A novel polypseudorotaxane composed of cyclic β-peptide as bead component†

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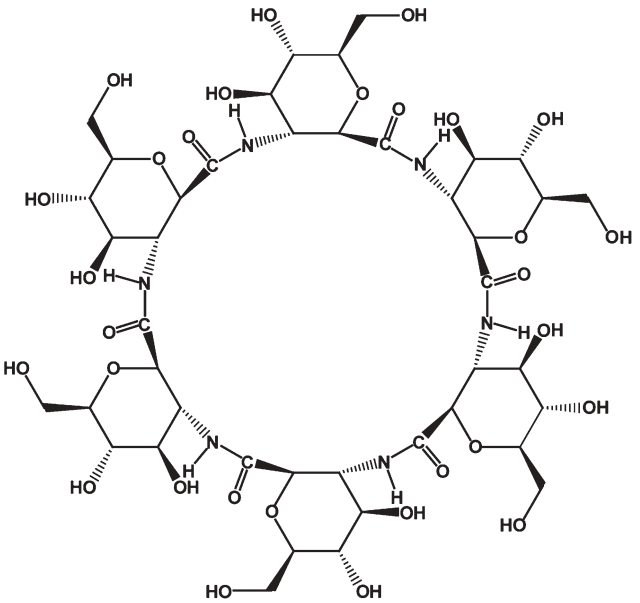
Received (in Cambridge, UK) 6th December 2006, Accepted 1st February 2007
First published as an Advance Article on the web 13th February 2007
DOI: 10.1039/b617768a

A polypseudorotaxane composed of cyclic hexamers of β-glucosamino acid and poly(ethylene glycol) (PEG) was constructed, where the beads were stacked unidirectionally together *via* hydrogen bonds to form a peptide nanotube.

Nano size molecular architecture has been of great interest in recent science and technology.[1] Polyrotaxane, in which cyclic molecules called "beads" are threaded by a linear "string", are attractive due to their unique properties. A variety of applications of rotaxanes have been reported. For example, a rotaxane having cyclobis(paraquat-*p*-phenylene) showed redox-activated switching and acted as an efficient electron mediator.[2] Another polyrotaxane having cyclodextrins was applied to gene delivery due to the effective binding to a plasmid DNA.[3] Since the properties of rotaxanes are strongly dependent on the kind of bead component, various beads, for instance, cyclodextrins,[4] crown ether,[5] cucurbiturils,[6] and cyclic α-peptide,[7] have been extensively investigated for polyrotaxanes.[8]

On the other hand, we have focused our attention on the cyclic β-peptides,[9] especially those composed of β-amino acids having a cyclohexane or pyranose ring on the side chain. These cyclic β-peptides spontaneously stack together to form a tubular structure *via* intermolecular hydrogen bonds between amide groups.[10] Notably, the cyclic peptides align in a unidirectional orientation in the nanotube. It should be challenging and interesting to thread the peptide nanotube with a polymer chain, because the assembly may be stable without stoppers due to the multivalent interactions between the nanotube and the polymer chain. In this case, the large association energy between the bead and the string will not be required for threading, in contrast to general rotaxane formation using strong binding mutual recognition sites on each component (*e.g.* electrostatic or hydrogen bond interaction). We chose a cyclic hexa-β-peptide of β-glucosamino acid because of its large inner pore of 5.1 Å and its water solubility. Hydrophobic interactions may force a poly(ethylene glycol) (PEG) chain to reside in the nanotube of the cyclic hexa-β-peptide. Another unique feature of this polypseudorotaxane is its large dipole moment due to the unidirectional stacking of the cyclic peptides. The molecular architecture with a dipole will have interesting applications such as a vectorial electron mediator as shown with helical peptides.[11]

The novel cyclic hexa-β-peptide **1** was synthesized from the acetyl protected hexamer[12] by deprotection. The product was identified by NMR and MALDI-TOF MS.[13] The cyclic trimer composed of the same sugar units was soluble only in H_2O, while compound **1** was widely soluble in polar solvents such as DMSO, DMF, TFA, MeOH and H_2O.



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The FT-IR spectrum of **1** in the solid state showed amide I (C=O stretching mode) and amide II absorption (mainly N–H bending and C–N stretching modes) at 1675 cm^{-1} and 1539 cm^{-1}, respectively. The appearance of the amide II band indicates that the amide groups in compound **1** took the *trans* configuration.

