Polymer Journal, Vol. 35, No. 9, pp 714—720 (2003)

Polysaccharide–Polynucleotide Complexes Part 17. Solvent Effects on Conformational-Transition of Polydeoxyadenylic Acid in the Complexes with Schizophyllan

Masami MIZU, Kazuya KOUMOTO, Taro KIMURA,*
Kazuo SAKURAI,† and Seiji SHINKAI**

Department of Chemical Process & Environments, The University of Kitakyushu, 1–1 Hibikino, Wakamatu-ku, Kitakyushu, Fukuoka 808–0135, Japan
**Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center, 1465–5 Aikawa, Kurume, Fukuoka 839–0861, Japan*
***Faculty of Engineering Department of Chemistry & Biochemistry, Graduate School of Engineering, Kyushu University, 6–10–1 Hakozaki, Higashi-ku, Fukuoka, Fukuoka 812–8581, Japan*

(Received May 9, 2003; Accepted July 7, 2003)

ABSTRACT: This paper examines the relationship between the conformation and solvent for the poly(dA)/s-SPG complex using circular dichroism in the 240–300 nm wavelength region. At the low temperature and low salt concentration, poly(dA) in the complex is estimated to take *C2′-endo* with a different torsional angle from the *anti* form. With increasing temperature, the circular dichroism (CD) spectrum starts to bear characteristics of *C3′-endo* and *anti* as a minority among the majority of *C2′-endo* and *anti*. When the salt concentration is increased, the spectrum becomes almost identical to that of the high temperature form under the non-salt condition. Therefore, addition of salt provides the identical effect to increasing temperature. D_2O induces the same effect as decreasing temperature.

KEY WORDS Polysaccharide–Polynucleotide Complex / Thermodynamics / Solvent Effect / β-1,3-Glucan / Circular Dichroism / Polynucleotide /

Conformational changes in polynucleotides play important roles in biological systems.[1] A replication fork in DNA undergoes a conformational change when it interacts with a single-stranded polynucleotide binding protein (SSB).[2] This change disables the replication fork to retrieve hybridization, which is crucial to DNA replication. Powell and Gray[3] explored the conformational change induced by SSB, using poly(dA) as a model single-stranded DNA (ssDNA). Their circular dichroism (CD) data demonstrated that SSB induces the same conformational change in poly(dA) as that of heating poly(dA) or protonating the polymer at low pH. It seems that ssDNAs conformational response to external stimuli is one of the crucial issues to understand the relationship between ssDNA conformation and biological roles.[1,2]

Sakurai and Shinkai[4] are the first to demonstrate that a neutral β-1,3-glucan schizophyllan (SPG) can bind single homo-polynucleotides to change the conformation dramatically. Here, SPG is produced by *Schizophyllun commune* and belongs to the β-1,3-glucan family (Figure 1).[5] SPG exists in a triple helix in water and a single chain (s-SPG) in dimethyl sulfoxide (DMSO).[6,7] When a s-SPG/DMSO solution is diluted with water (renature), SPG can gain the triple helical conformation again.[8] When some polynucleotide

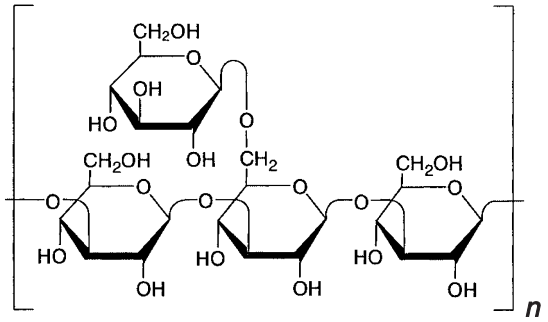


Figure 1. Chemical structure and repeating unit of schizophyllan.

is present in the renaturing process, a new triple helix consisting of one polynucleotide chain and two s-SPG chains is formed instead of reforming the original triple helix.[4] Mizu *et al.*[9] demonstrated that poly(dA) forms a complex with s-SPG and the complex exhibits the following features. (1) A critical base number is necessary to induce the complexation. (2) When the base number is > 60, the poly(dA) chain in the complex undergoes structural transition upon heating below the dissociation temperature (T_m) of the complex. This is a distinct conformational transition and we denote the low and higher temperature forms as HL and H, respectively. (3) The hypochromic effect once ceases at the transition temperature and appears again. Such complicated CD spectral changes have never been observed for single-stranded RNAs such as poly(C), poly(A), and

†To whom correspondence should be addressed.

