

Nanoribbons Self-Assembled from Triblock Peptide Polymers and Coordination Polymers**

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Triblock peptide copolymers (made by biological expression of a designed DNA template), organic bisligands with two terdentate groups, and Zn^{2+} ions associate spontaneously into well-defined nanoribbons (width: 20–25 nm; thickness: 2–3 nm; lengths in the μm range) when dissolved at proper ratios in water at moderate pH values. These nanoribbons are stabilized by an extraordinary concerted effect of distinct noncovalent interactions: metal–ligand complexation (yielding supramolecular coordination polymers), hydrogen bonding, hydrophobic interactions (supporting secondary and tertiary structure of the peptide copolymer), and polyelectrolyte complexation between coordination polymers and the charged blocks of the peptide copolymers. Combining complementary experimental results and established knowledge on related peptide polymers, we have arrived at a model for the molecular structure of the nanoribbons.

Self-assembly and spontaneous formation of complex, well-defined structures driven by noncovalent interactions has become a major theme in chemistry, material science, and

nanotechnology.^[1] Much of the inspiration for synthetic building blocks, supramolecular structures, and nanostructured materials comes from biology. For example, one of the most successful “work horses” of supramolecular chemistry, the ureidopyrimidinone quadruple-hydrogen-bonding group,^[2] was inspired by the nucleotide bases. Inside and between natural proteins a subtle interplay of many different noncovalent interactions yields an enormous variety of well-defined molecular structures and aggregates. It is attractive to utilize naturally occurring peptide sequences, with known noncovalent interactions, to create new supramolecular structures. Because of recent developments in molecular biology, it is now feasible to design and produce new peptide copolymers by biological expression of a designed DNA template.^[3–6] The peptide copolymers can be regarded as intermediates between synthetic polymers and natural proteins. As their amino acid sequence can be tailored, they can be regarded as interesting models for natural proteins, in which the degree of complexity can be controlled. Moreover, these monodisperse, perfectly defined copolymers may also prove useful as constituents of nanostructured materials.

Herein we discuss the spontaneous formation of well-defined supramolecular nanofibers, in which a “biosynthetic” polypeptide is a key constituent. We describe the self-assembly of nanoribbons based on a combination of a biosynthetic peptide polymer and an oppositely charged organometallic supramolecular polymer.

The triblock peptide copolymer **1** (Scheme 1 a, $\text{pI} = 6.86$ where $\text{pI} = \text{pH}$ at isoelectric point) was produced by expression of a designed DNA template.^[6] The two outer blocks, which are nearly entirely neutral and hydrophilic, form random coils in aqueous solutions at any pH value. The middle block is positively charged at low pH ($\text{pH} < \text{pI}$) owing to the presence of a histidine residue at every eighth position. It is designed to fold into β sheets, with the histidine residues at the turns. The Tirrell group pioneered biosynthetic polypeptides, which are quite similar to our middle block, but instead of histidine residues they incorporated other amino acids.^[4,5]

The ligand consists of two terdentate ligand groups (L, pyridine-2,6-dicarboxylic acid) connected at the 4-position of the pyridine ring by a spacer of four ethylene oxide (EO) units (deprotonated form: $\text{L}_2\text{EO}_4^{4-}$, Scheme 1 b). This compound was synthesized as described in the literature.^[7,8] Divalent metal ions such as Zn^{2+} can be coordinated by two negatively charged terdentate ligand groups. Overall, such a complete coordination unit carries a 2– charge, as illustrated in Scheme 1 c. In aqueous solutions of a 1:1 mixture of Zn^{2+} and $\text{L}_2\text{EO}_4^{4-}$, linear coordination polymers with a high degree

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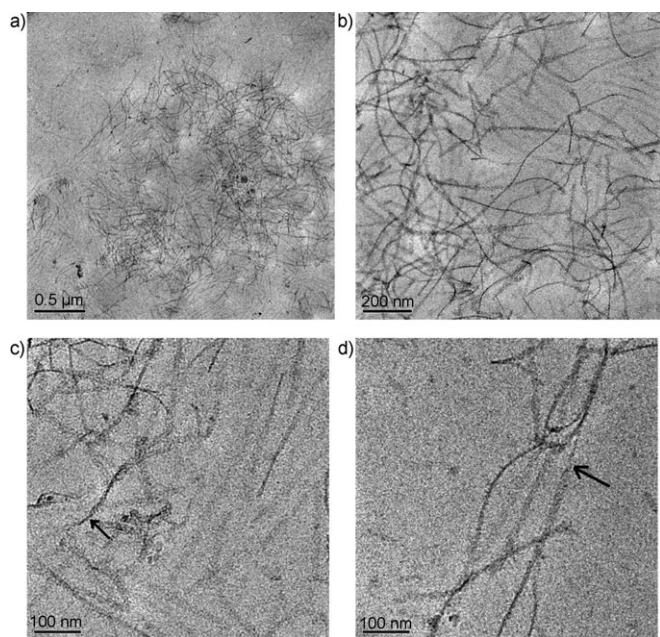
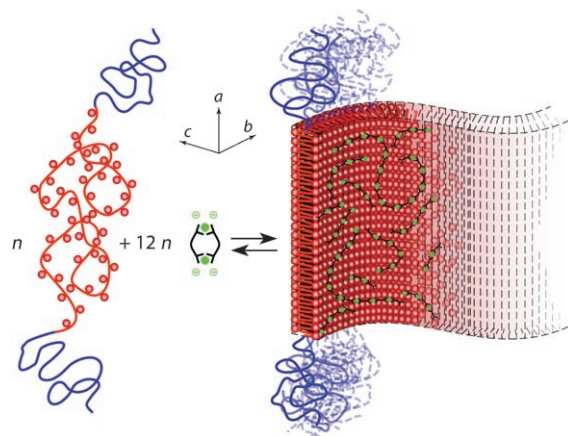


