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Thermoresponsive Self-Assembly of Short Elastin-like Peptides

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Short elastin-like penta- and deca- peptides have been found to self-assemble into nanofibers in a temperature dependent way. The peptides were synthesized by solid phase synthesis, modified at the N- and C- terminals respectively by acetylation and mannosylation, and analyzed in terms of thermoresponsive self-assembling. CD spectra of these peptides were indicative of conformational transition irrespective of terminal modification. Dynamic light scattering (DLS) suggested that the peptides formed molecular assembly of micrometer sizes at higher temperatures. The self-assembling transition temperature was dependent on the end group modification. TEM revealed that self-assembling peptides formed ordered supramolecular of nanofiber and sphere in shape. The supramolecules of these short elastin-like peptides will be useful as novel stimuli responsive materials.
Key words: elastin, self-assembly, peptide

1. INTRODUCTION

Material fabrication through supramolecular chemistry is paid much attention[1] to construct ordered nano-materials such as liquid crystals,[2] organogels,[3] and micelles[4] expressing sophisticated functions. These supramolecular assemblies were driven via electrostatic,[5] hydrogen bonding,[6] π-π stacking[7] and hydrophobic interactions.[8] Hydrophobic interactions in amphiphilic molecules are most important in biological processes. For example, amphiphilic peptides form helix bundles in aqueous solutions[9] and lipid bilayers.[10] Amphiphilicity in proteins induces folding based on hydrophobic interaction.

Design and control of the molecular amphiphilicity are important to fabricate supramolecular materials, and the stimuli responsive molecules are unique in terms of amphiphilicity.[11] For example, block copolymers of poly(*N*-isopropylacrylamide)-block-poly(acrylic acid) forms supramolecular structures like micelles with changing environments (temperature, pH and other chemical or physical changes).[12] These stimuli responsive supramolecules are enthusiastically studied and applied to drug delivery, molecular devices and so on.

Polymeric elastin-like peptides $(VPGVG)_n$ (V=*L*-valine, P=*L*-proline, G=glycine) was reported to cause stimuli-response due to temperature dependent coil to β-spiral transition.[13] At lower temperature the peptide is hydrophilic due to hydration of side chains, and at higher temperature the peptide becomes hydrophobic due to the dehydration. Most of works have focused on polymeric elastin-like peptides $(VPGVG)_n$ (n>10), but even short elastin-like peptide pentamer VPGVG was reported to induce stimuli responsive structural transition.[14,15,16] This inspired us to construct stimuli responsive supramolecules with short elastin-like peptides. Here we report the supramolecular formation and the temperature dependence of short elastin-like penta- and deca-peptides $(VPGVG)_n$ (n=1, 2) including those carrying α-D-mannose at the C-terminal and/or acetyl at the N-terminal (Fig. 1). The modification of the end group induces changes in amphiphilicity, which affects the molecular assembling.

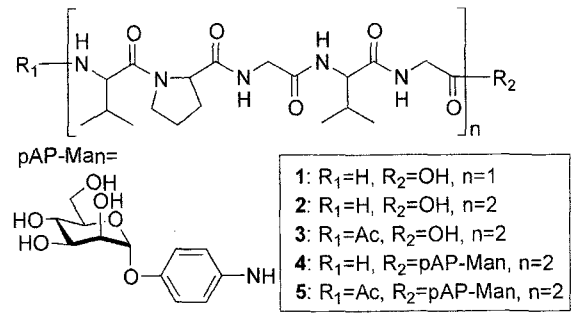

Fig.1 Molecular structure of short elastin-like peptides.

2. EXPERIMENTAL

2.1 Syntheses

Short elastin-like peptides were prepared by a conventional Fmoc procedure on Wang resin with the coupling reagent of BOP and HOBt in DMA. The peptides were removed from resin with 95 % TFA and 5% phenol. The N-terminal protected peptides and *p*-aminophenyl (pAP) α-mannoside were conjugated with HATU. The peptides were characterized by MALDI-TOF-MS (Voyager, Applied Biosystems, USA) and ^{1}H-NMR (Varian Inova 500). The purities were confirmed by HPLC and TLC with two different solvent systems.

