Formation of Nanofibrillar Aggregates by Water-Soluble β-Structural Oligopeptide, (L-Leu-L-Lys)$_8$

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The alternating oligopeptide Pyr-(LK)$_8$ doped by a pyrenyl group at the terminal formed helical tape-like aggregates in an aqueous solution at pH 7. Addition of NaCl induced random coil-to-β-structure transition to show the excimer peak of pyrenyl groups, although no similar result was observed by the corresponding random oligopeptide Pyr-(L_8K_8).

A self-assembling method with small molecules has been expected as a new method to create precise structures at the nano scale. It is a great advantage to build up nanoscale structures because not only physical structures can be controlled but also chemical functions can be selected by molecular design. On these view points, a self-assembling system with lipid aggregation has been widely investigated.[1] Hydrophobic effect, electrostatic interaction, and hydrogen bonding interaction are often included as driving forces for aggregation and thus aggregation morphology is remarkably influenced by them. For example, Kunitake et al. discussed the relationship between the chemical structure of synthetic lipids and their morphologies from globules to fibrillar aggregates.[2] We also reported that nanohelices and -tubular structures based on single-walled bilayer structures could be created by peptide lipids, whose morphologies were dependent on the secondary structures of lipid head groups.[3]

On the other hand, there are recent reports on aggregation structures induced by the secondary structures of poly(amino acid)s in the field of protein engineering.[4] In particular, β-structures might be favorable for nanostructure creation due to the stability and specific molecular orientation.[5] However, a multiple hydrogen bonding interaction such as β-structures often causes precipitation through strong interaction in the three dimensional range. Therefore, it has been assumed that it is quite difficult to produce nanostructures constructed in β-structure-forming polypeptide.[6]

In this communication, we introduce water-soluble nanofibrillar aggregates by β-structural sequential L-leucine and L-lysine oligopeptides Pyr-(LK)$_8$. This has three key characteristics: (1) the sequence is alternating with hydrophobic and hydrophilic amino acids; (2) the degree of polymerization is monodispersed. If β-structure is formed in an aqueous solution, it is expected that the monodispersity may prevent three-dimensional aggregation by amphiphilic property; (3) the terminal fluorescent chromophoric group enables us to evaluate the molecular orientation spectrophotometrically.

The alternating oligopeptide Pyr-(LK)$_8$ having a pyrenyl group at the terminal was prepared by a peptide synthesizer (P. Biosystems, Pioneer) according to the Fmoc solid phase method.[7] Pyr-L_8K_8 as the corresponding non-alternating oligopeptide was also prepared by the similar procedure. The sequence is LKLKKLLLKKLLKLKK. Both the Pyr-(LK)$_8$ and Pyr-L_8K_8 showed typical CD spectra for random coils in their aqueous solutions at pH 7.[8] The possible reasons will be derived from electrostatic repulsion among the residual amino groups of L-lysine. In fact, pH increase to promote the neutralization of the residual amino groups induced secondary structures.[9] Interestingly, it was observed that addition of NaCl at pH 7 promoted a random coil-to-β-structure phase transition in Pyr-(LK)$_8$ without pH increase. It is estimated that NaCl suppresses the electrostatic repulsion even at pH 7. Figure 1 showed the concentration effects of NaCl on $[\theta]_{202}$ at pH 7. Distinct change was observed by addition of 50–100 equimolar of NaCl against the L-lysine residue. On the contrary, the non-alternating Pyr-L_8K_8 showed a random coil-to-α-helix transition at pH 7 with NaCl. For example, the addition of 300 equimolar of NaCl per the residue increased the α-helix content by ca. 30%. Here, we succeeded to make a water-soluble β-structure-including oligopeptide at pH 7 by controlling the amino acid sequence.

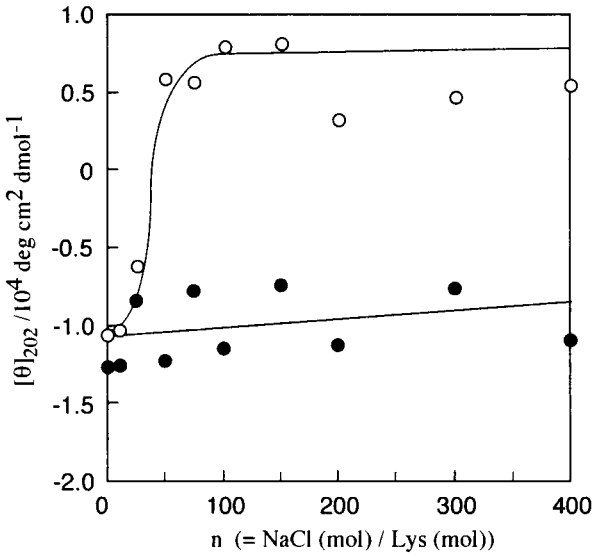

Figure 1. Effect of concentration of NaCl on molecular ellipticity at 202 nm with (○); Pyr-(LK)$_8$ and (●); Pyr-L_8K_8 (2.0 mM) in pH 7 aqueous solutions. [oligopeptides] = 2.0 mM.

