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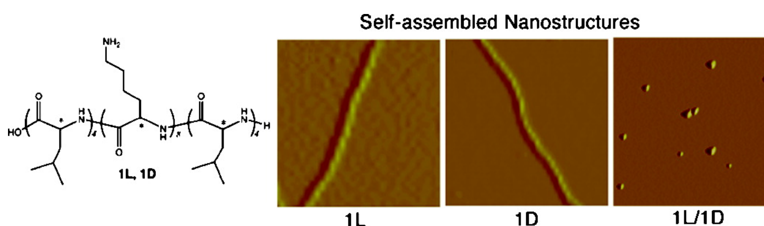

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Structural Control of Self-Assembled Nanofibers by Artificial β-Sheet Peptides Composed of D- or L-Isomer

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The self-assembly of peptides and proteins into β-sheet-rich fibrillar structures is currently the focus of biochemical and biophysical research because of their association with neurodegenerative diseases, such as Alzheimer's and Creutzfeldt-Jacob's.[1−4] Several proteins were identified in amyloid diseases,[5,6] and the amyloid fibrils were found to have typically unbranched and long morphology with diametric ranges of 4−10 nm, although amyloid precursor proteins did not share any sequence or structural homology. Nonpathogenic proteins and synthetic peptides also form nanofibers that structurally resemble in vivo fibrils.[7−12] Thus, the ability to form amyloid-like nanofibers from various synthetic peptides gives access to a large number of model systems with which to study the process of fibril formation in more detail. In particular, developing novel strategies that control the peptide self-assembly, including fibril formation, are attractive in order to understand the pathogenesis of and therapeutics for amyloid diseases. In addition, this is also important as a key technology for the generation of new nanostructured materials with potentially interesting properties.[13−15] In the present study, we describe a novel method for the control of peptide self-assembly using synthetic β-sheet peptides composed of L- or D-amino acid as building blocks. Amyloid-like nanofiber formation and its nanostructure could be regulated by the stereospecificity of the constituent peptide species.

Triblock-type amphiphilic oligopeptides, **1L** and **1D**, were designed to have tetra-L- or -D-leucine domains, which provide the hydrophobic driving force for self-assembly, flanking pH-responsive L- or D-(lysine)$_8$ segments, respectively (Figure 1A). These peptides were prepared by standard solid phase peptide synthesis using Fmoc chemistry and purified by reverse-phase HPLC. We demonstrated previously that the peptide **1L** could be self-assembled into nanofibers upon partial neutralization of the charge of Lys residues at around pH 9.[12] The conformational properties of **1L**, **1D**, and **1L**/**1D** binary mixture were first investigated by means of circular dichroism (CD) spectroscopy at pH 9.0 (Figure 1B). When the sample solutions were freshly prepared, the CD spectra of pure **1L** and **1D** gave mixed patterns of α-helix and random coil structures with two negative and positive maxima, respectively, at 220 and 202 nm (Figure 1B, a and c). The CD measurements revealed gradual changes typical for a β-sheet structure (single maximum at 215 nm) in both cases of **1L** and **1D**, although the chirality of β-sheet structure was opposite and the β-sheet contents were slightly different between **1L** and **1D** (**1L** contains more random structure than **1D**) (Figure 1B, b and d). Figure 1C also plotted the molar ellipticity at 215 nm ($[\theta]_{215}$) as a function of time. With the elapse of time, the $[\theta]_{215}$ values for **1L** decreased sigmoidally and reached a constant value at about 8 h. The time course of the $[\theta]_{215}$ changes for **1D** was similar to that for **1L**, namely, the conformational transition into β-sheet structure was also accelerated at around 4 h and reached saturation at 8 h. These results indicated that both L- and D-peptides formed β-sheet structures through the similar transition kinetics, but with opposite chirality. It should be noted that, at pH 7.0, both **1L** and **1D** were predominantly in the monomeric random coil conformation even after incubation for 24 h, at which the amino groups of Lys residues were protonated (data not shown). On the other hand, when **1L** was mixed with the **1D** at pH 9.0 (in 1:1 molar ratio), the CD spectrum for the binary mixture was almost flat close to the baseline just after sample preparation (Figure 1B, e), and the CD spectra were time-independent within the period of 24 h (Figure 1C). The observed results indicate either that the aggregated structure exists in optically pure macrodomains or that the two enantiomers aggregate non-specifically, or a mixture of these two extremes.

