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 electronic property 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.

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-polyethylene. After synthesis, the peptide was washed with methylene chloride and dried under vacuum for 2 h. The peptide was cleaved from the polymer and deproctected 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 PTAA-Li in deionized water was prepared. Twenty microliters of the polymer solution was mixed with 0, 20, or 40 µl of the positive peptide (JR2K) solution (2.2 mg/ml) and diluted with deionized water to a final volume of 300 µl. 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 µl containing 20 mM sodium phosphate or with deionized water to a final volume of 2,000 µl. 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 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 dichromism; ICD, induced CD.

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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 JR2K 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 (1–5), 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. 2. 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.

**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 JR2K 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 (1–5), 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. 2. 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.
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 \( \approx 190-195 \) nm, a strong negative peak at 202 nm, and a weaker negative peak at 223 nm, indicative of a random coil or a \( \beta \)-like structure. As mentioned
observed indicates that there is a transition from 
222 and 208 nm, suggesting that different polymer-to-peptide 
There is a change in the ratio of the mean residue ellipticities at 
polymer–peptide interactions between the nonpolar amino acids and the hydro-
helical structures are most likely stabilized by hydrophobic 
will induce an ordered helical structure of the peptides. The

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 hydro-
gen-bonded ion pair complex between the negatively charged 
carboxyl groups of the polymer side chains and the positively 
charged 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 
310-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 supramolecular 
structure. It is also of interest to investigate whether the polymer 
chain is inside or outside the helix bundles. For instance, if the 
peptide is sandwiched between the helix bundles, the super-
molecule 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 
zwiterionic 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 zwitte-
ric PT derivative with a similar helical conformation. The 
self-assembly between synthetic peptides and CPs has been shown 
before (23) and might offer a new 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 7.5) and after addition 
of JR2K are shown in Fig. 5. The assignment of the peaks was

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**Fig. 4.** Emission spectra of 120 nmol of PTAA-Li (on a monomer basis) in deionized water (□), 20 mM sodium phosphate (pH 7.5) (×), 20 mM sodium phosphate (pH 7.5) with 9.0 nmol of JR2K (○), and 20 mM sodium phosphate (pH 7.5) with 18 nmol of JR2K (·). The emission spectra were recorded with excitation at 400 nm.
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–O 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–H bending vibration and the C–C 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–C 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–C bond-stretching vibration for the water solution of PTAA-Li is also decreased, but the peak assigned to the C–C 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 deionized 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 biospecific 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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