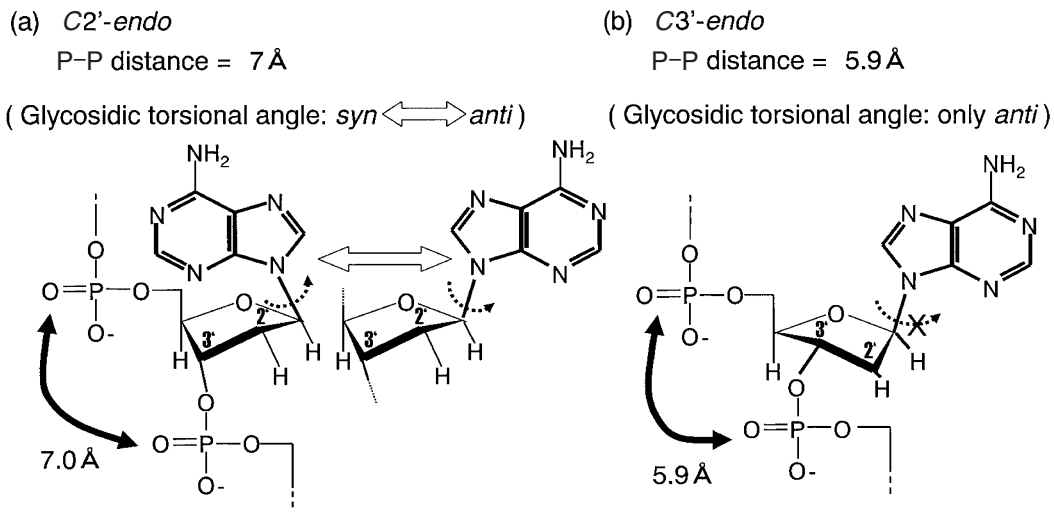


Figure 2. Schematic illustration of the conformational notation of polynucleotides. (a) *C2′-endo* puckering. For this puckering, the torsional angle of the glycosidic bond can be allowed to take two forms: *anti* and *syn*. The distance between the phosphate anions is 7.0 Å. (b) *C3′-endo* puckering. This puckering allows only *anti* form and the distance between the phosphate anions is 5.9 Å.

poly(U).[4,10] This spectral diversity is characteristic of poly(dA) and may be related to the conformational flexibility of DNA.[11] This paper examines the solvent effect of the complex made from poly(dA) and s-SPG to clarify the nature of this unique structural transition.

CONFORMATIONAL NOTATION OF POLYNUCLEOTIDES AND THE CD SPECTRUM FOR POLY(DA)

According to conformational studies on polynucleotides,[1,12] the ribose ring of ribonucleosides usually takes the *C3′-endo* puckering and the ribose in deoxyribonucleosides takes the *C2′-endo* puckering. Here, the *C3′-endo* puckering (or N-type) is a ribose puckering in which the *C3′* carbon is out-of-plane projecting out toward the same direction of the base moiety, and the *C2′-endo* puckering (or S-type) is defined in the same manner (see Figure 2). Torsional freedom around the glycosidic bond greatly differs between *C2′-endo* and *C3′-endo*. The *C3′-endo* puckering only takes the *anti* conformation which causes the Watson–Crick hydrogen-bonding sites to be directed far away from the ribose ring. The *C2′-endo* puckering enables the base to rotate rather freely and to take both *anti* and *syn* conformations. The *syn* form is an almost reverse position of the *anti* form, with Watson–Crick hydrogen-bonding sites now oriented towards the ribose. Generally, the *anti* form is more preferable than the *syn* form, however, the stability difference depends on the base molecules and the atmosphere surrounding the molecules. When the base is adenine, *anti* is more preferable than *syn*. The energy difference between those (*anti* and *syn*) is only 0.3 kcal mol^{-1},[12] small enough to convert each other. The electrostatic interaction between the phosphate anions plays an important role to determine the polynucleotide conformation, especially, the salt concentration dependence of the conformation. The distance between the adjacent phosphate anions is about 5.9 Å for the *C3′-endo* puckering, while 7.0 Å for the *C2′-endo* puckering.[1] Therefore, *C2′-endo* is more preferable in lower salt concentrations than *C3′-endo*, because electrostatic repulsion is less sealed in the lower salt concentration. The sugar puckering and the glycosidic bond angle are not defined as one conformation, but as a group of conformations having a similar steric parameter.[12] Polynucleotide chains contain *C2′-endo* and *C3′-endo* at the same time.

