Thermally induced conformational-transition of polydeoxyadenosine in the complex with schizophyllan and the base-length dependence of its stability†‡

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Received (in Cambridge, UK) 13th December 2000, Accepted 22nd January 2001
First published as an Advance Article on the web 13th February 2001

A single chain of schizophyllan, one of the β-1,3-glucan family, can form a stoichiometric complex with poly(dA) and the poly(dA)'s conformation and the complex stability strongly depends on the base length.

Conformational changes in polynucleotides play an important role in biological systems.[1] Powell and Gray[2] have explored the conformational change induced by a single-stranded polynucleotide binding protein (SSB), using poly(dA) as a model single-stranded DNA (ssDNA). Their CD data clearly demonstrated that SSB induces the same conformational change in ssDNA as that of heating poly(dA) or of protonating the polymer at low pH. More recently, Sakurai and Shinkai[3] found that a single chain of schizophyllan (s-SPG) can form a complex with single-stranded RNAs, such as poly(A) and poly(C), and RNA's conformation is altered by complexation.[3] Their finding led us to examine if s-SPG can interact with ssDNA. This report presents our preliminary results for an interaction observed between poly(dA) and s-SPG.

SPG (produced by *Schizophyllum commune* of the Basidiomycota) belongs to the β-1,3-glucan family.[4] SPG exists in a triple helix in water and a single chain in DMSO.[5] When s-SPG in DMSO solution is diluted with water (renature), SPG can gain the triple helical conformation again.[6] We found that when RNA such as poly(C) or poly(A) coexists in the renaturing process, a new triple helix consisting of one polynucleotide chain and two s-SPG chains is formed instead of the original triple helix reforming from three s-SPG chains.[7]

Fig. 1 demonstrates the base lengths (X_b) dependence of the CD spectra ([θ] is the molar ellipticity) for poly(dA) itself (left) and for the mixture of poly(dA) and s-SPG (poly(dA)+s-SPG) (right). The spectra for poly(dA) themselves are overlaid for X_b = 18–60 and that for X_b = 250 shows slight deviation from the others in the 260–280 nm region. Since the overall feature of the spectra seems identical, we can assume that both the helix content and conformation of poly(dA) do not depend on X_b.

On the other hand, the mixtures show that the conformation of poly(dA) strongly depends on X_b. In the case of X_b = 18, there is no difference between poly(dA) itself and the mixture, indicating that no significant interaction exists. From X_b = 30 to 45, the difference between the poly(dA) themselves and the mixtures becomes evident (*i.e.* increase at 260 nm and decrease at 250 nm). Thus complexation occurs at this base length, and becomes more favourable with increasing X_b. In the case of X_b = 60 and 250, the spectra are different from those of $X_b \leqslant 60$ (*i.e.* two new positive bands at 257 and 285 nm and one negative band at 267 nm), indicating that the longer poly(dA) exhibits a different conformation from the shorter poly(dA). As shown in the ESI†[8] this novel interaction is only observed for s-SPG and other polysaccharides (including triple-helix of s-SPG[7]) show no interaction at all.

Fig. 2 presents the temperature dependence of the CD spectra for both poly(dA) and the mixture at X_b = 250. For convenience, the spectra for the shorter wavelength (180–240 nm) and the longer (240–300 nm) are presented on different [θ] scales. For poly(dA) (the solid line), the CD spectrum at shorter wavelength does not change much upon heating. On the other hand, heating induces a dramatic change in that for poly(dA) of the complex. At T = 5 °C, there are two positive bands at 257 and 285 nm and one negative band at 267 nm in the complex. At T = 60 °C, however, there is a strong negative band at 250 nm, a strong positive band at 260 nm, and the 285 nm band disappears. At T = 40 °C, it seems that the spectrum is a hybrid of those at 5 and 60 °C. At T = 90 °C, there is no spectral difference between poly(dA) and the complex. This feature indicates that heating induces a conformational transition of poly(dA) in the complex between 40 and 60 °C, and that the complex is dissociated above 90 °C. Interestingly, the transition

