Orientation Control of Self-stacking D,L-Alternating Cyclic Octa-α-peptide through Multiple Electrostatic Interactions

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We designed D,L-alternating cyclic octa-α-peptides having two D-lysines and two D-glutamates as electrostatically interacting units in order to regulate the orientation of the peptide units upon stacking in an antiparallel manner. Spectroscopic analyses of the fluorescence probe on the peptide indicated the successful control of the orientation.

Recently, much attention has been paid to the development of device-oriented nanomaterials based on the self-assembled peptide nanotubes.[1] One of the examples is a one-dimensional functional molecular array reported by Ghadiri's group.[2a] They have shown that C_4-symmetric D,L-alternating cyclic octa-α-peptides (ACOPs) having a sequence of cyclo-[(L-Lys(NDI)–D-Lys)$_4$] (NDI = 1,4,5,8-naphthalenetetracarboxylic acid diimide derivative) stacks to each other to give quadruple one-dimensional NDI array. The successful formation of the NDI array might be attributed to the following characteristics of ACOPs: 1) the nearly flat structure of ACOP with the amino acid side chains extending to the outside of the ring is suitable for stacking of functional moiety, 2) the multiple hydrogen bonding among the ACOP units tolerates the loading of functional moieties without destruction of supramolecular structure, and 3) the antiparallel manner of the stacking makes the spatial arrangement of the amino acid side chains of the adjacent units predictable. However, a problem would arise if a monofunctionalized C_1-symmetric ACOP molecule was used as a building unit: the functional moieties in the adjacent units do not necessarily stack in an "eclipsed" orientation due to the C_4-symmetric nature of the hydrogen-bonding sites resulting in the formation of nanotubes with randomly oriented functional moieties. Solution to this problem would apparently increase the advantage of ACOP as a template. In this communication, we propose a method to control the stacking manner of ACOPs by introducing ionic interactions.

Ionic complementarity of the peptide, that is, a designed spatial arrangement of positively and negatively charged amino acid side chains, is now widely utilized to construct self-assembly based aggregates of α-helix and β-sheet peptides.[3] In the present study, we applied this principle to control the orientation of ACOP units in a peptide nanotube. We designed two ACOPs, AP and BP, having an identical amino acid composition including two D-Lys and two D-Glu, but are different in their sequence (Figure 1). (*S*)-2-Amino-3-(1-pyrenylcarbonylamino)propanoic acid (denoted as Z in Figure 1) was used as a spectroscopic probe for self-assembled state of ACOP molecules. Upon stacking in an antiparallel fashion, charged side chains in the neighboring ACOP units should come to close proximity, hence the multiple ionic interactions might regulate mutual arrangement. Accordingly, the pyrenyl (Py) groups in AP-derived nanotubes are expected to align on the one side, while those in BP-based

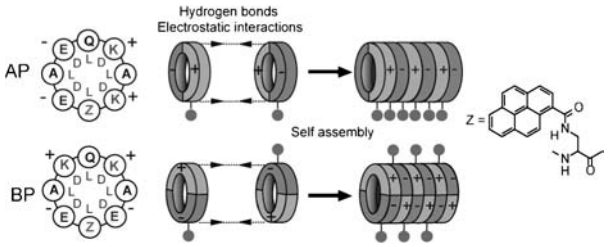

Figure 1. Design of ACOPs.

nanotubes should be located in an alternating manner. A related study has been reported by Karlström and Undén.[4] Their work was on the dimerization behavior of ion-complementarity-based heteromeric ACOPs having three glutamates on one unit and three lysines on the other. According to their design, only attractive interaction is possible for the two heteromeric units, thus some ambiguity remains for the orientation of the two units. In contrast, our design provides strict control of the orientation because both attractive and repulsive electrostatic interactions should cooperate during the stacking process. Peptides were synthesized by Fmoc solid-phase method and were cyclized on a resin according to the reported procedure.[5] All peptides were purified with reversed phase HPLC and identified by MALDI-TOF-MS analysis.

Morphology of the peptides in the solid state was observed using transmission electron microscope (TEM). For both AP and BP, TEM image showed the formation of bundles of rod-like filamentous structures (Figure 2). The diameter of the rod was 1.5 nm, which corresponds to the size of the ACOP unit. Thus, nanotube-forming ability of these ACOPs was confirmed. Infrared spectroscopic analysis showed that wavenumbers of the peaks in amide I and amide II region of AP and those of BP agreed with those reported by Ghadiri's group (Table S1).[2,6] This implies that the stacking of ACOP units occurs in an antiparallel manner.

To confirm the self-assembly of peptides in solution, we used centrifugal filter devices equipped with 100 kD and 10 kD molecular weight (MW) cut-off membrane.[6] The peptide samples in the buffer were applied on the 100 kDa MW cut-off filter and were concentrated by centrifugation. The majority of peptides (>74%) was recovered from the concentrated solution

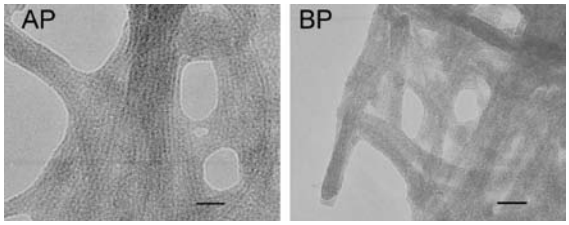

Figure 2. TEM images of ACOPs (scale bar, 20 nm).

