Water-soluble Polythiophene as an Optical Probe for Detection of the Helicity and Conformational Transition in Polysaccharides

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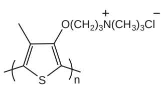
(Received July 5, 2005; CL-050870)

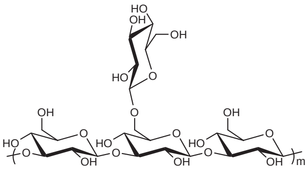
A conformation-sensitive optical method for monitoring the random coil–helix transition of polysaccharides has been developed by using a water-soluble polythiophene.

DNAs, polypeptides, and polysaccharides are typical biopolymers having the helical high-order structures and can enjoy the helix–random coil transformations. These changes can be conveniently monitored by ultraviolet–visible (UV–vis) absorption and circular dichroism (CD) spectroscopic methods for DNAs and CD spectroscopy for polypeptides, but for polysaccharides there is no effective method except optical rotatory dispersion (ORD), viscometry, and light scattering[1] which are not so sensitive, particularly in the low concentration region, to the conformational transition. Therefore, it is still a challenging subject to find new methods useful for facile monitoring of the conformational transition of polysaccharides. Recently, we demonstrated that supramolecular complex can be formed between a water-soluble polythiophene derivative (PT-**1**) and certain polysaccharide.[2] In the present work, we have focused on the use of this water-soluble PT-**1** as an optical probe for the detection of the helicity and conformational transition of polysaccharides induced by the changes in the medium conditions. Herein, we report an initial proof-of-concept demonstration about this idea.

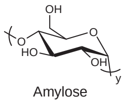
Natural polysaccharides schizophyllan (SPG) and amylose were chosen as model glucans to access the viability of this approach in view of two facts that their higher-order conformations are well-characterized and controllable by changing the medium conditions[3,4] and that they are important building blocks for the construction of functional supramolecular complexes.[5,6] SPG is a β-1,3-glucan and exists as a right-handed triple helix (t-SPG) in water but as a single random coil (s-SPG) in dimethyl sulfoxide (DMSO). When water is added to its DMSO solution (renaturation), s-SPG collapses owing to the hydrophobic interaction and retrieves its original triple helix structure.[3] Amylose is a α-1,4-glucan with left-handed helical conformation in crystal but exists as either a random coil or an interrupted loose helix in a neutral aqueous solution, whereas the predominant conformation in DMSO solution appears to be a tight helix.[4] As a typical experimental procedure, the PT-**1**/polysaccharide complexes were prepared by adding s-SPG (or amylose)/DMSO solution to PT-**1**/water solution, and then the obtained solutions were incubated for 12 h at 60 °C. The ratio of the PT-**1** and polysaccharide solutions was chosen so that, after mixing, the concentrations of PT-**1** and polysaccharides could become 1.5×10^{-4} and 6.0×10^{-4} M, respectively, and the volume fraction of water in the mixtures (V_w) was 0.95 unless specially stated.



PT-**1**



SPG



Amylose

Figure 1 compares absorption and CD spectra between PT-**1** and its mixtures with SPG and amylose at 20 °C. In the absence of polysaccharides, the absorption maximum of 403 nm is attributed to a random-coiled conformation of the PT backbone. The absorption maximum is red-shifted to 454 nm upon addition of s-SPG, which is associated with an increase in the effective conjugation length of the PT-**1**, demonstrating that the interaction between PT-**1** and s-SPG will force the PT-**1** backbone to adopt a more planar conformation. However, upon the introduction of amylose the absorption maximum is blue-shifted to 369 nm, which is related to a decrease in the effective conjugation length of the PT-**1**, indicating that the complex formation between PT-**1** and amylose will force the PT-**1** to adopt a more nonplanar conformation.