2.2 Circular dichroim (CD) spectra

CD spectra were recorded in a JASCO J-730 (JASCO, Tokyo, Japan) with a peltier temperature controller. The spectra were measured every 1 °C with elevating temperature. Data were processed using JASCO spectra manager v.1.51.

2.3 π-A isotherms

The π-A isotherms were collected with Miyata-type moving wall trough NL-BIO-40S-MWC (Nippon Laser & Electronics, Nagoya, Japan) at a constant reducing area of 1.0 cm^2/s. The peptide samples were dissolved in chloroform/methanol (9:1 v/v) at concentration of 1.0 x 10^{-4} M. The peptide solution was spread on the aqueous

phase by using a microsyringe, and equilibrated for 15 min before compression.

2.4 Dynamic light scattering (DLS)

DLS measurements were performed with raising temperature on a DLS-6600HK (Otsuka Electronics, Osaka, Japan) equipped with a 5 mW He-Ne laser. The apparent diameter of the peptide was calculated by cumulant analysis.

2.5 Transmission electron microscope (TEM) observation

The morphology of the peptides was analyzed by TEM using a JEM-2000 (JEOL, Japan). The sample was transferred to carbon-coated grid at 35°C. For negative staining, carbon-coated grids were floated on aqueous peptide solutions at 35°C for 30 sec. After blotting of the grids, the samples were stained with 2% uranyl acetate (pH 7.4).

2.6 Trapping efficiency[17]

The trapping efficiency of calcein in the short elastin-like peptide was determined by the following procedures at 35°C. Peptide (**2**) was dissolved in 2.5 μM calcein solution, and the concentration of the peptides was 1.0 x 10^{-4} M. The peptide solution (300 μL) was heated up to 35°C, and the temperature was kept at least 10 min. Then, calcein existing in the bulk phase was quenched by adding $CoCl_2$ solution (1mM, 7.5 μL). The fluorescence of the peptide solution was measured before and after addition of $CoCl_2$. Subsequently, Triton X-100 (10%, 25 μL) was added to destroy the peptide self-assembly and the fluorescence was measured again. The trapping efficiency of calcein in the peptide solution was calculated according to the following equations,

$$\text{Trapping efficiency (\%)} = \frac{(F_{in} - r \times F_{totq})}{(F_{tot} - r \times F_{totq})} \times 100$$

F_{in}, F_{tot}, F_{totq} and r represent the fluorescence after addition of $CoCl_2$ (the fluorescence from the internal compartment), the fluorescence before addition of $CoCl_2$, the fluorescence after addition of Triton X-100, and the dilution factor.

3. RESULTS and DISCUSSION

3.1 Conformation of peptides

The conformations of the short elastin-like peptides were investigated by CD spectroscopy.[14] The spectra of the peptides were measured from 5 to 55°C. Representative CD spectra of **2** and **4** were depicted in Fig. 2. The spectra of **2** were characteristic of a random coil conformation at low temperature: a minimum at 200 nm, a maximum around 210 nm, and a shallow minimum at 220 nm. With an increase of temperature, the minimum at 200 nm became shallow and the ellipticity at 220 nm was deepened, indicative of the structural change from random coil to type II β turn. The presence of isosbestic point at 214 nm suggests the conformational transition with temperatures. The CD spectra of **1** and **3** were similar to that of **2**. These results indicated that CD spectra of the short elastin-like peptides without mannosylation gave the similar profile to those of poly(VPGVG).

On the other hand, mannosylated peptide **4** showed negative peaks around 200 and 220 nm, and an isosbestic point at 214 nm. The peak at 220 nm was deepened with

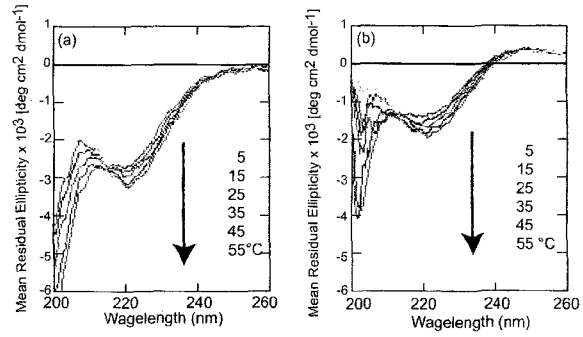

Fig. 2 CD spectra of (a) peptide **2** and (b) peptide **4** from 5 to 55°C at 2.0 x 10^{-4} mol/L