Circular dichroism in the 240–300 nm wavelength range reflects the spatial relationship between the adjacent bases along polynucleotides through the interactions between the induced dipole moment of the bases. Olsthoorn *et al.*[13,14] extensively studied the relationship between the CD spectra and the chain conformation of poly(dA) determined by NMR, and found that the poly(dA) chain consists of *C2′-endo* and *anti* (S_{dA}) as a major species and *C3′-endo* and *anti* (N_{dA}) as a minor one, and with increasing the chain length, the population of N_{dA} essentially vanishes.[15] Since the population of N_{dA}–N_{dA} should be negligible, only two combinations for the adjacent bases essentially determine the CD spectra, that is, S_{dA}–S_{dA} and S_{dA}–N_{dA} sequences. The CD spectra for S_{dA}–S_{dA} and S_{dA}–N_{dA} sequences are characterized to have a strong negative band at 250 nm and a weak positive band at 280 nm, and a strong negative band at 250 nm and a strong positive band at 260 nm, respectively.

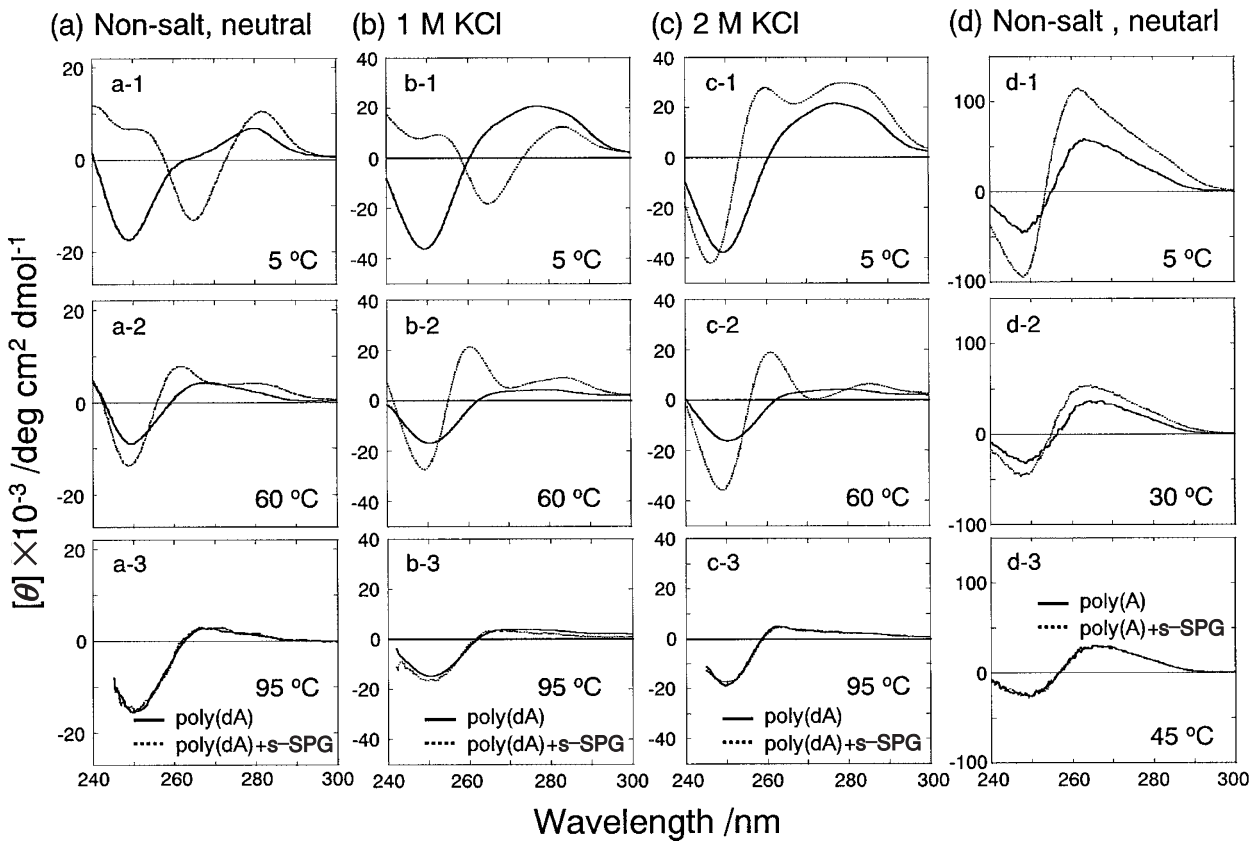


Figure 3. Comparison of the CD spectra in three solutions containing poly(dA) (non-salt and neutral: (a), 1 M KCl: (b), and 2 M KCl: (c)) at 5, 60, and 95 °C. For reference, poly(A) is shown in panel (d). In all panels, the solid and dotted lines represent the CD spectra for poly(dA) and the mixtures of poly(dA) and s-SPG.

EXPERIMENTAL

High-purity grades of NaCl, KCl, DMSO, D_2O, and formamide were purchased form Wako Chemical. Poly(dA) and poly(dT) were obtained from Amersham Phamacia and the degrees of polymerization, based on reported sedimentation velocities, were about 250 and 300, respectively. According to the previous work,[9] the poly(dA) base number of 250 was considered sufficient to ignore the chain end effect. The schizophyllan sample was kindly provided by Taito Co. in Japan. The molecular weight of s-SPG was 1.5×10^5, *i.e.