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Columnar Assembly Formation and Metal Binding of Cyclic Tri-β-peptides Having Terpyridine Ligands

Futoshi Fujimura[†] **and Shunsaku Kimura***[,†]

Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Kyoto-Daigaku-Katsura, Nishikyo-ku, Kyoto 615-8510, Japan

shun@scl.kyoto-u.ac.jp

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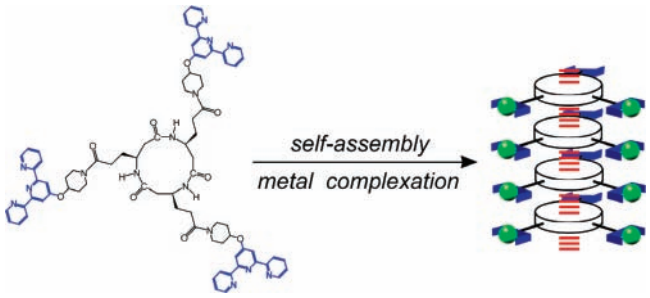
ABSTRACT



A novel cyclic tri-β-peptide having terpyridine (tpy) metal ligands was synthesized to investigate its assembly formation and metal complexation. Microscopic observation revealed that this cyclic peptide formed a rod-shaped molecular assembly. The assembly was able to bind Cu(II) because the tpy ligands covered the surface of the crystal, keeping the tpy plane parallel to the ring plane of the cyclic tri-β-peptide.

Organic molecular nanowires fabricated by molecular self-assembly have attracted considerable attention in recent years for possible applications to nanoelectronics.[1] Molecular nanowires are required to be controllable in size and length. In light of this point, peptide nanotubes (PNTs) composed of cyclic peptides stacking in a column via intermolecular hydrogen bonds are one of the most suitable molecular objects, because PNTs have several variables for molecular design such as ring size, α-,[2] β-,[3] δ-,[4] and ϵ-peptide,[5] and side chains, which make the molecular assemblies adjustable for each purpose. We previously demonstrated parallel assembly of dipolar columns of cyclic tri-β-peptide,[6] which is composed of three *trans*-2-aminocyclohexylcarboxylic acid (ACHC) residues familiarized by Gellman et al.[7] Since all amide groups of PNTs composed of cyclic β-peptides orient

* To whom correspondence should be addressed. Tel: +81-75-383-2400. Fax: +81-75-383-2401.

[†] Department of Material Chemistry, Kyoto University.

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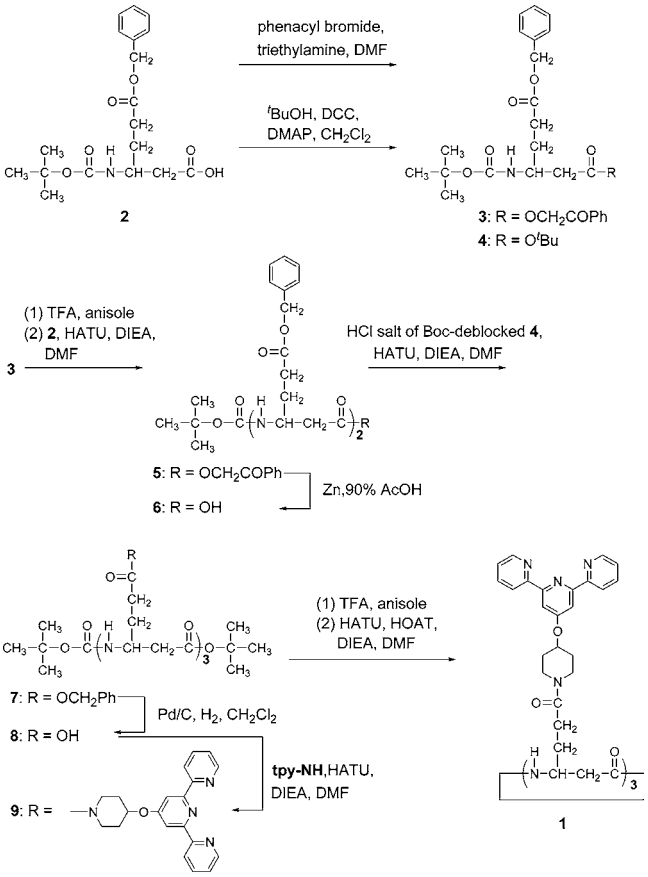
in parallel to the tube axis, a large dipole will be generated in the PNTs, which may have a potential application to molecular devices. However, PNTs generally possess low electronic conductivity,[8] which may restrict their application. To improve the electronic conductivity of PNTs, incorporation of π-systems and/or metal ions to the PNT may be effective. Therefore, the PNT bearing the π-systems with the ability to form a metal complex was prepared in this work. 2,2′:6′,2″-Terpyridine (tpy) was chosen as the ligand. Tpy is one of the most versatile building blocks in supramolecular chemistry as demonstrated by Lehn et al.[9] This tridentate ligand is able to form complexes with various transition metal ions with high binding constants.[10] The present paper reports on the molecular assembly formation and metal binding property of cyclic tri-β-peptide having tpy ligands at the side chains.

