests the induction of asymmetric structure of the dye.

De novo designed proteins are now attracting much attention. A few designed small proteins, e.g., amphiphilic 4-helix bundles, have been explored.[1-5] We have designed and synthesized the proteins, **P2**, **P4**, and **P6**, shown in the following scheme with one-letter abbreviations[6] for amino acids. They are constituted with 2, 4, and 6 amphiphilic helices, respectively, and the linear linkage parts. The helical parts, which are represented with underlines, have amino acid compositions similar to Ho et al.[1] but the sequences are different from theirs. For the linkage parts glycine-rich sequences and/or cystine were employed to afford flexible mobility which is expected to bring about induced-fitting feature.

(**P2**) AGELKKLLEELKKLLEEAKGKPGGLKKLEELLKKLEELLG

(**P4**) CGELKKLLEELKKLLEEAKGKPGGLKKLEELLKKLEELLG
|
CGELKKLLEELKKLLEEAKGKPGGLKKLEELLKKLEELLG

(**P6**) CGGELKKLLEELKKLLEGKPGGELKKLLEELKKLLEEAKGKPGGLKKLEELLKKLEELLG
|
CGGELKKLLEELKKLLEGKPGGELKKLLEELKKLLEEAKGKPGGLKKLEELLKKLEELLG

These peptides were synthesized by the modified solid-phase method.[7] They were purified by ion-exchange chromatography and confirmed by amino acid analysis. The circular dichroism(CD) spectra of the peptides showed typical α-helix bands, of which the intensities were in accord with the theoretical values taking into consideration that the underlined sequences form α-helices.[7] The denaturation experiments with guanidine hydrochloride showed that the peptides form helix bundles involving a

coiled-coil structure, which is considered to be constructed by hydrophobic interaction between helices.[7)]

The formation of inclusion complex was examined with acridine orange-10-dodecyl bromide(AODB), which is known as a fluorescence probe for hydrophobic micro-environment. The fluorescence of AODB was enhanced with increase in the concentration of the peptides as shown in Fig.1. Significant enhancement was observed for **P6**. The extent of fluorescence enhancement was not proportional to the number of helices constituting each protein. It is suggested that AODB is included in hydrophobic sites of the helix bundle proteins because AODB has high hydrophobicity due to dodecyl group as well as aromatic rings. As the CD intensity of **P2** is dependent on its concentration, **P2** is assumed to form dimer $(\mathbf{P2})_2$. The double-reciprocal plots (Fig.1) gave association constants, $1.1\times10^4 M^{-1}$, $1.4\times10^4 M^{-1}$, and $4.4\times10^4 M^{-1}$ for $(\mathbf{P2})_2$, **P4**, and **P6**, respectively. The almost equal values of association constants for $(\mathbf{P2})_2$ and **P4** also supports that these inclusion complexes have a similar structure. The association constant for **P6** is about three times larger than the others, suggesting that not only 4 helices but also other two helices in **P6** take part in complex formation.

Induced circular dichroism was found for these protein-AODB complexes at the absorption wavelength of achiral AODB (Fig.2(A)). The band intensities increased with an increasing concentration of the peptide. The patterns of CD spectra are almost similar among these proteins. However,

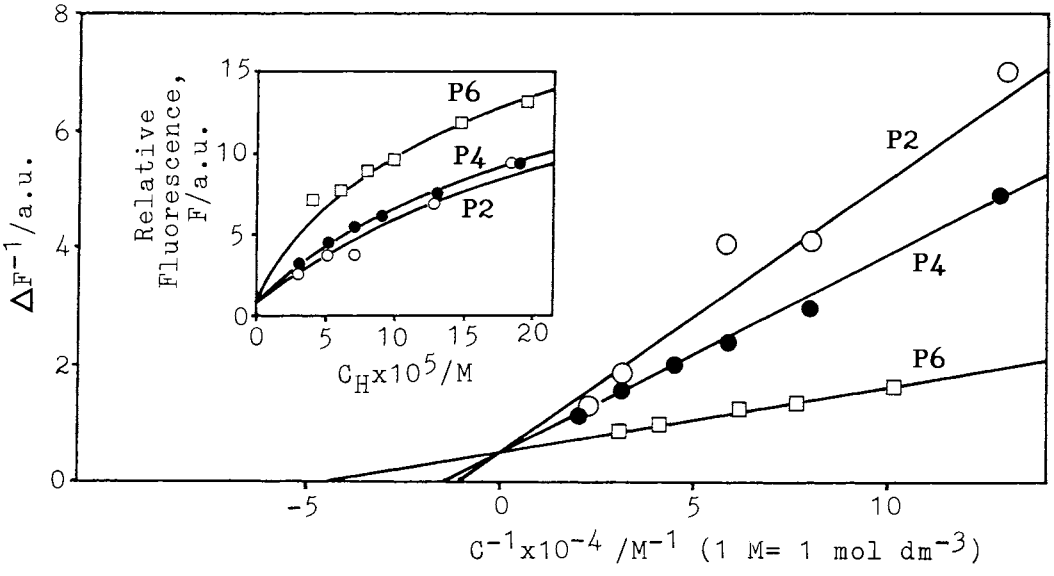

Fig.1. Double reciprocal plots of the increment of fluorescence intensity(ΔF) versus the concentration of the proteins(C) under the condition of constant AODB concentration(2.0×10^{-6}M). The inset figure exhibits the relation between observed fluorescence intensity(F) and the concentration of a helical unit(C_H).

