Twisting macromolecular chains: Self-assembly of a chiral supermolecule from nonchiral polythiophene polyanions and random-coil synthetic peptides

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The self-assembly of a negatively charged conjugated polythiophene derivative and a positively charged synthetic peptide will create a chiral, well ordered supermolecule. This supermolecule has the three-dimensional ordered structure of a biomolecule and the electronic properties of a conjugated polymer. The molecular complex being formed clearly affects the conformation of the polymer backbone. A main-chain chirality, such as a predominantly one-handed helical structure induced by the acid–base complexation between the conjugated polymer and the synthetic peptide, is seen. The alteration of the polymer backbone influences the optical properties of the polymer, seen as changes in the absorption, emission, and Raman spectra of the polymer. The complexation of the polythiophene and the synthetic peptide also induce a change from random-coil to helical structure of the synthetic peptide. The supermolecule described in this article may be used in a wide range of applications such as biomolecular devices, artificial enzymes, and biosensors.

The development of chiral conjugated polymers (CPs) with a well defined structure is of great interest because of their potential for being used in optoelectronic devices, sensors, and catalysis. In particular, polythiophenes (PTs) (1–8) with an optically active substituent in the 3 position have been studied for these purposes. These studies have been focused mainly on the chiral behavior of the PTs depending on solvent (solvatochromism) or temperature (thermochromism). Chiral PTs normally exhibit optical activity in the π – π^* transition. This phenomenon derives from the main-chain chirality when chains are aggregated to form a supramolecular, π -stacked self-assembly due to intermolecular interactions in a poor solvent or at low temperature, whereas they show no activity in the UV-visible region in a good solvent or at high temperatures (5, 9–12). Recent studies (13–16), using optically inactive CPs that become chiral after addition of a chiral guest, show that chirality introduction can also be a result of a main-chain chirality, such as a predominantly one-handed helical structure induced by an acid–base complexation between the CP and the chiral guest.

Natural biopolymers such as protein and DNA frequently have helical conformations that contribute to the three-dimensional ordered structure and the specific function of the biopolymer. As a part of the cell signaling pathways and enzymatic reactions, conformational alterations of biomolecules are very important in biological systems. Likewise do the conformational alterations of CPs allow direct connection between the geometry of chains and the resulting electronic structure and optical processes. Hence, it would be of great interest to combine helical biomolecules and optically inactive PTs. Such combinations have been reported (17, 18), and an induced chirality in the PTs due to the helical biomolecules was detected. To make a hybrid supermolecule between an optically inactive PT and a biomolecule with a random-coil conformation is still a challenge. This hybrid supermolecule will have optical properties and three-dimensional ordered structure derived from the complexation of the PT and

the biomolecule. The supermolecule would have the three-dimensional ordered structure of a biomolecule and the electronic properties of a PT and therefore may be used in a wide range of applications such as biomolecular devices, artificial enzymes, and biosensors. In this article, we report such a hybrid molecule (Fig. 1) derived from self-assembly of an optically inactive PT derivative and a synthetic peptide with a random-coil formation. The simple and noncovalent assembly, the induction of chirality, and the chromic response of the complexation are merits in the further utilization of these supermolecules.

Materials and Methods

Polymer and Peptide Synthesis. The synthesis of the nonregioregular poly(thiophene acetic acid) (PTAA)-Li (Fig. 1) was reported elsewhere (19). The peptide JR2K (Fig. 1) was synthesized by using a Pioneer automated peptide synthesizer (PerSeptive Biosystems, Framingham, MA) with standard fluorenylmethoxycarbonyl (Fmoc) chemistry protocol and Fmoc-Gly-polyethylene glycol-polystyrene resin. After synthesis, the peptide resin was washed with methylene chloride and dried under vacuum for 2 h. The peptide was cleaved from the polymer and deprotected with trifluoroacetic acid (19 ml), triisopropylsilane (0.5 ml) and H₂O (0.5 ml), per gram of polymer, for 3 h at room temperature, precipitated by cold diethyl ether and lyophilized. They were purified by reversed phase HPLC on a semi preparative C-8 Hichrome column. JR2K was eluted isocratically with 29% 2-propanol in 0.1% trifluoroacetic acid at a flow rate of 10 ml/min. The purity was checked by analytical HPLC and the peptide was identified by matrix-assisted laser desorption ionization/time-of-flight MS.