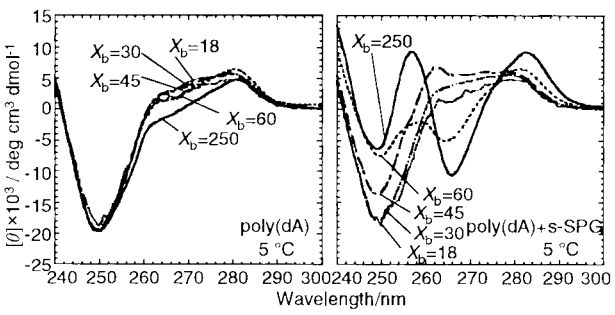

Fig. 1 The base length (X_b) dependence of the CD spectra for poly(dA) (left) and for the mixture made from poly(dA) and s-SPG (right) measured at 5 °C.

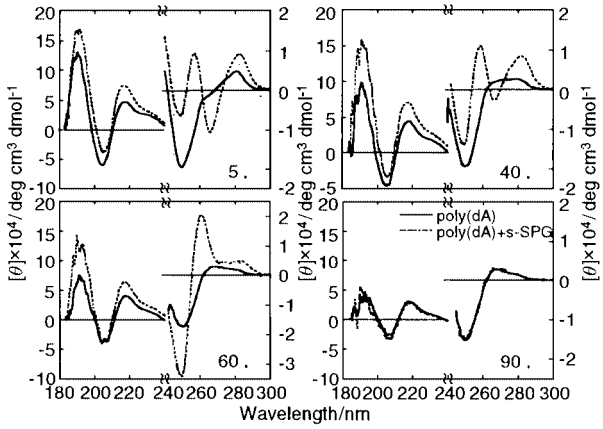

Fig. 2 Spectral change upon heating (from 5 °C, 40 °C, to 90 °C) for both poly(dA) (solid line) and its complex (broken line) for X_b = 250. The spectra at longer wavelengths and at shorter ones are plotted on separate vertical scales to make comparison easy.

† Electronic supplementary information (ESI) available: structure of s-SPG, experimental method, gel electrophoresis of poly(dA) plus dextran (Fig. S1) and s-SPG (Fig. S2). See http://www.rsc.org/suppdata/cc/b0/b009943k/

from 'positive 257 nm + negative 267 nm bands at 5 °C' to 'negative 250 nm + positive 260 nm bands at 60 °C' is correlated with a reverse of the Cotton effect. This feature resembles the feature observed for the B- to Z-form transition in DNA duplexes.[9]

One method to classify polynucleotide conformations is to examine the CD bands related to the phosphoric ester around 170–200 nm. Three typical conformations in DNA duplexes can be characterized as follows: a small negative band at 210 nm and a very large positive band at 190 nm in the A-form, a large positive band (smaller than the A-form) at 190 nm and a fairly small band at 210 nm in the B-form and a relatively large negative band at 185–195 nm and a large positive band at 180 nm in the Z-form. Namely, inversion of the Cotton effect is usually observed between the Z- and B- (or A-) forms. This criterion has been used to study the influence of the ionic strength on the conformational transition from the B- to Z-form[10] and the medium polarity on the conformational transition from the B- to A-form[11]

We apply the above criteria to the present system.[12] By comparing the left hand spectra in each panel in Fig. 2, we can conclude that the poly(dA)'s helicity does not undergo any transition. From the similarity between the spectra of poly(dA) and the complex at 5 °C and the fact that poly(dA) itself takes the B-form (C_2'-*endo* of the ribose and *anti* of the adenosine) in aqueous solution,[1,13] it can be considered that poly(dA) in the complex at 5 °C also takes the B-form. Even for the same type of conformation, the spectra in 240–300 nm are different between poly(dA) and the complex. This difference can be attributed to the difference in the conformation or the electronic state in adenosine, because the complexation can alter both. At 60 °C, the 190 nm band of the complex is larger than that of poly(dA), while the intensities of the 210 nm band are same. This feature can be interpreted in two ways. One possible rationale is that poly(dA) in the complex takes the A-form. This speculation is based on the fact that the CD spectrum in 240–300 nm resembles that of poly(A)[14] which is already known to take the A-form.[1,15] Another rationale is that poly(dA) in complex retains the B-form and the spectral change in 240–300 nm is due to a conformational transition of the adenosine such as *anti* to *syn*. However, the final assignment of the spectral change needs more extensive work using NMR spectroscopy.

