Morphological Modulation of Self-assembled Peptide by Aggregation-induced α-Helix/β-Sheet Transition

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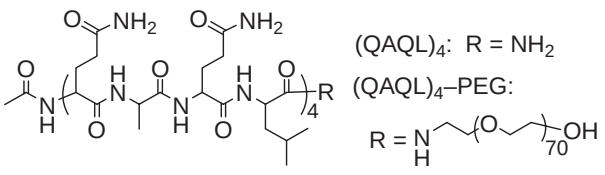
Poly(ethylene glycol) (PEG)-appended peptide with a binary sequence of hydrophobic and hydrophilic amino acids with 16 residues gave grain-like aggregate in aqueous solution. The peptide segment in the aggregate adopted β-sheet structure, while the peptide itself originally adopted α-helix structure. In the hydrophobic domain constructed by peptide segment, hydrogen bonding rearranged to give secondary structural transition. The α-helix/β-sheet transition simultaneously gave morphological transition from a grain-like state to a fibrous object.

Programmed-assembly of peptides has attracted much attention, because the phenomenon possesses emerging potential in material science for nanostructural devices.[1,2] One of the most contributive features of peptides for these applications would be the transition ability of secondary structure such as α-helix/random coil, random coil/β-sheet, and α-helix/β-sheet transitions.[3–5] Especially, the β-sheet structure often provides unique self-assembled objects such as fibrils, ribbons, and barrels based on interstrand hydrogen bonding.[6–9] Designing poly(ethylene glycol) (PEG) segments with peptides has influence on solubility and amphiphilicity of the peptide segment. Thus, PEGylated peptide shows unique self-assembled structure incorporating self-assembling properties of the peptide segment and phase-separation between PEG and peptide.[10,11]

We have focused on an amphiphilic copolymer constructed with a peptide as a hydrophobic and PEG as a hydrophilic segment. The peptide segment is composed of 16 residues, which contains a repetitive sequence of Gln–Ala–Gln–Leu (QAQL) for four times (Scheme 1). The peptide was prepared by a solid-phase method using a 9-fluorenylmethoxycarbonyl (Fmoc) strategy.[12] It is well known that peptide sequences constructed by alternative polar and nonpolar amino acids adopt β-sheet structure.[13] The peptide segment, $(QAQL)_4$ having binary sequence would adopt a stretched conformation as a stable conformation. In this report, aggregation of $(QAQL)_4$–PEG and secondary structural transition of the peptide segment was investigated. The morphological transition by mixing the $(QAQL)_4$–PEG with $(QAQL)_4$ was also investigated.

The peptides dissolved in 2,2,2-trifluoroethanol (TFE) were diluted in pure water to 2.6×10^{-6} mol L^{-1} including 10% TFE.



Scheme 1.

The secondary structure of $(QAQL)_4$ and $(QAQL)_4$–PEG was investigated by CD spectroscopy (Figure 1a). $(QAQL)_4$–PEG showed a negative peak at 217 nm, which is typical of β-sheet structure. On the other hand, $(QAQL)_4$ showed negative peaks at 222 and 208 nm in addition to a negative peak at 217 nm, indicating coexistence of a right-handed α-helix structure with β-sheet. It was suggested that the PEG segment obviously contributes to the arrangement of the secondary structure of the peptide segment.

This result can be explained by aggregation of peptide-induced rearrangement of the hydrogen bonding to show secondary structural transition, as is often discussed in reports based on kinetic studies of amyloid protein.[4,14] Since eight of the sixteen amino acids in the peptide segments are hydrophobic, $(QAQL)_4$ is hydrophobic rather than the PEG segment. Thus, the PEGylated peptide possesses amphiphilicity. This amphiphilicity of the peptide–PEG moiety caused microphase separation to give aggregation of peptide segments by hydrophobic interaction. In the hydrophobic domain, the peptide segments packed tightly to show rearrangement of hydrogen bonding from intramolecular to intermolecular manner. Then, intermolecular hydrogen bonding induced β-sheet transition.

By casting a drop of $(QAQL)_4$–PEG solution on mica, the aggregate was observed by an atomic force microscope (AFM) after drying the substrate. Grain-like aggregates were observed as shown in Figure 1b, while aggregates based on β-sheet structure generally appear as fibrous objects. The result suggests that $(QAQL)_4$–PEG aggregate in the aqueous solution to form hydrophobic domain with several tens β-strands (Figure 1c). PEG seg-

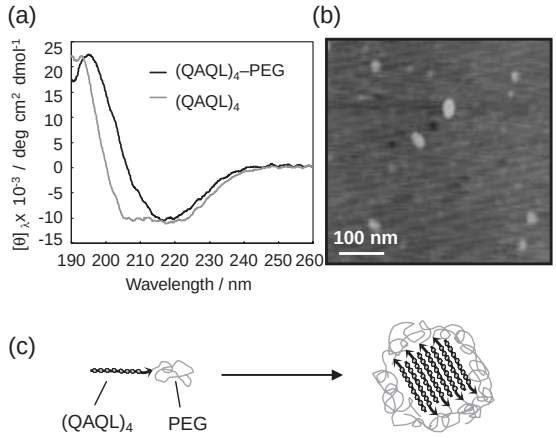


Figure 1. (a) CD spectra of $(QAQL)_4$–PEG and $(QAQL)_4$, (b) AFM image of $(QAQL)_4$–PEG aggregate obtained by casting a drop of the peptide solution, and (c) schematic representation of $(QAQL)_4$–PEG aggregates.

ments, which inevitably accompany the periphery of the peptide domain, would physically interfere with β-sheet propagation of the peptide segment.