*, 231 repeating units. A mixture of poly(dA) and s-SPG was prepared by adding a DMSO solution of s-SPG to an aqueous solution of poly(dA). Final concentrations of poly(dA) and s-SPG were 5.3×10^{-3} g dL^{-1} and 5.0×10^{-2} g dL^{-1}, respectively. The volume fraction of water in the mixture was 0.91 for all samples. Before measurements, all samples were annealed at 5 °C for 6–10 d. The CD and ultraviolet absorbance (UV) spectra in the 240–300 nm region were measured at 0–95 °C on a Jasco J-720WI spectropolarimeter and Jasco V-570 UV/VIS/NIR spectrophotometer, respectively. The CD and UV spectra depended only on the salt concentration, and the difference in cations did not provided any change. T_m was determined according to the established method.[4, 9, 10]

RESULTS AND DISCUSSION

Conformational Transition on Heating in Non-Salt and Neutral Solutions

Figure 3 presents the CD spectra in three solutions containing poly(dA) (non-salt and neutral: (a), 1 M KCl: (b), and 2 M KCl: (c)) at 5, 60, and 95 °C. For comparison, the poly(A) system is presented in panel (d). In all panels, the solid and dotted lines represent the CD spectra for poly(dA) and the mixtures of poly(dA) and s-SPG. Here, the mixture does not necessarily means the complex, but it may just consist of s-SPG and polynucleotide, individually. Hereinafter, we denote the complex and mixture as poly(dA)/s-SPG and poly(dA) + s-SPG, respectively. s-SPG is CD inactive at this wavelength, so that all change in CD should be ascribed to conformational changes in poly(dA).