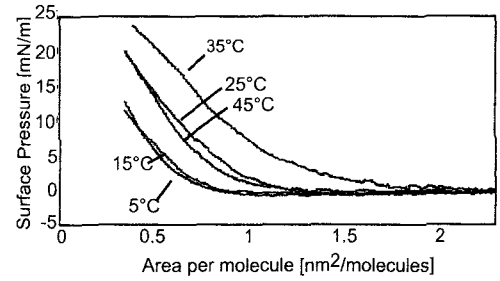

Fig. 3. π-A istherms of peptide **2** from 5 to 45°C.

raising temperature similarly to **2**, but the ellipticity was much smaller than that of **2**, and the peak shape at 200 nm and 250 nm was different. The peaks in CD spectra at 200 and 250 nm were due to the phenyl group in the peptide. The CD spectra of **5** were similar to that of **4**. Despite the difference of CD spectra between **2** and **4**, the stretching peaks of carbonyl (C=O) in FTIR were almost the same, indicating that the conformation of mannosylated peptide (**4**) was similar to that of short elastin-like peptide of **2**.[16, 19]

3.2 π-A isotherms

We studied supramolecular formation of short elastin-like peptides. First we investigated the Langmuir layer formation of peptide **2** at the air-water interface with changing temperatures (Fig. 3).[20, 21] The maximum surface pressure of peptide **2** was below 15 mN/m at 5 and 15 °C. The isotherm was shifted to larger molecular area with raising temperature to 25 and 35 °C. At 45°C, the π-A isotherm shrank again compared to that at 35°C. Expansion of the isotherm occurred mainly from 15 to 35 °C due to an increase of hydrophobicity, and shrinkage occurred from 35 to 45 °C due to a change of molecular packing. The π-A isotherms suggested the formation of thermoresponsive supramolecules at the air-water interface.

3.3 DLS measurements

Thermoresponse of pseudo-diameters (size of self-assembly) of peptides in aqueous solution was investigated by DLS (Fig. 4). Elastin-like peptides **1** and **2** increased their diameter around 30 °C. Theremoresponsive self-assembly of peptide diameters was consistent with conformation changes seen in the CD spectra. Diameter change of 10-mer peptide **2** was more

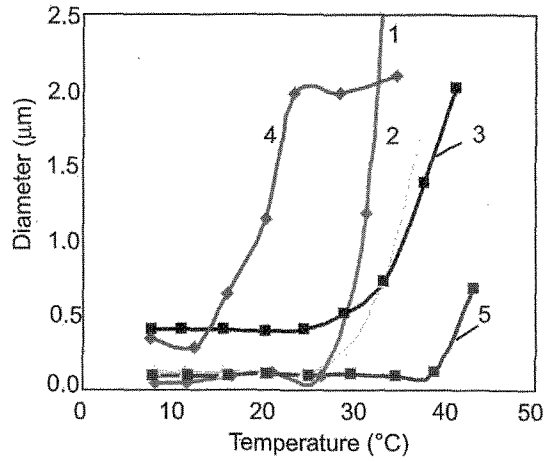

Fig. 4 Temperature dependence of the molecular size estimated by DLS at 1.0 x 10^{-4} mol/L.

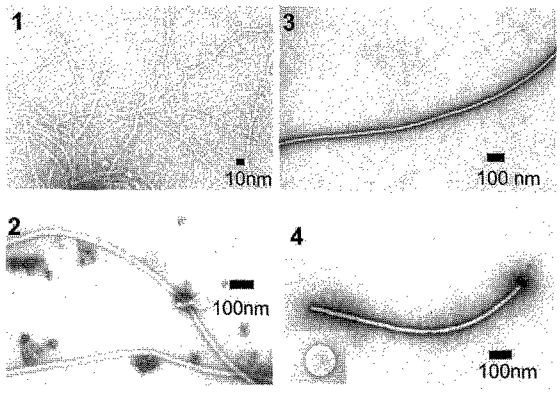

Fig. 5 TEM observation cast at 35°C, and stained with 2% uranyl acetate.

sensitive to temperature than that of 5-mer peptide **1**, and the diameter of **2** became larger than that of **1**, indicating molecular interactions depending on peptide chain length.[22]

Self-assembling of the modified elastin-like peptide **3**, **4** and **5** in aqueous solution were also examined by DLS.[22] Peptides **3** and **4** spontaneously formed molecular assemblies of 400-500 nm diameter at room temperature. Diameters of peptide **3**, **4** and **5** increased with heating, but the diameter change of mannosylated peptide **4** and **5** occurred at different temperatures from **1**, **2** and **3**. Modifications of the N- and C-terminals affected intramolecular interactions, resulting in change of self-assembling temperatures.