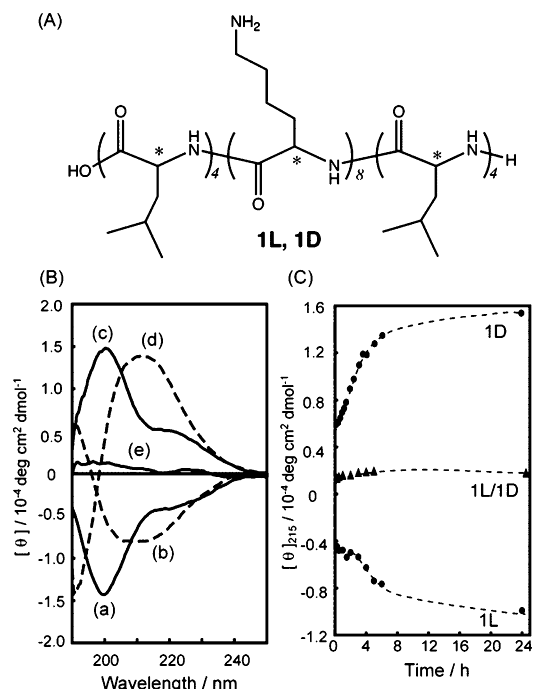

Figure 1. (A) Chemical structures of the enantiomeric amphiphilic peptides, **1L** and **1D**, used for self-assembly. (B) CD spectra of **1L** (a and b), **1D** (c and d), and **1L**/**1D** equimolar mixture (e) in Tris/HCl buffer (containing 5% TFE) at pH 9.0. The spectra were measured just after preparation of sample solutions (a, c, and e) and after incubation for 6 h (b and d). The total peptide concentration was 40 μM. (C) Time dependences of the molar ellipticity at 215 nm ($[\theta]_{215}$) at this condition.

The nanostructures of the peptide assemblies were characterized by atomic force microscopy (AFM). Figure 2 shows the tapping-mode AFM images of **1L**, **1D**, and **1L**/**1D** mixture obtained after incubation for 6 h at pH 9.0. AFM images obtained from pure **1L** and **1D**, in which both peptides took the β-sheet form, revealed the presence of amyloid-like nanofibers (Figure 2A and C). These nanofibers possess nearly uniform diameters of ca. 6 nm and lengths in excess of 1 μm. As shown in Figure 2B (amplitude image),

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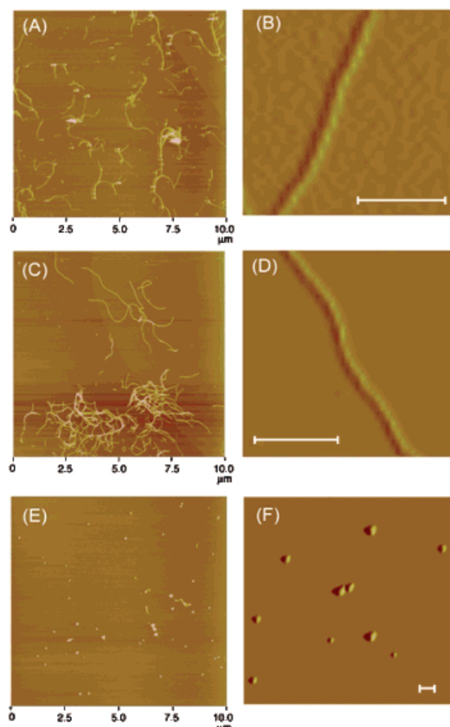