In panel (a-1), there is considerable spectral difference between poly(dA) and poly(dA)/s-SPG. The spectrum of poly(dA) exhibits a typical feature for S_{dA}–S_{dA}, showing a strong negative band at 250 nm and a

weak positive band at 280 nm. Poly(dA)/s-SPG demonstrates an exciton-couple type band, showing a strong positive band at 283 nm and a new negative band at 265 nm. In the previous communication,[9] this spectrum was denoted as HL (see appendix) and, as far as we know, we have never seen such a spectral shape for poly(dA) and its derivatives. This is characteristic of the poly(dA)/s-SPG complex. On considering the origin of the CD spectrum in this range, the appearance of the new spectra suggests that the spatial relationship between the adjacent adenine moieties is drastically altered upon the complexation. The movement of the adenine in poly(dA) is achievable because the puckering in poly(dA) is *C2′-endo*. Thus the rotation around the glycosidic bond is allowed. Although determination of the exact torsional angel is impossible from CD data, the vanishing 250 nm negative band (characteristic of *anti* for both *C2′-endo* and *C3′-endo*) suggests that the *syn* form is one possibility for this novel CD pattern in the complex. We can thus conclude that the complexation induces the movement of the adenine from *anti* to a completely new form (maybe *syn*); as a consequence, the CD spectrum is drastically changed on the complexation. The spectral changes upon the complexation for poly(dA) are quite contrast with poly(A) (d-1) in which the complexation does not change the spectral shape but it only enlarges the intensity. This contrast between poly(A) and poly(dA) can be ascribed to the difference in the ribose puckering between poly(A) and poly(dA); therefore, the puckering in poly(A) is *C3′-endo* so that only *anti* is allowed.

At 95 °C in the non-salt and neutral solution, the poly(dA) in the complex undergoes conformational transition HL to H before the dissociation of the complex. Panel (a-2) presents the CD spectrum for H at 60 °C (dotted line), clearly showing considerable difference from HL (dotted line in a-1). Comparing with the CD spectrum of S_{dA}–N_{dA} reported by Olsthoorn *et al.*,[13] H is identical to their spectrum, having a strong negative band at 250 nm and a strong positive band at 260 nm. Therefore, H can be ascribed to a poly(dA) conformation containing *C3′-endo* and *anti* as a minority among the majority of *C2′-endo* and *anti*.

Salt Concentration Dependence of the Poly(dA) Conformation

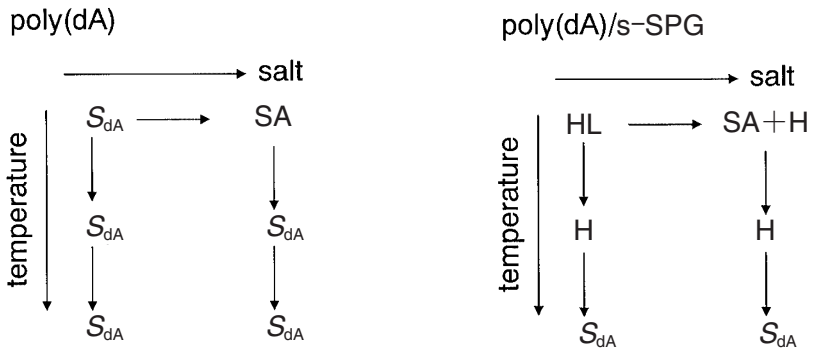
When we increased KCl concentration at 5 °C (compare (a-1), (b-1), and (c-1) in Figure 3), the poly(dA) spectrum (solid line) increases the intensity around 275 nm with maintaining the 250 nm negative band. This indicates that there is a new conformation in the higher salt solution. Hereinafter, we label it as SA. In comparison of the solid lines between (c-1) and (d-1), the overall spectral shape of SA is quite similar to that of poly(A). In the previous work,[1] poly(A) takes *C3′-endo* and *anti*, so that the similarity suggests that poly(dA) also takes *C3′-endo* and *anti* in the 2 M and 1 M KCl solution. This is a reasonable conclusion from the standpoint that the salts reduce the electrostatic repulsion between the phosphate anions. The distance between the adjacent phosphate anions is about 5.9 Å in *C3′-endo*, while it is 7.0 Å in *C2′-endo*. Therefore, in the non-salt solution, *C2′-endo* is more preferable than *C3′-endo* and this seems the main reason why poly(dA) takes *C2′-endo* in the non-salt solution. When a suitable amount of salts is added, the electrostatic repulsion becomes less important and other factors determine the conformation.