the CD spectra are much different from that of acridine orange-poly(α-glutamic acid) system.[8] This may be attributed to the difference in dye-peptide interaction, that is, the interaction of our system is hydrophobic and that of the latter system is ionic. With the increase of wavelength the sign of Cotton effect turns from minus to plus. It is known that acridine orange forms stacking dimer in a concentrated solution.[9] For protein-AODB systems UV spectra exhibit the peak related to the stacking of acridine orange parts (Fig.2(B)). On the basis of UV spectra it is considered that the CD spectrum is made up of positive Cotton effect

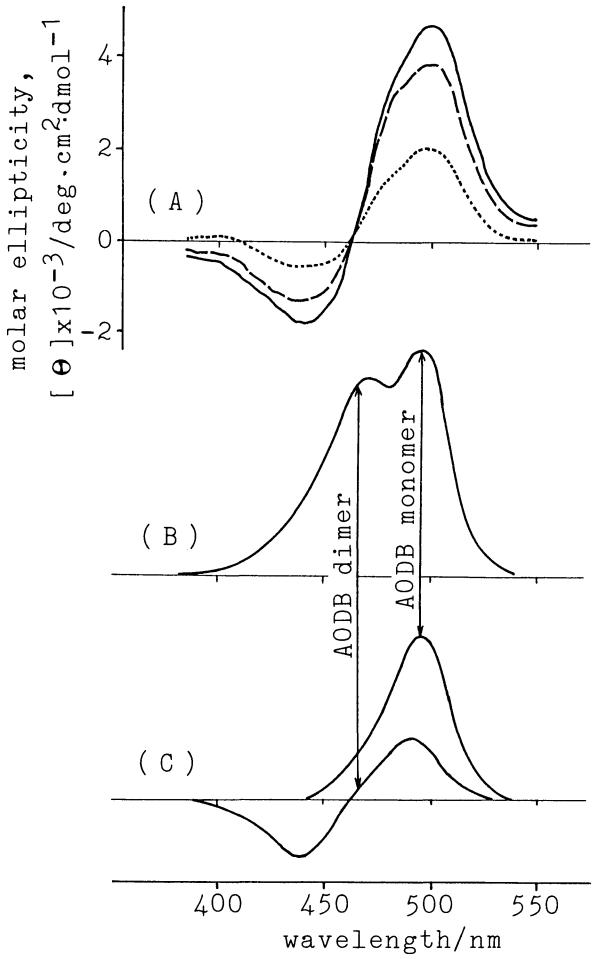

Fig.2. (A)The observed CD spectra of P4-AODB system; [protein]=3.3x10^{-5}M(——), 2.5x10^{-5}M(----), 1.3x10^{-5}M (·····); [AODB]=8x10^{-6}M. (B)The corresponding UV spectrum; [protein]=3.3x10^{-5}M. (C)The components of induced CD spectra estimated with the peak shape of UV spectrum.

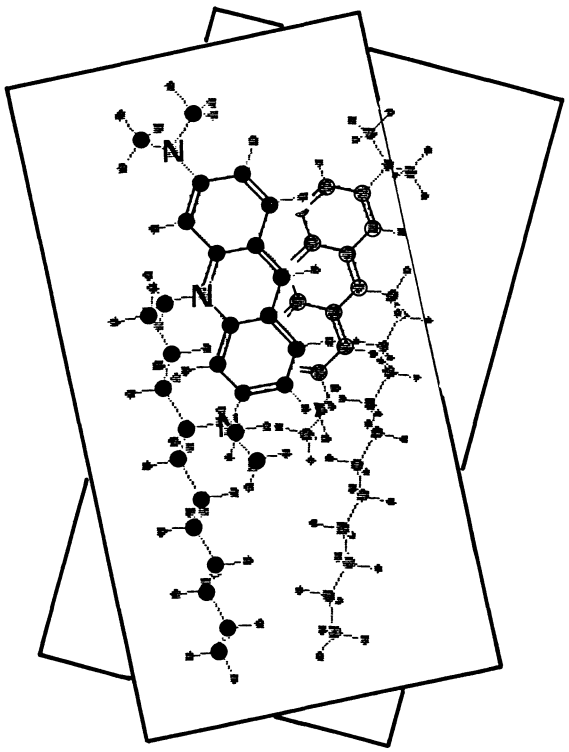

Fig.3. Estimated twisting sense of aromatic part of stacked AODB dimer included in the proteins. The orientation of dodecyl group in the figure is one of the probable cases.

corresponding to monomeric AODB and S-curved CD spectrum due to exciton interaction of AODB dimer (Fig.2(C)) because the magnitude of the band at about 490 nm is more intensive than that of the band at about 440 nm.

The findings of induced CD for both monomer and dimer of AODB mean that the AODB molecules are submitted to inclusion in chiral environment because AODB itself has no chirality. The resembled CD spectra for these proteins, **P2**, **P4**, and **P6**, lead to the consideration that these sites have similar structure. By means of the exciton chirality method[10] it is revealed that the chirality of AODB dimer is left-handed twisted mode in viewing along the longitudinal axis (Fig.3). It is considered that the AODB dimer not only exists in chiral surroundings but also causes chiral deformation of stacking. Interestingly, the AODB dimer of this chirality mode can fit in with left-handed coiled-coil structure of protein.[11] The chiral inclusion site may be formed along the twisted and hydrophobic interior of associated helices. Further precise drawing for the steric structure of these proteins will be reported in near future.

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6) Abbreviations for amino acids are: A,Ala; C,cysteine; C-C,cystine; E,Glu; G:Gly; K,Lys; L,Leu; P,Pro.
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