PT-**1** itself is optically-inactive, so that no CD pattern was detected, indicating that the PT-**1** backbone adopts an achiral random-coiled conformation in the mixed solvent. Interestingly, upon addition of s-SPG or amylose, a split-type induced CD (ICD) was detected in the π–π^* transition region. Normally, optically-active PTs bearing chiral β-substituents can form intermolecularly π-stacked chiral aggregates in poor solvents at low temperature, accompanied by the appearance of fine vibronic bands at the longer wavelength.[7] In our case, upon addition of s-SPG or amylose, no vibronic bands were detected, and the obtained complexes scarcely exhibited the linear dichroism. These results support the view that the chirality introduction in the PT-

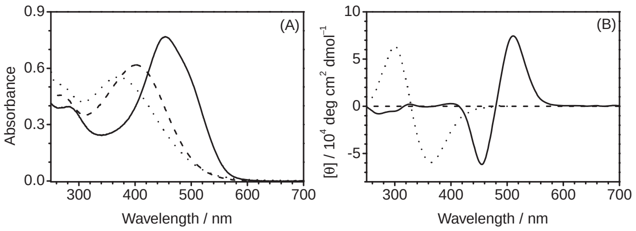


Figure 1. Absorption (A) and CD (B) spectra of PT-**1** in the absence (dashed line) and the presence of s-SPG (solid line) and amylose (dotted line, the CD intensities are magnified 5 times for comparison) in $V_w = 0.95$ water–DMSO mixed solvent. $[\text{PT-}\mathbf{1}] = 1.5 \times 10^{-4}$ M; $[\text{s-SPG}] = 6.0 \times 10^{-4}$ M; $[\text{amylose}] = 6.0 \times 10^{-4}$ M.

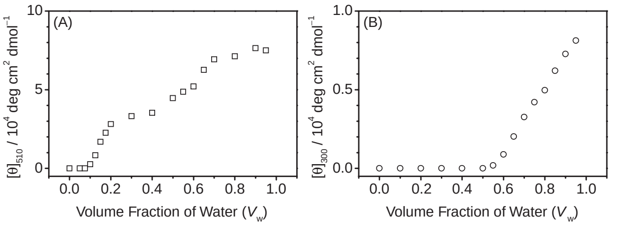


Figure 2. Composition dependence of the CD relative intensity of PT-**1** in the mixtures with s-SPG (A) and amylose (B). [PT-**1**] = 1.5×10^{-4} M; [s-SPG] = 6.0×10^{-4} M; [amylose] = 6.0×10^{-4} M.

1/polysaccharide complexes is most probably caused by intrachain effects rather than by interchain π-stacking effects, and a predominantly one-handed helical structure of PT-**1** is induced by interpolymer complexation with s-SPG or with amylose. Most interestingly, the shape and sign of the ICD patterns in the PT-**1**/polysaccharide complexes are characteristic of a right- (PT-**1**/SPG) or left-handed helix (PT-**1**/amylose) of the PT backbones,[8] reflecting the stereochemistry of SPG and amylose, respectively, which also tend to adopt a right- or left-handed helical structure. These results indicate that the conformation-sensitive PT-**1** will be a potential optical probe for monitoring the helicity and conformational transitions of polysaccharides.

To evaluate the capability of PT-**1** as an optical probe for the detection of the conformational transition in polysaccharides, the composition-dependent CD spectra of PT-**1** were estimated in the presence of s-SPG or amylose (Figure 2). The conformational transition of SPG dissolved in water–DMSO mixed solvents has been studied by light scattering.[3] It has been concluded that the thermodynamically stable form of SPG in aqueous DMSO is triple helices at V_w above 0.163 and single strand coils at V_w below 0.109 at 25 °C; whereas triple helices and single random coils coexist in the region of $V_w = 0.109$–0.163. As shown in Figure 2a, for the PT-**1**/s-SPG system, PT-**1** is CD-silent at V_w below 0.10 and becomes CD-active at $V_w = 0.10$. In the region of $V_w = 0.10$ to 0.20, the ICD intensities drastically increase, and then increase gradually with moving to the water–richer region and eventually level off at V_w above 0.8. Interestingly, the onset composition for the supramolecular chiral complex formation is very close to the point where the single strand coil of SPG begins to renature to the triple helix. This coincidence indicates that the renaturation process is a key factor to induce the complex formation and PT-**1** can be used as an optical probe to monitor the conformational transition of SPG. The results shown here also give a hint that the hydrophobic interaction between the PT-**1** backbone and SPG is dominant for the PT-**1**/s-SPG complex formation.

To show the versatile ability of PT-**1** as a probe for detection of the polysaccharide conformation, another important natural glucan, amylose, was also investigated. Even though amylose adopts a tight helix in DMSO, one cannot expect the significant interaction between amylose and PT-**1**, which makes this system CD-silent. It can be seen from Figure 2b, however, that in the mixture of PT-**1** and amylose, PT-**1** becomes CD-active at V_w above 0.50 and the ICD intensities increase gradually with increasing water content, indicating that the hydrophobic interaction is the main driving force for the complex formation.