Optical Measurements. A stock solution containing 1.0 mg·ml^{−1} PTAA-Li in deionized water was prepared. Twenty microliters of the polymer solution was mixed with 0, 20, or 40 μ l of the positive peptide (JR2K) solution (2.2 mg·ml^{−1}) and diluted with deionized water to a final volume of 300 μ l. After 15 min of incubation, the samples were diluted with a stock buffer solution (sodium phosphate, pH 7.4) to a final volume of 2,000 μ l containing 20 mM sodium phosphate or with deionized water to a final volume of 2,000 μ l. The samples were incubated for 10 min at room temperature, and the emission spectra were recorded with an ISA spex FluoroMax-2 apparatus (Jobin Yvon, Longjumeau, France). All spectra were recorded with excitation at 400 nm. The circular dichroism (CD) spectra were recorded with an ISA Jobin Yvon CD6 (5-mm quartz cell), and a

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Abbreviations: CP, conjugated polymer; PT, polythiophene; PTAA, poly(thiophene acetic acid); CD, circular dichroism; ICD, induced CD.

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Fig. 1. Structural data for JR2K and PTAA-Li. (a) Amino acid sequence for JR2K. (b) Chemical structure of the repeating unit of PTAA-Li. (c) Schematic drawing of the conformational changes and the supramolecular assembly of the synthetic peptide JR2K and PTAA-Li. JR2K does not form homodimers because of electrostatic repulsion.

Perkin-Elmer Lambda 9 UV/visible/NIR spectrophotometer was used for the absorption measurements.

Raman Measurements. A stock solution containing 1.0 mg·ml⁻¹ PTAA-Li in deionized water was prepared. Fifty microliters of the polymer solution was mixed with 0 or 50 μ l of the positive peptide (JR2K) solution (2.2 mg·ml⁻¹ in deionized water) and diluted with deionized water to a final volume of 300 μ l. After 15 min of incubation, the samples were diluted with a stock buffer solution (sodium phosphate, pH 7.4) to a final volume of 600 μ l containing 20 mM sodium phosphate or with deionized water to a final volume of 600 μ l. The samples were incubated for 10 min at room temperature. The Raman unit used was a FRA 106 Raman Fourier transform spectrometer (Bruker, Billerica, MA) with a resolution of 4 cm⁻¹, the light source was an Nd:YAG laser operating at 1,064 nm and a power of 450 mW. To get a good signal-to-noise ratio, 500 scans were averaged over 14 min. Care was taken so that the samples would not be damaged by the high-intensity radiation.

Results and Discussion

Absorption Measurements. The absorption spectra of PTAA-Li (120 nmol on a monomer basis) in deionized water and after 10 min of

incubation in a buffer solution (20 mM sodium phosphate, pH 7.4) are shown in Fig. 2. An absorption maximum of 397 nm is related to a nonplanar conformation of the polymer backbone, and an absorption maximum of 437 nm is related to a certain degree of planarization of the polymer backbone, suggesting that the deprotonation of the side chain in the alkaline buffer solution will induce a planarization of the polymer backbone. These results are in agreement with an earlier study (20) that suggested that internal hydrogen bonding between adjacent carboxyl groups is responsible for the nonplanar conformation. The more planar conformation of the polymer in the alkaline solution is most likely caused by electrostatic repulsion forces that act between the carboxylate groups, forcing the polymer chains to stretch.