When the peptide adopting α-helix structure mixed with the PEGylated peptide solution, the peptide was introduced into the hydrophobic domain of the PEGylated peptide aggregate to show β-sheet transition. Mixtures of $(QAQL)_4$ and $(QAQL)_4$–PEG in several molar ratios of PEG/$(QAQL)_4$ were prepared. The ratios of PEG/$(QAQL)_4$ at 0.09, 0.17, 0.25, 0.33, and 0.91 are corresponding to the mixing ratio of $(QAQL)_4$ and $(QAQL)_4$–PEG at 10/1, 5/1, 3/1, 2/1, and 1/10, respectively. The secondary structure was estimated by CD spectroscopy (Figure 2a). Spectra corresponding to α-helix transformed into β-sheet structure as PEG/$(QAQL)_4$ increased. It was suggested that secondary structure of peptide segment arranged according to the content of PEG segment in the mixture. The value of $[\theta]_{208}/[\theta]_{215}$ as a ratio of α-helix/β-sheet content is plotted in Figure 2b. It was clearly confirmed that β-sheet transition occurred at PEG/$(QAQL)_4 = 0.17$. At this ratio, morphological observation was investigated by casting a drop of the solution on mica substrate. Fibrous objects were observed in the AFM image (Figure 2c). $(QAQL)_4$, which proceeded α-helix/β-sheet transition by introduction into the hydrophobic domain, also influenced the aggregation of the PEGylated peptide to give morphological transition. The peptide introduced into the peptide domain of the grain aggregates showed α-helix/β-sheet transition to give enlargement of the peptide domain. Then, the interference of PEG segments on the periphery of the peptide domains was reduced. Intermolecular hydrogen bonding was allowed to show propagation of the β-sheet fibrils. A drop of $(QAQL)_4$ solution resulted in no characteristic morphology, because the peptide with α-helix structure would be highly disperse (data not shown). It supported the proposal that the obtained morphological transition was dependent on the microphase separation between PEG and peptide segments.

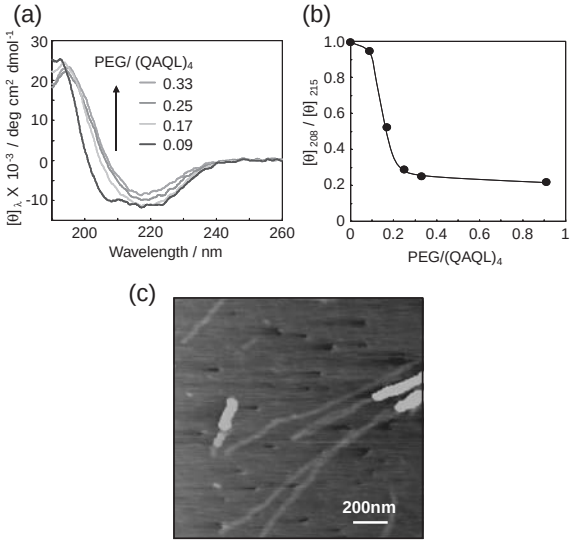


Figure 2. (a) CD spectra of the mixture of $(QAQL)_4$–PEG and $(QAQL)_4$. (b) Plots of $[\theta]_{208}/[\theta]_{215}$ vs. the molar ratio of PEG and $(QAQL)_4$. (c) AFM image shows the obtained aggregate from the mixture at PEG/$(QAQL)_4 = 0.17$.

In summary, we have synthesized $(QAQL)_4$–PEG, which self-assembled into grain-like aggregation on the basis of amphiphilicity. When mixing $(QAQL)_4$ into the PEGylated peptide aggregate, the peptide was introduced into the hydrophobic domain to show the α-helix/β-sheet transition. The peptide addition also had influence on the morphology of the aggregate. The grain-like aggregate transformed into fibrous objects by the balance of the interference of the PEG segment on the periphery of the peptide domain. Such morphological transition by mixing PEGylated peptide and the corresponding peptide will provide a novel strategy for construction of the nanoarchitecture by programmed-assembly.

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12 $(QAQL)_4$ was synthesized on CLEAR-Amide resin (Peptide Institute. Inc.) by using 3 equivalents of Fmoc–amino acid derivatives. $(QAQL)_4$–PEG was prepared by the same procedure with TentaGel PAP resin (RAPP Polymere) having preloaded PEG chain (MW 3000) instead of CLEAR-Amide resin. Cleavage of the peptides from the resin and deprotection of the side chain were carried out with trifluoroacetic acid/1,2-ethanedithiol/thioanisole/water (84:8:4:4). The peptides were precipitated and washed with diethyl ether.
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