When we examine the salt-concentration dependence of the spectrum for poly(dA) at 5 °C [solid lines in (a-1), (b-1), and (c-1) in Figure 3], the spectrum changes from S_{dA} to SA between 0 and 1 M KCl. The complex maintains HL at 1 M KCl and the spectral change occurs between 1 and 2 M. In 2 M KCl, the spectrum of the complex seems to consist of H and SA. This indicates that the poly(dA) chain in the complex takes almost *C3′-endo* and *anti* in 2 M KCl. When we increased temperature in 2 M KCl, H was maintained and the 275 nm positive band disappeared, indicating that at 2 M KCl, the H conformation dominates at all temperatures. Therefore, there is no conformational transition on heating in the 2 M KCl solution.

Figure 4 summarizes the salt concentration dependence of the CD spectrum of poly(dA)/s-SPG, compared with that of poly(dA). At the low temperature and low salt concentration, the poly(dA) in the complex takes the HL form, which is estimated to be *C2′-endo* with the different torsional angle from the *anti* form. With increasing temperature, HL changes to H, which bears characteristics of *C3′-endo* and *anti* as a minority among the majority of *C2′-endo* and *anti* (*i.e.*, S_{dA}–N_{dA}). When the salt concentration is increased, the spectrum changes to combination of SA and H, with disappearing HL at 5 °C and H form is dominated at 60 °C.

Melting Behavior of the Complex and Comparison with Poly(dA)/Poly(dT) Duplex

Figure 5 compares the temperature dependence of UV absorbance at 257 nm for poly(dA), poly(dA) + s-SPG in non-salt and neutral, and poly(dA) + s-SPG in 2 M NaCl. For poly(dA), the absorbance increases with increasing temperature, due to the decrease in the helix content in the original *C2′-endo* and *anti* form.[1, 12, 13, 16] For poly(dA) + s-SPG in non-salt and neutral, the absorbance at 0 °C is lower than that of poly(dA) because



S_{dA}: *C2'-endo and anti* for poly(dA) chain
N_{dA}: *C3'-endo and anti* for poly(dA) chain
HL: high molecular weight and low temperature form, *C2'-endo* in that the torsion of the glycosidic bond is different from S_{dA}, it can be *syn*
H: high temperature form, *C3'-endo and anti* as a minority among the majority of *C2'-endo and anti*
SA: conformation of poly(dA) itself in higher salt concentrations

Figure 4. Summary of the salt concentration dependence of the CD spectrum of poly(dA)/s-SPG, compared with that of poly(dA).

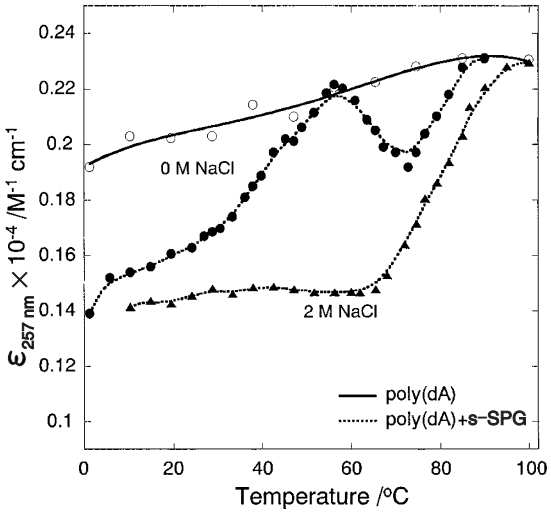


Figure 5. Temperature dependence of UV absorbance at 257 nm for poly(dA), poly(dA) + s-SPG in the non-salt and neutral solution, and poly(dA) + s-SPG in 2 M NaCl.