The ^1H NMR spectrum of **1** in DMSO-d_6 at room temperature showed two signals for N–H and O–H at C-3. However, it is notable that the other protons showed only one signal. This indicates that **1** takes two C_6 symmetric conformations which are similar structures in most parts. Indeed, computational geometry optimization of **1** using Molecular Mechanics program 2 (MM2) and the semiempirical Austin Model 1 (AM1) method revealed that two types of intramolecular hydrogen bonds are possible for the planar C_6 symmetric conformations; one is between O–H at C-3 and pyranose oxygen and the other between O–H at C-3 and O–H at C-6.[13] According to NMR measurement at elevated temperature, these two conformations were averaged on the NMR

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† Electronic supplementary information (ESI) available: Experimental details of synthesis and characterization. MALDI-TOF MS spectrum of cyclic peptide **1**. NMR spectra of **1**. Result of geometry optimization. CD spectra of **1** and the mixture of **1** and PEG. AFM image of **1**. See DOI: 10.1039/b617768a

timescale to give signals of one C_6 symmetric conformation.[13] On the basis of the molecular model of the C_6 conformation, the diameter of the inner pore was estimated to be 5.1 Å.[13] Since the van der Waals diameter of a PEG chain is 3.1 Å,[14] it is expected that these two components will form an inclusion complex as shown with cyclodextrins and a PEG chain (Fig. 1).

Circular dichroism (CD) and ^{1}H NMR spectroscopies were used to study polypseudorotaxane formation between **1** and a PEG chain. First, the nanotube formation of **1** was examined by changing the solvent composition from water to a water–ethanol solution (1/1 v/v). A shift of the peak wavelength to a longer wavelength and an increase of the peak intensity were observed transiently in a range between 15% and 30% of ethanol content.[13] This result suggests that the ethanol addition induced a transition from a monomeric solution to a molecular assembly of the cyclic peptides. The NMR measurement of a solution of water and ethanol (1/4 v/v) of **1** showed signal broadening especially at the H-3 proton, which also agrees with the interpretation of the assembly formation.[13]

Second, polypseudorotaxane formation was examined by using PEG of average molecular weight 600 (PEG600). The addition of PEG600 to a solution of **1** in water and ethanol (1/1 v/v) did not change the CD spectra immediately after the mixing, indicating no threading of PEG to the peptide assembly. On the other hand, when PEG was added to an aqueous solution of **1**, signal intensities in CD spectra decreased dramatically, suggesting threading of a PEG chain into the cyclic peptides.[13] The decrease of signal intensities is ascribed to precipitation of the peptides even though the solutions for the CD measurements were apparently transparent. The precipitation could be induced by dehydration of the peptide assembly by absorption of PEG chains on the surface of the assembly. However, this possibility is excluded by the following observations: i) a small amount of PEG was enough to observe the CD change, and ii) even in a solution of water and ethanol (1/1 v/v) the CD signal intensities decreased after treatment of the mixture of the cyclic peptide and PEG with sonication and standing at room temperature for 24 h. These results indicate that a PEG chain can hardly thread into a tubular structure of **1** formed in advance in a solution of water and ethanol, but a polypseudorotaxane should be easily formed in water *via* threading sequentially one cyclic peptide after the other. Further, the

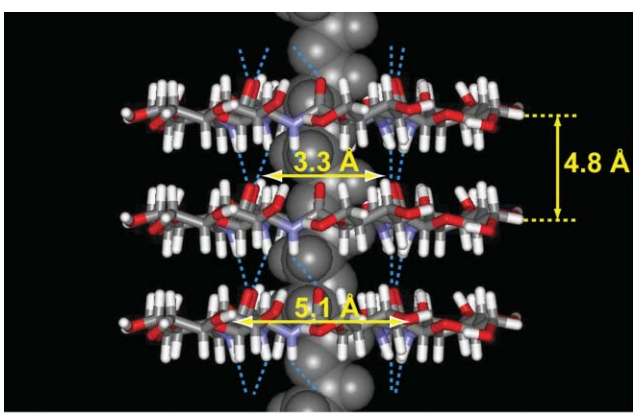

Fig. 1 3-D model of the novel polyrotaxane composed of cyclic peptide **1** and a PEG chain (shown in grey). Blue dashed lines represent the hydrogen bonds between amide groups of the cyclic peptide.