Fig. 3 shows the temperature dependence of $[\theta]_{250}$ ($[\theta]$ at 250 nm) in the left panel and of the extinction coefficient at 257 nm (ε_{257}) in the right one, comparing poly(dA) and the mixture. Table 1 summarizes schematically the temperature dependence of the poly(dA) conformation for each X_b. Poly(dA) for all X_b values shows a monotonous increment in both $[\theta]_{250}$ and ε_{257}. We consider that this change is due to decrease in the helix content in the original B-form. On the other hand, for X_b = 250 in the complex, $[\theta]_{250}$ drastically decreases in the range T = 20–60 °C, then increases in the range T = 60–80 °C, and finally merges into the data of poly(dA) itself. The data for the ε_{257} change are clearly correlated with those of CD. This temperature dependence confirms that the poly(dA) in the complex

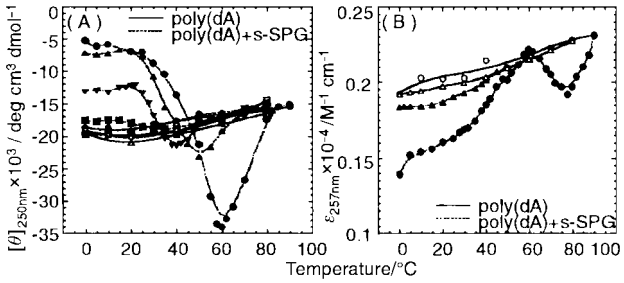

Fig. 3 The temperature dependence of $[\theta]$ at 250 nm (A) and ε at 257 nm (B) for poly(dA) (solid line and unfilled marks) and their mixtures with s-SPG (broken line and filled marks). X_b = 250 (○ and ●), X_b = 60 (△ and ▲), (x_b = 45 (▽ and ▼), x_b = 30 (■ and □) and X_b = 18 (◇ and ◆), respectively.

Table 1 The conformational transition for poly(dA) and poly(dA) in the complex

Poly(dA)	Conformation of poly(dA)			
Poly(dA)	B	→		
Complex				
X_b = 18	B	→		
X_b = 30	LL	→ 22 °C		
X_b = 45	LL	→ 23 °C	H	→ 45 °C
X_b = 60	HL	→ 30 °C	H	→ 65 °C
X_b = 250	HL	→ 30 °C	H	→ 80 °C

B = B-form. LL = conformation of a low molecular weight (Mw) and low temperature (T). HL = conformation of a high Mw and low T. H = conformation of high T.

undergoes a conformational transition (*i.e* a high molecular weight (*Mw*) and low *T* conformation (HL) to a high *T* conformation (H) as denoted in Table 1) before the complex dissociation. With decreasing X_b, H becomes less obvious. At X_b = 45, HL disappears and a low *Mw* and low *T* conformation (LL) is cooperatively dissociated at 35 °C and a small amount of H appears. These features indicate that the presence of this conformation is characteristic of long poly(dA) chains. As discussed above, HL is considered to be the B-form and H can be the A-form or a conformational transition of adenosine. At this moment, however, the origin of LL is not clear yet. In conclusion, we have substantiated that s-SPG forms a novel macromolecular complex with poly(dA). This novel interaction between s-SPG and poly(dA)[16] should provide new insight into the polysaccharide–DNA interactions, frequently important in biological systems.

Notes and references

‡ Polysaccharide–polynucleotide complexes (V).

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16 As shown in Fig. S2 (ESI),† we determined the stoichiometric ratio of the complex, using gel electrophoresis. The ratio we found for this system is that two s-SPG repeating units interact with three bases, which is the same result as that for the poly(C) and the poly(A) systems.