Peptide Design and Synthesis. Metal ions such as Ru(II) and Fe(II) form complexes with tpy ligands preferably taking six-coordinate geometry. In the present study, this geometry will put a severe constraint on a tubular structure of stacking cyclic tri-β-peptides when two tpy ligands of adjacent cyclic peptides in the tube are forced to accommodate the metal ion. We therefore selected Cu(II), which prefers four- or five-coordinate geometry with one tpy ligand.[11] The long piperadine spacer was inserted between the cyclic skeleton and the tpy ligand in the molecule to avoid steric hindrance and electrostatic repulsion between the neighboring ligands either in free or complex form. When these two factors are avoided, the cyclic tri-β-peptide will take a planar structure including the side chain keeping the regular PNT structure even after complexation. The cyclic tri-β-peptide having three tpy ligands was prepared by a liquid-phase method (Scheme 1). Boc-[β-Glu(OBzl)]-O^{t}Bu and Boc-[β-Glu(OBzl)]-Opac were synthesized from [β-Glu(OBzl)]-OH. Chain extension reactions to the linear di- and tripeptides were carried out using HATU as a coupling reagent. In the case of the synthesis of the trimer, the Boc group of Boc-[β-Glu(OBzl)]-O^{t}Bu was selectively removed without cleavage of *tert*-butoxy ester by 4 N HCl-dioxane according to the previous report.[12] After the chemical conversion from benzyl ester to the tpy derivative at the side chain and removal of the protecting groups at the both terminals of the main chain with treatment of TFA, cyclization reaction was carried out using HATU, HOAT and DIEA in DMF (0.1 mM). ^{1}H NMR spectrum of the trimer in DMSO-d_6 clearly indicates that the predominant conformation is C_3 symmetric on a NMR time scale. The spin coupling constants between amide proton and H$_\beta$ in the cyclic skeleton was found to be 9.0 Hz, which gives the dihedral angle of θ (H−N−C$_\beta$−H$_\beta$) of 172° according to the Karplus equation.[13] The dihedral angle indicates the anti relationship between the NH and H$_\beta$, which is similar to the result of the other cyclic tri-β-peptide.[6,14]

Scheme 1. Synthetic Route of Cyclo[β-Glu(tpy)$_3$][a]



[a] Abbreviations: DMAP, *N*,*N*-dimethylaminopyridine; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium haxafluorophosphate; DIEA, *N*,*N*-diisopropylethylamine; HOAT, 1-hydroxy-7-azabenzotriazole; tpy-NH, 4′-(2-piperidin-4-yloxy)-2,2′:6′,2″-terpyridine.

Assembly Formation. The trimer showed high solubility for a variety of mixed solutions of polar and nonpolar solvents such as $CHCl_3$−MeOH, THF−CH_3CN, and even THF−H_2O.[15] This unprecedented solubility of the cyclic tri-β-peptide may be ascribed to high solubility of tpy ligands and the difficulty in formation of intermolecular hydrogen bonds to grow into a tubular structure probably due to the introduction of the bulky tpy ligands at the side chains. However, we succeeded in preparing a straight needle-like assembly under a proper assembling condition. Figure 1 shows the crystals of cyclo[β-Glu(tpy)$_3$] which were recrystallized from THF−CH_3CN. When the crystals were ob-

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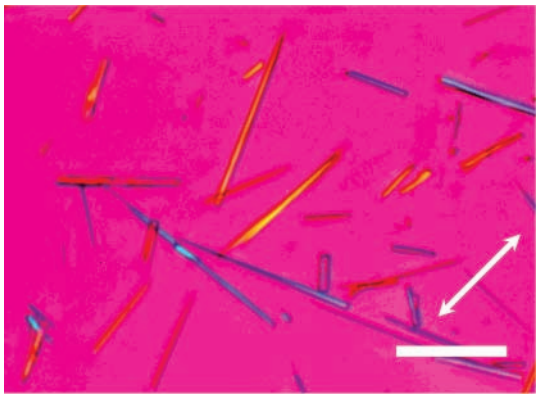