Figure 2. Cryo-TEM images of the mixture of **1** and $[\text{Zn-L}_2\text{EO}_4]^{2-}$ at $f_- = 0.5$ and pH 5.4, in which the concentration of **1** is 0.65 g L^{-1} ($[+] = 0.47 \text{ mM}$). a) Overview of ribbons. b–d) Enlarged views of the ribbons. The arrows in (c) and (d) indicate twisting of the ribbons.

modes. The persistence length is the characteristic length scale at which a fiber or polymer remains straight: the larger it is, the stiffer the fiber. As expected, the persistence length is smallest for bending in the direction normal to the broad surfaces of the ribbon (the c direction; see also Scheme 2, in which we adopted the labeling of directions as in reference [4]) and can be estimated as about 100 nm. Bending in the direction of the broad surface (the a direction) is much more difficult, and the associated persistence length is at least an order of magnitude larger. The persistence length for twisting can be estimated to be a few hundred nanometers.

To probe the secondary structure of the peptide in the aggregates observed by light scattering and cryo-TEM, we measured circular dichroism (CD) spectra of the mixed system and compared them with solutions of **1** without Zn^{2+} and $\text{L}_2\text{EO}_4^{4-}$ at different pH values. As seen in Figure 1 b, the CD spectrum at acidic pH value (e.g. pH 5.4) exhibits a deep minimum in the 195–200-nm region (bars in Figure 1 b), which is characteristic for a combination of the outer and middle blocks in an unordered state.^[5] In contrast, at basic pH value (e.g. pH 11), the negative minimum shifts to 206 nm (squares). After subtraction of the contribution of the outer blocks, we obtain a negative minimum at around 207 nm and a positive one at 215–220 nm (circles), thus indicating formation of β turns (triple helices) but not β sheets.^[10,11] This result is as expected: The middle block of **1** is unfolded at $\text{pH} < 6$ owing to repulsion between the charges of the histidine residues. At $\text{pH} > 9$, the middle block folds owing to discharging of the histidine residues. It appears that discharging **1** by adjusting the pH value is insufficient for true β -sheet formation. Furthermore, we found that a negatively charged covalent polyelectrolyte, such as sodium polyacrylate (PANa), was also not capable of inducing predominant β -



Scheme 2. Ribbon formation at pH 5.4 in the mixed system of peptide copolymer **1** and $[\text{Zn-L}_2\text{EO}_4]^{2-}$. The outer blocks of **1** are drawn in blue, the middle blocks in red, with the positively charged histidine residues as red spheres. The ligand molecules, Zn ions, and the coordination complexes that they form are drawn as in Scheme 1. At the concentrations and pH values used, the peptide copolymers are coils, and the coordination complexes are predominantly small rings. For clarity not all end blocks of the peptide copolymers in the ribbon are drawn, only a few coordination polymers are drawn (there is one coordination center for every two positive histidine units), and the 2– charges of the coordination centers are not indicated in the ribbon. As in reference [4], the directions are named as follows: a : direction of the hydrogen bonds within the β sheet, b : direction of β -sheet stacking, c : direction of peptide-chain axis within the β sheet.