Here, it is worthy to emphasize that the ICD intensities of the PT-**1**/s-SPG complexes are much stronger than those of the PT-**1**/amylose complexes over the whole range of the water–DMSO mixtures, indicating that PT-**1** has the more regular helical (or twisted) structure in the former complexes. In addition, the ICD intensities for both complexes are gradually intensified with increasing water concentration. These differences can be rationalized by the following explanations: i) for the supramolecular chiral complex formation between s-SPG (or amylose) with guest molecules, the hydrophobic interaction is the main driving force, ii) it is known that s-SPG tends to retrieve the triple helix regardless of the solvent compositions at V_w above 0.10. The coincidence of these two effects facilitates the formation of the PT-**1**/s-SPG complex featuring the highly ordered structure. However, the situation of amylose is in contrast to that of SPG, because with increasing water concentration, amylose undergoes the conformational transitions from tight helix–loose helix–random coil in water–DMSO mixtures, which retards the complex formation, resulting in the poor regularity of PT-**1** in the amylose complex in comparison with that in the SPG complex.

In summary, we have demonstrated that a conformation-sensitive water-soluble polythiophene derivative (PT-**1**) can be used as a probe to detect the helicity of polysaccharides. Particularly important is a new finding that PT-**1** is capable of following the conformational transition of schizophyllan in the water–DMSO mixtures over the whole range of compositions. We believe that the present results will provide a new approach for facile monitoring of the helicity and the helix–random coil transformation of polysaccharides. In addition, the resultant chiral interpolymer complexes may be regarded, from a supramolecular viewpoint, to be a new alternative for optically-active conjugated polymers.

We are grateful to JST SORST program for financial support and to Mitsui Sugar Co. for providing SPG samples.

References and Notes

1 D. M. Goodall and I. T. Norton, *Acc. Chem. Res.*, **20**, 59 (1987).
2 C. Li, M. Numata, A.-H. Bae, K. Sakurai, and S. Shinkai, *J. Am. Chem. Soc.*, **127**, 4548 (2005).
3 T. Yanaki, T. Norisuye, and M. Fujita, *Macromolecules*, **13**, 1462 (1980); T. Sato, T. Norisuye, and H. Fujita, *Macromolecules*, **16**, 185 (1983).
4 Y. Nakanishi, T. Norisuye, A. Teramoto, and S. Kitamura, *Macromolecules*, **26**, 4220 (1993); N. W. H. Cheetham and L. Tao, *Carbohydr. Res.*, **35**, 287 (1998).
5 K. Sakurai and S. Shinkai, *J. Am. Chem. Soc.*, **122**, 4520 (2000); M. Numata, M. Asai, K. Kaneko, T. Hasegawa, N. Fujita, Y. Kitada, K. Sakurai, and S. Shinkai, *Chem. Lett.*, **33**, 232 (2004); M. Numata, T. Hasegawa, T. Fujisawa, K. Sakurai, and S. Shinkai, *Org. Lett.*, **6**, 4447 (2004); A.-H. Bae, M. Numata, T. Hasegawa, C. Li, K. Kaneko, K. Sakurai, and S. Shinkai, *Angew. Chem., Int. Ed.*, **44**, 2030 (2005); M. Numata, M. Asai, K. Kaneko, A.-H. Bae, T. Hasegawa, K. Sakurai, and S. Shinkai, *J. Am. Chem. Soc.*, **127**, 5875 (2005).
6 A. Star, D. W. Steuerman, J. R. Heath, and J. F. Stoddart, *Angew. Chem., Int. Ed.*, **41**, 2508 (2002); O.-K. Kim, J. Je, J. W. Baldwin, S. Kooi, P. E. Phehrsson, and L. J. Buckley, *J. Am. Chem. Soc.*, **125**, 4426 (2003).
7 B. M. W. Langeveld-Voss, R. A. J. Janssen, and E. W. Meijer, *J. Mol. Struct.*, **521**, 285 (2000).
8 The positive and negative first Cotton effects are related to a right- and left-handed helix, respectively, see: Ref. 7.

Published on the web (Advance View) September 10, 2005; DOI 10.1246/cl.2005.1354