After the addition of 1.0 or 2.0 eq, on a monomer basis, of a positively charged peptide (net charge of +11 at neutral pH), JR2K (Fig. 1), the absorption maximum is blue-shifted to 402 and 389 nm, respectively. These shifts are associated with a decrease of the effective conjugation length of the polymer backbone, demonstrating that the interaction between PTAA-Li and JRK will force the polymer backbone to adopt a more nonplanar conformation. We suggest that the negatively charged carboxyl groups of the polymer side chains will most likely interact electrostatically with the positively charged lysine (K) groups of the peptide side chains, and these interactions will force the polymer backbone to adopt a nonplanar conformation. The peptide has been designed to form a four-helix-bundle heterodimer (21–23) with a negatively charged peptide, and homodimers are not formed due to electrostatic repulsion. Because the interactions between the negatively charged side chains of the polymer and the positively charged groups of the peptide will disrupt the electrostatic repulsion forces between the peptide molecules, helix-bundle homodimers are allowed to form. Hence, the formation of the polymer-peptide complex might induce the conversion from a random-coil to a more ordered helical structure of JR2K. An addition of 4.0 eq, on a monomer basis, of JR2K was also added to the PTAA solution and no further blue shift was seen, indicating that all the binding sites on PTAA are saturated after the addition of 2.0 eq, on a monomer basis, of JR2K.

CD Measurements. As reported (1–8), optically active PTs exhibit a split-type induced CD (ICD) in the π - π^* transition region. The CD spectra of PTAA-Li in deionized water and after 10 min of incubation in a buffer solution (20 mM sodium phosphate, pH 7.4) are shown in Fig. 3. PTAA-Li is optically inactive, and no characteristic ICD pattern in the π - π^* transition region can be seen for these solutions. The absence of CD signals followed by the different shifts of the absorption maxima suggest that the polymer backbone adopts a nonplanar random-coil conformation in deionized water and a more planar achiral conformation in alkaline buffer solution.

Interestingly, after the addition of different amounts of JR2K, split-type ICDs in the π - π^* transition region are induced. Normally, π -stacked chiral aggregations of conjugated thiophene polymers, as seen in poor solvents, are accompanied by a color change from yellow-orange to purple (5, 9–12). This change is caused by a transition from a disordered coil-like form to a rod-like, π -stacked one to give a chiral supramolecular aggregate with interchain interactions of transoidal PTs. In our case, the blue shifts in absorption, induced by different amounts of JR2K, are accompanied by an increase in the ICD, indicating that chirality introduction may not be derived from π -stacked chiral aggregation of the polymer. Instead, the ICDs can be a result of main-chain chirality such as a predominantly one-handed helical structure induced due to the interaction between PTAA-Li and JR2K (13–16, 24). It is interesting that a similar induced chirality has been seen for a free amino acid functionalized PT derivative (24), suggesting that the acid-base complexation between the carboxyl group of the polymer side chains and the amino groups of the lysine side chains in the peptide is responsible for the induced chirality of the polymer

Fig. 2. Absorption spectra of 120 nmol of PTAA-Li (on a monomer basis) in deionized water ($\square$), 20 mM sodium phosphate (pH 7.5) ($\times$), 20 mM sodium phosphate (pH 7.5), with 9.0 nmol of JR2K ($\diamond$), and 20 mM sodium phosphate (pH 7.5), with 18 nmol of JR2K ($\triangle$). a.u., Arbitrary units.

backbone. The shape and sign of the ICD pattern are characteristic of a right-handed helical form of PT (10, 11).

The CD spectrum in the far-UV region (Fig. 3) shows that

JR2K has a positive CD signal of ≈ 190 – 195 nm, a strong negative peak at 202 nm, and a weaker negative peak at 223 nm, indicative of a random coil or a β -like structure. As mentioned

Fig. 3. CD spectra of 120 nmol of PTAA-Li (on a monomer basis) in deionized water ($\square$), 20 mM sodium phosphate (pH 7.5) ($\times$), 20 mM sodium phosphate (pH 7.5) with 9.0 mol of JR2K ($\diamond$), and 20 mM sodium phosphate (pH 7.5) with 18 mol of JR2K ($\triangle$). CD spectra of the JR2K solutions, without the polymer and incubated under the same conditions, are marked with filled symbols.