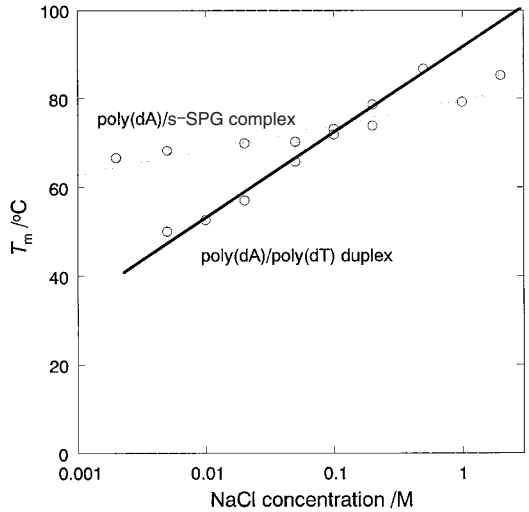


Figure 6. Comparison of NaCl concentration dependence of T_m for poly(dA)/poly(dT) duplex and poly(dA)/s-SPG complex.

of the hypochromic effect due to the complexation. On heating, it stays at a lower value than that of poly(dA) at T = 0–50 °C, then increases to the same value with poly(dA), and again shows the lower absorbance than that of poly(dA) in T = 60–95 °C before the dissociation of the complex. The disappearance of the hypochromic effect is consistent with the conformational transition before melting. In sharp contrast to the non-salt and neutral solution, the complex in 2 M NaCl dose not show such disappearance. There is thus no conformational transition.

Figure 6 plots T_m against NaCl concentration comparing between poly(dA)/s-SPG and poly(dA)/poly(dT). When NaCl concentration is less than 5 mM, there is no hybridization for the poly(dA) + poly(dT) mixtures, Because both poly(dA) and poly(dT) are polyanions, so that the electrostatic repulsion between the cations has to be shielded by salts in order to form the duplex.[1,17,18] With increasing NaCl concentration ([NaCl]), T_m increases 100 °C at [NaCl] = 2 M. T_m of the complex is insensitive to [NaCl], compared to the poly(dA)/poly(dT) duplex. For the complex, T_m increases from 65 to 75 °C when [NaCl] increases from 0 to 2 M. This may be the schizophyllan chain having no electrical-charge so that there is no electrostatic repulsion between s-SPG and poly(dA). The small increment in T_m in the complex is contrast to that of PNA/DNA complex, in that T_m decreases slightly with increasing [NaCl].[19,20] PNA is a peptide nucleic acid in which the phosphodiester backbone has been replaced by a pseudo-peptide chain, so that there is no electronic