Figure 1. Optical microscopic image of cyclo[β-Glu(tpy)$_3$] crystals under the cross-nicol configuration with a sensitive tint plate. The double-headed arrow shows the orientation of z' axis of a tint plate. Scale bar = 100 μm.

served under the cross-nicol configuration with a sensitive tint plate, they displayed the orange or yellow color in the case that the long axis of the crystal coincides with the z' axis of the tint plate and blue color in the case that they are perpendicularly oriented. The result indicates that the refractive index along the short axis of the crystal is larger than that along the long one. There are two possible chromophores in the molecule, making the refractive index large: amide groups and tpy ligands.

The crystal structure was analyzed by electron diffraction. The diffraction pattern obtained by the incident electron beam perpendicular to the rod axis showed a unit cell with an axial spacing of ca. 4.8 Å, which is agreeable with the interpretation that the cyclic tri-β-peptides stack up to form a tubular structure through 1-D intermolecular hydrogen bond network as described with other cyclic β-peptides in the previous reports.[6,14] Amide groups in the crystals should therefore

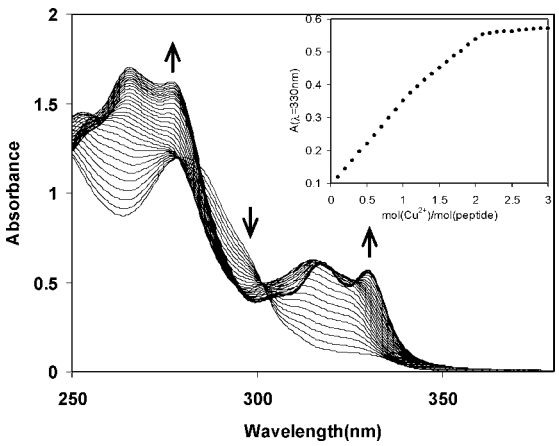

Figure 2. UV−vis absorption spectra obtained during the complexation reaction between cyclo[β-Glu(tpy)$_3$] and Cu(II). The inset shows the increases in absorbance at 330 nm.

align along the long axis to contribute to the refractive index along the long axis. Then, in order to explain the larger refractive index along the short axis of the crystal, the tpy ligands should orient perpendicular to the long axis of the crystal with keeping the tpy plane in the ring plane of the cyclic tri-β-peptide. The latter condition is indispensable for the cyclic peptides to stack up without steric hindrance. Further, the columnar stacking of the tpy ligands with this style should be stabilized by π-π interaction in the tubular structure.

Metal Binding Property. To examine the ability of the tpy ligands in cyclo[β-Glu(tpy)$_3$] to bind Cu(II), the complex formation was studied by UV−visible absorption spectroscopy. A typical spectral change in the UV−vis absorbance of the peptide solution upon the addition of $CuCl_2$ is shown in Figure 2. When each tpy ligand in cyclo[β-Glu(tpy)$_3$] forms complex with Cu(II) independently, the stoichiometry of the complex should be $mol_{peptide}/mol_{metal}$ = 1:3. However, the absorbance increase at 330 nm with the addition of Cu(II) shows saturation at the addition of two equivalents of Cu(II) as shown in the Figure 3 inset. On the other hand,

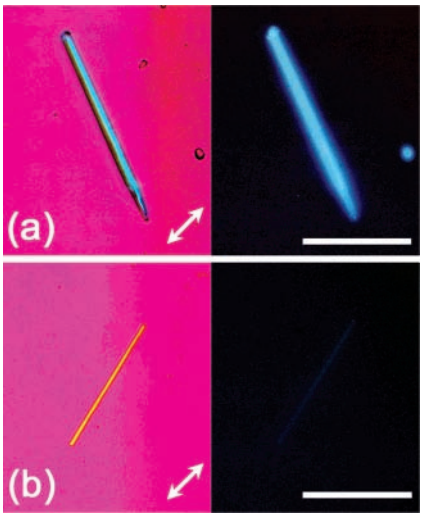

Figure 3. Cross-polarized optical (left) and fluorescence (right) microscopic images of cyclo[β-Glu(tpy)$_3$] crystals with (a) and without (b) Cu(II). The double-headed arrow shows the orientation of z' axis of a tint plate. Scale bar = 100 μm.