sheet structures (Figure S4 in the Supporting Information). However, upon mixing **1** with $[\text{Zn-L}_2\text{EO}_4]^{2-}$, the CD spectrum of the middle block exhibits positive ellipticity at 200 nm and negative minima at 210 nm and 220 nm (triangles). The spectrum can be attributed to a mixture of β sheets and a set of turns,^[12,13] such that the turns occur at the histidine site of **1**. Owing to the existence of turns in **1**, the characteristic CD spectrum of **1** can never be completely the same as that of pure β sheets, which shows a deep minimum of larger than $-5 \text{ kdeg dmol}^{-1} \text{ cm}^2$ at about 215 nm.^[14] The value of the negative minimum for the CD spectrum of **1** in the presence of $[\text{Zn-L}_2\text{EO}_4]^{2-}$ is $-4.2 \text{ kdeg dmol}^{-1} \text{ cm}^2$ at 215 nm, which is less than values usually found for β -sheet formation (at least $-5 \text{ kdeg dmol}^{-1} \text{ cm}^2$). The lower value in our experiment is probably caused by the “spectra-flattening” effect.^[15] If dimers or multimers occur, as they do in our case, the molecules closest to the light source will absorb nearly all the light and cast a molecular shadow on the molecules in the same multimer that are more distant from the light source. Thus, the effective molar ellipticity and the effective extinction coefficient of the multimer are significantly lower than the molar ellipticity or extinction coefficient of the monomolecularly dissolved species.^[15] In addition, the CD spectra of **1** were also measured in the presence of only Zn^{2+} or $\text{L}_2\text{EO}_4^{4-}$ and show similar features with the random-coil conformation (see Figure S2 in the Supporting Information), thus indicating that Zn^{2+} or L_2EO_4 alone does not produce any β -sheet conformations. The results indicate that the coordination polymer is exceptionally effective in inducing β -sheet formation, probably owing to the adaptable polymerization degree of these coordination polymers.^[9]

These observations, in combination with what is known on peptide polymers like **1**, lead us to a model for the self-assembled ribbons (see Scheme 2). From X-ray data on similar polypeptides in the crystalline state, the dimensions of a β sheet are estimated to be about $3 \times 23 \text{ nm}^2$.^[4] These dimensions agree within experimental uncertainty with the thickness and width of the nanoribbons observed by cryo-TEM (Figure 2c,d). This finding suggests that the nanoribbons are single-stranded, unbundled β stacks resembling the crystal structure.^[4] At pH < 5.4, the histidine residues in the middle block of molecule **1** are positively charged. In the presence of an equivalent amount of negatively charged coordination complex $([\text{Zn-L}_2\text{EO}_4]^{2-})_n$, these histidine residues form electrostatic complexes with the middle block of **1**. This compensation of the positive histidine charges enables the middle blocks to simultaneously fold into β sheets and to stack. In this process linear coordination polymers form out of small, ring-shaped oligomers, owing to the interactions with histidine-rich surfaces of the β stacks. The transformation of small rings to linear chains upon formation of electrostatic self-assemblies was reported in our previous results.^[9] The broad sides of the ribbons expose the positively charged histidine residues at the turns of the β sheets. The surface density of the histidine residues and, hence, that of the positive elementary charges at the surfaces of the ribbons can be estimated by assuming that 24 histidine residues occupy an area given by the width of the ribbon (about 23 nm) times the average intersheet spacing (about 0.5 nm, assuming that it is similar to the spacing in the crystal structure of analogous peptide polymers): about one elementary charge per 0.5 nm^2 (there are 48 histidine residues in a middle block, and 24 on each “long edge” of a β sheet). If these positive elementary charges are exactly compensated by adsorbed coordination polymers, there should be one coordination center per two histidine charges, that is, about one coordination center per nm^2 . Hence, the broad surfaces of the ribbons are densely covered with adsorbed coordination polymer.

The appearance of the nanoribbons demonstrates an extraordinary concerted effect of at least four distinct non-covalent interactions: The secondary structure (β sheets) and ternary structure (stacking) of the peptide middle blocks are stabilized by 1) hydrogen bonding and 2) hydrophobic interactions. Formation of these secondary and ternary structures is made possible by neutralization of the positive histidine charges by the negatively charged supramolecular coordination polymers. This neutralization involves 3) complexation of oppositely charged polyelectrolytes and 4) formation of the supramolecular coordination polymers out of multidentate ligands and Zn^{2+} ions by metal coordination. According to recent theoretical predictions, adsorption of reversible supramolecular polymers can strongly promote their degree of polymerization.^[16,17] In our system this applies for the coordination polymers adsorbed at the histidine-rich surfaces of the β stacks. However, formation of these β stacks is induced by the interaction of the middle blocks of **1** with the coordination polymers. In this system two supramolecular “polymerizations” induce each other: formation of the coordination polymers, and formation of long β stacks.

The nanoribbons appear to be “single stranded”, which must mean that they are sufficiently stabilized against bundle formation. Edge-to-edge association of the ribbons is prevented by the coiled hydrophilic outer blocks of the peptide copolymer. Face-to-face association is prevented by the hydrophilic nature of the histidine-rich surfaces covered with coordination polymer.

In summary, we report on well-defined hybrid nanoribbons spontaneously formed from a biosynthetic triblock peptide copolymer and an oppositely charged coordination polymer. Folding and stacking of peptide polymers induced by polyelectrolyte complexation rather than by pH changes provides new possibilities for the control of self-organization in silklike peptide copolymers. The nanoribbons can themselves be considered as huge supramolecular polymers. An interesting feature is that they are formed with the help of another smaller-scale supramolecular polymer (the $([\text{Zn-L}_2\text{EO}_4]^{2-})_n$ coordination polymer), which becomes an integral part of its structure. Hence, in this system, two reversible “polymerizations” induce each other mutually. We have not been able to find another example of this principle in the literature.

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