Fig. 4. Emission spectra of 120 nmol of PTAA-Li (on a monomer basis) in deionized water ($\square$), 20 mM sodium phosphate (pH 7.5) ($\times$), 20 mM sodium phosphate (pH 7.5) with 9.0 nmol of JR2K ($\diamond$), and 20 mM sodium phosphate (pH 7.5) with 18 nmol of JR2K ($\triangle$). The emission spectra were recorded with excitation at 400 nm.

above, the JR2K sequence was designed to form a heterodimeric four-helix-bundle motif after the addition of a negatively charged peptide (23), and the CD spectrum shows that no homodimeric four-helix bundles are formed because of electrostatic repulsion between the positively charged lysine (K) groups.

The CD spectra in the far-UV region for the PTAA-Li-JR2K solutions show a strong positive CD signal at ≈ 190 – 195 nm and strong negative peaks at 208 and 222 nm, indicative of a helical structure. Hence, the interaction between JR2K and PTAA-Li will induce an ordered helical structure of the peptides. The helical structures are most likely stabilized by hydrophobic interactions between the nonpolar amino acids and the hydrogen-bonded ion pair complex between the negatively charged carboxyl groups of the polymer side chains and the positively charged lysine (K) groups of the peptide side chains. A closer look at the CD spectra in the far-UV region for the two different polymer-peptide solutions reveals an interesting observation. There is a change in the ratio of the mean residue ellipticities at 222 and 208 nm, suggesting that different polymer-to-peptide ratios will alter the shape of the helices. The change in ratio observed indicates that there is a transition from α -helix to 3^{10} -helix with increasing amount of peptide. A similar observation has been seen in a previous study (23) using a zwitterionic PT derivative and a heterodimeric four-helix bundle.

A proposed mechanism of the formation of the supermolecule is shown in Fig. 1. Thus far, we have not determined the stoichiometric relationship between the polymer and the peptide, and we do not know whether the peptide forms a two-helix bundle, a four-helix bundle, or an even greater supermolecular structure. It is also of interest to investigate whether the polymer chain is inside or outside the helix bundles. For instance, if the polymer is sandwiched between the helix bundles, the supermolecule can be seen as a nanotube having a transducing polymer core with an insulating peptide cover.

Fluorescence Measurements. The conformational changes of the polymer chains will also alter the emission spectra for the polymer solutions (Fig. 4). In deionized water, light with an emission maximum of 558 nm is emitted, and after 10 min of incubation in a buffer solution (20 mM sodium phosphate, pH 7.4), a slight red

shift of the emission maximum, 563 nm, is seen. It is interesting that the intensity of the emitted light from the polymer is increased in the buffer solution, indicating that the polymer chains are more separated in the buffer solution. This separation is probably caused by electrostatic repulsion between the negative carboxyl groups of the polymer side chains. A recent study (24) of a zwitterionic PT derivative has shown a similar phenomenon as the charge of the zwitterionic side chain is altered. The intensity of the fluorescence for the aggregated phase of PT derivatives compared with the fluorescence for the single-chain state has been shown previously to be weaker by ≈ 1 order of magnitude (10, 12), and the fluorescence is probably decreased because of nonradiative deexcitation. This new channel for deexcitation is created by contact between polymer chains (24). An earlier study (25) also showed an increased intensity of the emitted light caused by the effect of different solvents being used.