the titration using a reference compound (4′-(2-piperidin-4-yloxy)-2,2′:6′,2″-terpyridine/tpy-NH) and Cu(II) shows complexation of a 1:1 ratio at the same concentration range. The tpy groups of the cyclic tri-β-peptide are confirmed to be involved in the complexation with Cu(II), because the absorbance around 710 nm, which is assigned to the metal−ligand charge-transfer band of the complex type of [Cu(tpy)]-Cl_2, appeared and increased the intensity until the addition of two equivalents of Cu(II).[16] The unusual stoichiometry of the 1:2 complex was also observed by MALDI-TOF/MS spectroscopy, which showed a signal of $[(M + (CuCl_2)_2 + H)^+]$ (m/z = 1638) as a highest molecular mass component

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for the sample prepared by mixing peptide **1** and $CuCl_2$ at molar ratio of 1:3.3. Since replacement reaction of two Cl atoms in $[Cu(tpy)]Cl_2$ with another tpy is considered unlikely to occur under the normal condition,[11] the complex formation with more than two cyclic peptides can be excluded. The third tpy ligand of cyclic peptide after complexation with two Cu(II) ions is thus somehow disturbed. Increase of the total plus charges of the complex may reduce the binding ability of the third ligand. Or the carbonyl groups in the cyclic skeleton could participate in the complexation. The reason remains to be solved. However, it is notable that $[Cu(tpy)]Cl_2$ takes a trigonal bipyramidal geometry with ca. 4 Å in length between two Cl atoms, which is less likely to affect the molecular stacking of cyclic peptides because the distance between adjacent cyclic peptides in the column is 4.8 Å.

Construction of PNT with Metal Complexes on the Surface. The surface of nanotube assemblies of cyclic tri-β-peptide should be covered with tpy ligands, which will provide binding sites for Cu(II). The complex of the peptide nanotube with Cu(II) is interesting in terms of the conductive property in a shape of the nanorod. The complex formation was analyzed by fluorescence microscopy. As shown in Figure 3, the crystals of cyclic tri-β-peptide were observed as fluorescent rods with excitation around 330 nm due to the tpy ligands. However, after the crystals were immersed in a MeOH solution of $CuCl_2$, they were weakly fluorescent, keeping the rod structure, indicating complexation of Cu(II) on the surface of the nanorods to quench the emission from tpy ligands. Since the observed rod structure was an assembly of many PNTs, tpy ligands at the interior of the bundle could not form complex with Cu(II). However, Figure 3(b) shows that the fluorescence of the assembly was quenched drastically. The result suggests that the tpy ligands located only at the external surface may be intensively fluorescent and the tpy ligands at the internal region are self-quenched due to high local concentration.

The observation suggests clearly that the molecular column of cyclic peptide having tpy ligands can bind Cu(II) without disruption of the original shape. Interestingly, under the optical microscope observation with polarizers and a tint plate, the anisotropic optical retardation properties were not changed upon complexation with Cu(II), indicating that the orientations of amide groups and tpy ligands in the assembly were not affected by metal binding at the exterior of the nanorods. Because Cu(II) prefers four- or five-coordinate geometry with a tpy ligand as described above, the binding of this metal to the columnar assembly did not destroy the intermolecular hydrogen bonds in the assembly. We tried to prepare a PNT with metal complex from Cu(II)-coordinated cyclo[β-Glu$(tpy)_3$], but regular assemblies were not obtained, suggesting that the carbonyl groups in the cyclic skeleton may participate in the complexation with Cu(II) in a solution to disturb formation of a PNT when the cyclic peptide was in advance incubated with Cu(II).

In summary, we synthesized the novel cyclic β-peptide having tpy ligands and investigated its assembly formation and metal binding property. The optical microscopic observation reveals that the trimer forms rod-shaped crystals and that amide groups and tpy ligands orient parallel and perpendicular to the rod axis, respectively. Moreover, fluorescence microscopic observation suggests that tpy ligands in the assembly bind Cu(II) ions. Since the prepared assembly may possess both high electronic conductivety and a large dipole, this assembly may have a variety of applications in the organic electronics field. This is now under investigation.

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Supporting Information Available: Synthetic details, characterization data, and ^1H NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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