The fluorescence spectroscopy gave us significant information to estimate the effect of NaCl on the conformational change. The microenvironmental information can be estimated by determining the fluorescence spectra of the pyrenyl group at the terminals of Pyr-(LK)$_8$ and Pyr-L_8K_8. Figure 2 shows that Pyr-(LK)$_8$ provided a monomer luminescence at 470 nm (λ_{ex} = 350 nm) without NaCl but a new luminescence peak

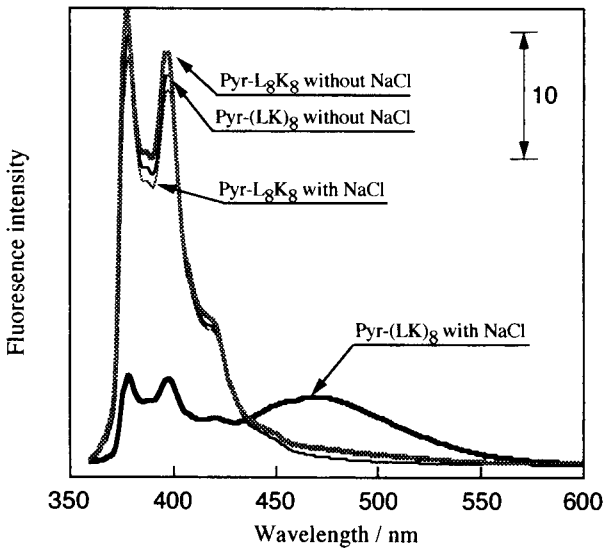

Figure 2. Effect of concentration of NaCl (100 equiv.) on fluorescence spectra (λ_{ex} = 350 nm) with Pyr-$(LK)_8$ and Pyr-L_8K_8 (2.0 mM) in a pH 7.0 aqueous solution at 25 °C.

was induced at around 470 nm by addition of 100 equivalent of NaCl. This new peak is attributable to excimer formation.[10] On the contrary, Pyr-L_8K_8 provided only monomer luminescence without or with NaCl. The big difference between Pyr-$(LK)_8$ and Pyr-L_8K_8 is undoubtedly induced by those in their conformations and thus it is estimated that the β-structure formation in Pyr-$(LK)_8$ promotes the chromophore interaction suitable for excimer formation. It is surely impossible in α-helical conformation.

TEM observation revealed their aggregation structures.[11] As shown in Figure 3, fibrillar aggregates were observed in Pyr-$(LK)_8$ whereas no similar aggregates were detected in Pyr-L_8K_8. A close observation of the fibrillar structure indicated that the aggregates were made up of smaller helical tape-like aggregates which were 12–15 nm in the width and 100 nm in the twisted pitch of the narrowest part. The exact aggregation structure is not yet specified but the minimum width of 12 nm indicates that Pyr-$(LK)_8$ produces molecular layer structures because Pyr-$(LK)_8$ is estimated to be 5.3 nm in the length when it forms complete β-structure.[12] As above-mentioned, Pyr-$(LK)_8$ forms excimer when

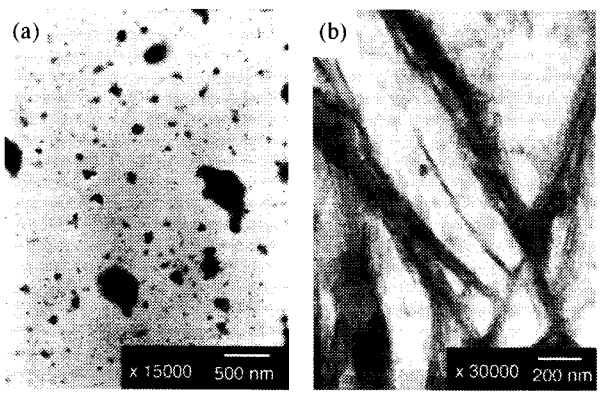

Figure 3. Transmission electron micrographs of (a); Pyr-L_8K_8 and (b); Pyr-$(LK)_8$ in aqueous solution with 200 equiv. NaCl. Initial concentration is 2.0 mM.

it is in a β-structure. These findings explain that Pyr-$(LK)_8$ can form highly-oriented aggregates through β-structure formation and subsequent intermolecular interaction.

In conclusion, we have demonstrated that water-soluble nanofibriller aggregates including β-structures can be realized by the alternating sequential oligopeptide composed of hydophobic and hydrophilic amino acids. This unusual water solubility is closely related to the fact that the oligopeptide is probably monodispersed in the molecular length. This property prevents three-dimensional aggregation to cause precipitation. Also the fluorescence and CD studies have revealed that the aggregates consisted of highly-oriented structures similar to molecular layer structures. Therefore, the alternating oligopeptides such as Pyr-$(LK)_8$ are very attractive as a component unit for nanostructure creation and furthermore their possible applicability will be expanded by choosing amino acids.

References and Notes

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7 Pyr-$(LK)_8$ was synthesized using the Fmoc chemistry on a peptide synthesizer. After the final step of deprotection, the resin was dried with *t*-butanol. The N-terminal was end-capped by 2-carboxypyrene with a DEPC method.[13] The target oligopeptide was obtained by cleavage with 10 mL of TFA/water (= 9:1 vol/vol) mixture solution. It was concentrated to about 1 ml and then added to diethyl ether. After the precipitate was collected by filtration and dried under reduced pressure, the white powders were obtained by freeze-drying from a pH 7.0 aqueous solution.

8 The oligopeptides were dispersed to be 10 mM aqueous solutions with ultrasonication for 1 min. The mother solution was diluted to be a proper concentration with water and the pH was adjusted by 0.01 N NaOH aqueous solution to 7.0. After further sonication for 3 min, the resultant clear solution was used for measurements.

9 Pyr-$(LK)_8$; $[\theta]_{217}$ = −11000 cm deg $dmol^{-1}$, Pyr-L_8K_8; $[\theta]_{217}$ = −6000 cm deg $dmol^{-1}$, at pH 11.

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