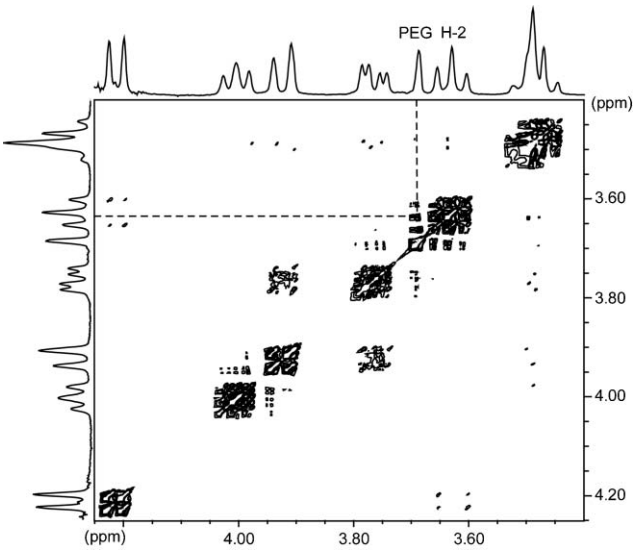

Fig. 2 2-D NOESY spectrum of the complex of **1** and PEG600 in water.

threading was confirmed by a two-dimensional NOESY NMR experiment of a mixture of PEG600 and **1** in water (Fig. 2). The spectrum clearly showed a cross-peak between the H-2 proton of **1** and the methylene protons of PEG, indicating the spatial closeness between these protons due to the threading of a PEG chain into the cyclic peptide.

PEG of average molecular weight 6000 (PEG6000) could also induce the CD change of **1** in water. The solution was deposited on a mica substrate and subjected to high resolution atomic force microscopy (AFM) under vacuum. Fig. 3(a) exhibits the AFM topographic image, which shows a few rod-shaped clusters in a

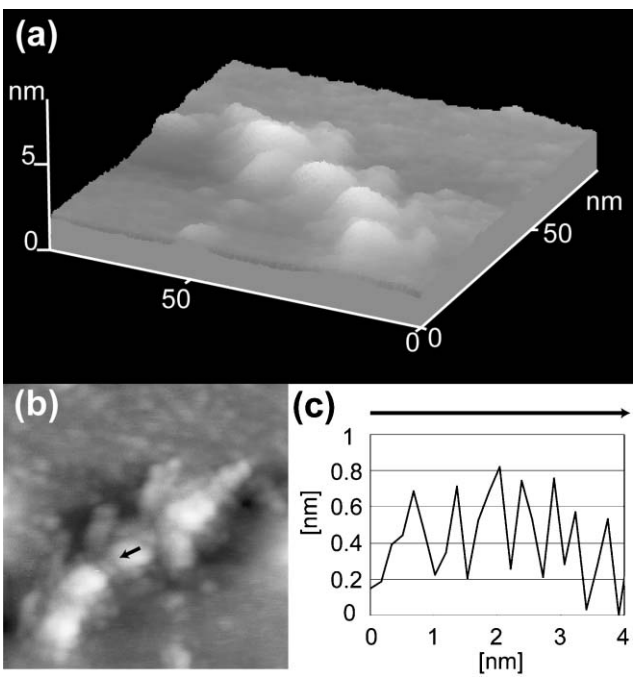

Fig. 3 (a) 3-D and (b) 2-D AFM images (81 nm × 81 nm) of the prepared polypseudorotaxane; (c) the height profile along the arrow shown in (b).

series with a length of *ca.* 50 nm in total and a height of 2 nm. These values agree well with the length of PEG6000 and the outer diameter of cyclic peptide **1**, respectively. Further, the height profile of one of the rod-shaped clusters revealed a periodicity of 0.5 nm (Fig. 3(c)), which just coincides with the stacking distance between the cyclic β-peptides in a nanotube structure.[10] On the other hand, AFM observation of compound **1** in the absence of PEG revealed disk-like assemblies with a diameter of *ca.* 30 nm and a thickness of 1 nm; cyclic β-peptides should lay down on the mica surface to form a double layer. Taken together, a polypseudorotaxane is formed by the peptide nanotube of **1** with a PEG chain as a string.

In summary, we prepared a novel polypseudorotaxane composed of a PEG chain and cyclic hexa-β-peptides having sugar units. A notable feature of this polypseudorotaxane is the self-assembling of the beads into a peptide nanotube. In the peptide nanotube, the beads align in a unidirectional orientation to form a large dipole moment. It will be interesting to prepare a polypseudorotaxane of the cyclic hexa-β-peptides and a conducting polymer, which may show a high electronic conductivity with a diode property due to the large electric field generated by the peptide nanotube. The possibility of replacement of PEG with other polymers is now under investigation.

This work is supported partly by Grants-in-Aid for Scientific Research B (18350063), and 21st century COE program, COE for an approach to New Materials Science, from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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