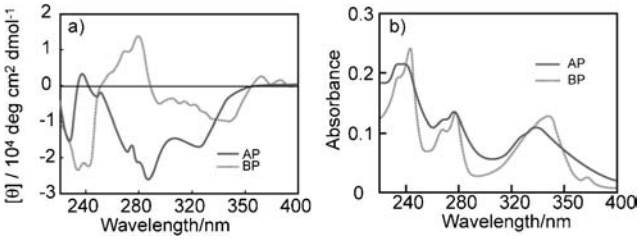

Figure 3. a) CD spectra and b) UV–vis spectra of ACOPs in 20 mM Tris-HCl (pH 7.4). [Peptide] = 10 μM.

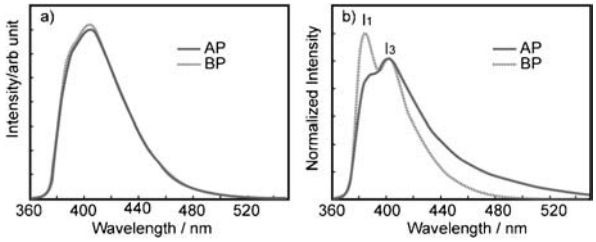

Figure 4. Fluorescence spectra of 10 μM ACOPs excited at 345 nm in 20 mM Tris-HCl buffer (pH 7.4) in the presence (a) and the absence (b) of 6 M GuHCl. For the spectra in b), height of the peaks were normalized at 402 nm.

and only a small amount of peptide was in the flow-through (Table S2).[6] On the other hand, most of the peptides passed through 10 kDa MW cut-off filter in the presence of denaturant 6 M guanidine hydrochloride (GuHCl). These results suggests that the peptide AP and BP self-assembled into large-sized aggregates (100 kDa is equivalent to ca. 90 mer) in the buffer.

Another evidence supports the self-assembly of peptides in solution; both AP and BP showed induced circular dichroism (ICD) signals at the pyrene π–π^* region in the buffer (Figure 3a). The signals must stem from the Py groups in the aggregated ACOPs because these ACOPs were ICD silent in the presence of GuHCl. Furthermore, the CD spectral curves for AP and BP were considerably different to each other. This indicates that the Py groups on the side chains were in entirely distinct microenvironment for these two peptide aggregates.

UV–vis spectra of ACOPs in the buffer were also different between AP and BP (Figure 3b), while those measured in the GuHCl were essentially superimposable (data not shown). The λ_{max} values corresponding to the S_2–S_0 transition of Py moieties in AP and BP were 341 and 349 nm, respectively. The λ_{max} value is generally affected by mutual arrangement between electronic transition moments of neighboring chromophores as shown in the H- and J-aggregates of porphyrin derivatives.[7] Presumably, also in our case, the Py groups in AP and BP are arranged with different orientations on the peptide nanotubes.

Fluorescence spectral study provided additional information on the microenvironment of Py groups in the peptide aggregates. In 6 M GuHCl, the spectral shapes of the two ACOPs were almost identical, whereas they were different in the buffer (Figure 4). The spectrum of AP in the buffer was accompanied with a shoulder at around 480 nm probably due to the excimer formation by pyrene moieties on adjacent ACOP units, although the intensity of the emission was quite low. Less efficient excimer formation between pyrenes spatially arranged in close proximity has been reported in the literature, and was explained by the restricted motion of Py groups in the molecular organization.[8] This might be the case for our peptides.[9] It should be noted that fluorescence spectrum of BP did not show excimer shoulder, and this is in accordance with proposed aggregate structure of this peptide. In the fluorescence spectrum of the buffer solution, two distinct peaks were observed at 384/401 nm for BP. Although somewhat ambiguous, the corresponding peaks are likely to be 386 and 401 nm for AP. These two peaks can be assigned as two vibronic bands derived from Py skeleton, I_1 and I_3.[10] An I_3/I_1 value is sensitive to the polarity of microenvironment, and the lower value means more polar environments. The values are 1.25 and 0.83 for AP and BP, respectively. The higher value for AP means that the Py groups in this aggregate are in more hydrophobic environment, and this is consistent with our design of the supramolecular structure in which a Py group is sandwiched by another Py groups in the neighboring units. The Py groups in BP should be apart from each other, hence more exposed to polar solvent.

Altogether, we can reasonably conclude that the orientation of the C_1-symmetric ACOP in nanotube was successfully controlled by electrostatic interactions between appropriately placed positively and negatively charged amino acid side chains. This principle might be applicable for rational design of the materials based on one-dimensional functional molecule array. A study toward the development of such materials including those having two kinds of functional molecule arrays on the opposite sides of the nanotubes is now underway in this laboratory.

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References and Notes

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