3.5 TEM analyses

The morphology of the self-assembly of elastin-like peptides was observed by transmission electron microscope (TEM) (Fig. 5). The peptides in aqueous solution were cast on TEM grid at 35°C and stained with 2 % uranyl acetate.

Short elastin-like peptides without modification (**1** and **2**) formed homogeneous nanofiber along long axis. The width of 10-mer **2** was 18-20 nm, and the lengths were in the micrometer order. The width of 5-mer **1** was 6.5 nm and smaller than **2**. The nanofiber formation of **1** and **2**

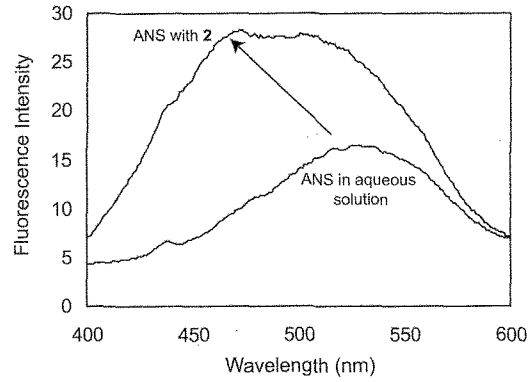

Fig.6 Fluorescence spectra of **2** containing environmental fluorescent probe of ANS (**2** : 10 mM, ANS : 0.1 mM, Excitation wavelength : 380 nm) .

clearly showed the self-assembling property of short elastin-like peptides and the dependence on the peptide chain length to self-assembly.

The morphology of the modified elastin-like peptides (**3**, **4** and **5**) was also examined. It was revealed that **3** formed nanofiber with 19-20 nm width and micrometer order length like **2**, that **4** formed nanofiber with 20 nm width and spherical objects with about 130 nm diameters, and that **5** formed non-uniform spherical objects. Morphology of **1**, **2**, **3** and **4** indicated that short elastin-like peptides tend to form uniform nanofiber which is similar to the elastin-like biopolymers.[23, 24, 25] Peptides of 10-mer **2**, **3** and **4** (except **5**) formed the uniform nanofiber with about 20 nm width despite terminal groups. The width of the nanofiber was depending on the peptide chain length. The spherical assembly of **4** and **5** suggested that the terminal mannosylation changes the morphology of the peptide from nanofiber to spherical assembly. These results suggested that the terminal modification affects the self-assembling property of the elastin-like peptide.[14] The control of the self-assembling property by adding amphiphilicity is under way.

3.4 Physicochemical analyses of self-assembling property.

A hydrophobic (ANS)[3] and a hydrophilic (calcein)[17] fluorescent probes were used to detect the self-assembling mechanism and structure. When ANS was added to peptide **2** in aqueous solution, the emission maximum shifted from 530 nm to 470 nm (Fig. 6). In contrast, calcein was added to the solution of **2** to detect the existence of inner water layer. Trapping efficiency of calcein with peptide **2** at 35°C was 11 %. The fluorescent probes showed the supramolecular formation by the hydrophobic interaction of the elastin-like peptide with wrapping inner water layer. Large van der Waals interactions of peptide side chains and hydrogen bonding formation of amide may also contribute the self-assembly.

4. CONCLUSION

We have demonstrated that short elastin-like peptides responded to temperature and self-assembled into uniform nanofibers. Peptides self-assembled into

nanofibers in the micrometer-order, while mannosylated peptides formed fibers and spherical objects. Morphology of peptide self-asssmbly was dependent on peptide chain length and amphiphilicity. The terminal mannoslyation of the peptide changed the self-assembling structure, and gave the lectin recognition abilities. Short elastin-like peptides are promising building blocks for the construction of well-defined stimuli responsive supramolecules.

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