After the addition of different amounts of JR2K, the emission maximum is blue-shifted and the intensity of the emission is increased, suggesting that the polymer backbone becomes more nonplanar and that separation of polymer chains occurs. The alteration of the polymer backbone and the separation of the polymer chains are most likely a result of the complexation of PTAA-Li and JR2K. The complexation, caused by the hydrogen-bonded ion-pair complex between the negatively charged carboxyl groups of the polymer side chains and the positively charged lysine (K) groups of the peptide side chains, will force the polymer chains to separate and induce a chirality of the polymer backbone. Similar emission properties were seen in an earlier study (24) of a zwitterionic PT derivative with a similar helical conformation. The self-assembly between synthetic peptides and CPs has been shown before (23) and might offer a route for the design of photonic devices, because well ordered polymers are of great importance for the performance of optoelectronic devices.

Raman Spectroscopy. To continue this spectroscopic investigation of the different backbone conformations of PTAA-Li, Raman spectroscopy was used. The Raman spectra (excitation at 1,064 nm) for the polymer in deionized water after 10 min in a buffer solution (20 mM sodium phosphate, pH 7.4) and after addition of JR2K are shown in Fig. 5. The assignment of the peaks was

Fig. 5. Raman spectra of 300 nmol of PTAA-Li (on a monomer basis) in deionized water (black line), 20 mM sodium phosphate (pH 7.5) (red line), and 20 mM sodium phosphate (pH 7.5) with 22 nmol of JR2K (blue line). The assignment of the peaks is shown in the spectra.

carried out on the basis of previous studies (26–29), and the main changes occur as follows.

The shift toward higher frequencies, seen for the polymer in deionized water and together with the JR2K peptide, of the stretching vibrations of the $C^{\alpha}=C^{\beta}$ bond suggests a more localized electronic density on these bonds that is associated with a decrease of the effective conjugation length along the polymer backbone. It is interesting that the peaks assigned to the $C^{\beta}-H$ bending vibration and the $C^{\beta}-C^{\beta}$ stretching are not seen in the Raman spectrum for the PTAA-Li-JR2E solution. The disappearance of peaks usually indicates a loss of symmetry in the molecule. Although the intensity of the $C^{\alpha}=C^{\beta}$ bond-stretching vibration is also decreasing for this sample, the other peaks still might be there but are not seen because of the noise level. On the other hand, the intensity of the $C^{\alpha}=C^{\beta}$ bond-stretching vibration for the water solution of PTAA-Li is also decreased, but the peak assigned to the $C^{\beta}-C^{\beta}$ stretching is not altered. To evaluate these observations further, more and precise experiments with an internal standard have to be performed. The peaks are also quite broad, probably as a result of interactions with the solvent and the fact that the polymer is not regioregular. However, the results from the Raman experiments are in agreement with the other optical measurements, clearly showing that the polymer backbone adopts three different conformation in demonized water, in buffer alkaline buffer solution, and in complex with JR2K.

Conclusions

We have shown that the self-assembly of a negatively charged conjugated PT derivative and a positively charged synthetic

peptide will create a chiral, well ordered supermolecule. This supermolecule has the three-dimensional ordered structure of a biomolecule and the electronic properties of a PT. The molecular complex being formed clearly affects the conformation of the polymer backbone, and a main-chain chirality, due to predominantly one-handed helical structure induced by the acid–base complexation between the CP and the synthetic peptide, is seen. The alteration of the polymer backbone has been detected thus far by optical measurement, but electrical detection of these transitions are most likely possible. The complexation of the PT and the synthetic peptide will also induce a change from random-coil to helical structure of the synthetic peptide. Because conformational alterations and well ordered structures of biomolecules are necessary for bio-specific interactions and enzymatic reactions in biological systems, the simple construction of such a system is of great importance. We suggest that the supermolecule described in this article may be used in a wide range of applications such as biomolecular devices, artificial enzymes, and biosensors. Assembly of electronic devices with the help of synthetic peptides is one of the tantalizing possibilities; using semiconducting polymer as prosthetic groups or coenzymes in synthetic biocatalysts may be another.

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