Figure 2. Tapping-mode AFM images of **1L** (A and B), **1D** (C and D), and **1L/1D** equimolar mixture (E and F) obtained after incubation for 6 h at pH 9.0; *z*-scale = 20 nm. Amplitude AFM images corresponding to the **1L** nanofiber (B), **1D** nanofiber (D), and **1L/1D** globules (F), respectively. Scale bar corresponds to 200 nm.

interestingly, the nanofiber made of **1L** has a clearly visible left-handed twist that repeats along the fiber length. The periodicity of this repeat is ca. 50 nm, and the fiber height oscillates between 5.5 and 6.5 nm. This twist and its handedness are probably due to the right-handed twist along the backbone of a β-strand made of L-amino acids.[16] It is considered that the packing of right-handed β-strands causes a left-handed spiral in the β-sheet.[17] On the other hand, the nanofiber formed by **1D** showed identical periodicity and was also a clear twist, but the twist direction was right-handed (Figure 2D). Therefore, it can be concluded that the helicity of self-assembled nanofibers could be easily controlled by the chirality of the amino acids in the constituent peptide species. More interestingly, such nanofiber formation was not observed for **1L**/**1D** mixed system, and the racemic mixture formed only globular aggregates (Figure 2E and F). A stereospecific polymerization into amyloid fibril has been suggested for amyloid β peptide and β_2-microglobulin,[18,19] in which the acceleration of the fibril formation was observed only when the fibrillar seed material was formed by the same enantiomer as the protofibrils. Thus, the cross-reaction between L- and D-peptides into a β-sheet may not be primarily so predominant, probably due to destabilization of the continuous hydrogen bonds between both enantiomers. In our case, it is supposed that the peptides **1L** and **1D** form co-aggregates rapidly, owing to the relatively strong hydrophobic interaction, and the resultant **1L**/**1D** complex at the initial stage probably prevents the control growth of the peptides required for nanofiber formation. In fact, such aggregate formation between **1L** and **1D** was strongly supported by the results of fluorescence resonance energy transfer (FRET) experiments. For the FRET study, we prepared nitrobenzofurazan (NBD)-labeled **1L** and Rhodamine B (RhB)-labeled **1D** to use as donors and acceptors, respectively. Fluorescence spectra of the mixture of **1L**-NBD and **1D**-RhB were measured at pHs 9.0 and 7.0 (see Supporting Information). As a result, efficient FRET was found to occur at pH 9.0, as evidenced by a quenching of the donor emission at 533 nm and an increase in the acceptor fluorescence at 568 nm ($I_{RhB}/I_{NBD} = 2.08$), as compared with the result at pH 7.0 ($I_{RhB}/I_{NBD} = 1.34$), at which both **1L**-NBD and **1D**-RhB were in monomeric random coil form. In addition, FTIR analyses showed that the **1L/1D** mixture took mainly a random coil (ca. 80%) and β-sheet structure (ca. 20%) after 6 h incubation, although the pure peptides formed a β-sheet structure (>70%). These results clearly show the complexation between **1L** and **1D** at pH 9.0 and also agree with the results of AFM and CD studies.

In this communication, we have reported on a method to control the nanostructure of self-assembled nanofibers by the intrinsic chirality of constituent peptide building blocks. We have also demonstrated that complex formation between the enantiomeric peptides at the initial stage for peptide self-assembly depresses subsequent aggregation into amyloid-like nanofiber, even at further incubation of the solution. We believe that this kind of work opens a new vista not only for fabricating novel nanostructured functional biomaterials but also for developing effective therapeutic agents of fibrillogenesis.

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Supporting Information Available: Experimental section, including the synthesis of the peptides used in this study. Fluorescence resonance energy transfer (FRET) experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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