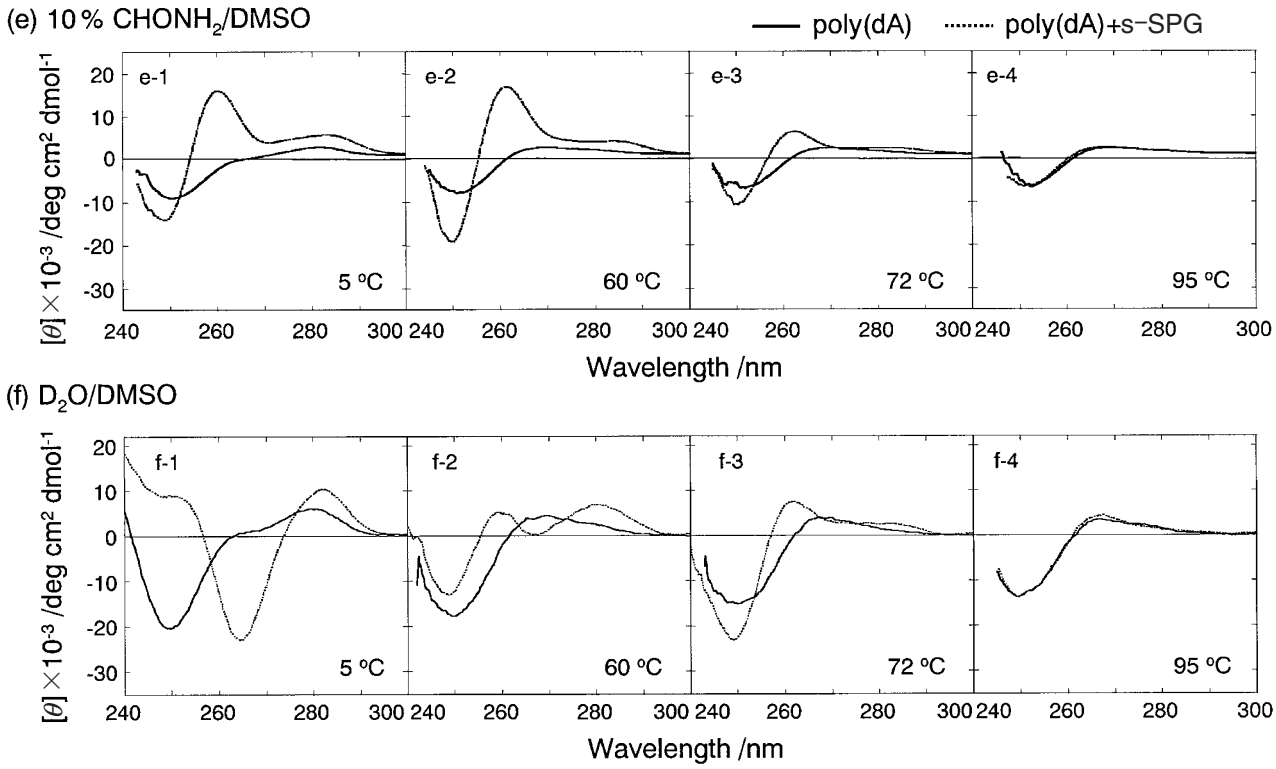


Figure 7. Temperature dependence of the CD spectra for poly(dA) and poly(dA) + s-SPG in (e) 10% formamide and (f) D_2O solution.

change in the chain.[21] The reason for increase T_m with increasing [NaCl] is not clear, but possibly related to the conformational change in poly(dA).

Addition of D_2O or Formamide

Figure 7 shows the temperature dependence of the CD spectra for poly(dA) and poly(dA) + s-SPG in aqueous solution containing 10 vol% formamide/DMSO and in D_2O/DMSO. Formamide cleaves the hydrogen bonding. Poly(dA) decreases the CD intensity at 250 nm, suggesting decrease in the helix content. As shown in (e-1), the CD spectrum of the complex in the formamide solution at 5 °C is quite similar to that of the non-salt and neutral solution at 60 °C. On comparing (e-1) and (a-2), formamide induces the same effect as increasing temperature. The addition of formamide enlarges the H conformation range.

When we exchanged the solvent for D_2O (Figure 7 (f-1)), the CD spectrum at 5 °C is essentially the same as that of the H_2O system. However, the HL form is not replaced by the H form at 60 °C (see (f-2)) and the transition temperature form HL to H can be evaluated to be 72 °C, which is 12 °C higher than that of the H_2O system. Therefore, D_2O induces the same effect as decreasing temperature.

SUMMARY

The relationship between the conformation and solvent for the poly(dA)/s-SPG complex was examined. The diverse and complicated CD spectra for the poly(dA)/s-SPG complex can be explained by the conformational diversity of the ribose puckering and the freedom of the torsional angle of the glycosidic bond for the adenine. This new sight should broaden our horizon to understand the novel interaction between polysaccharides and polynucleotides.

APPENDIX

Figure 8 shows the CD spectral change on heating for the poly(dA)/s-SPG complex. The spectra at 0 °C are different from those reported in the previous paper. The previous HL spectrum had a small negative band at 250 nm, whereas, the present one, a positive shoulder around 250 nm. The low temperature spectrum for poly(dA)/s-SPG (*i.e.*, HL) was subsequently found to depend on annealing conditions. The longer annealing time provides the smaller negative band at 250 nm and one week is sufficient for saturation. In this work, all samples were left at 5 °C for at least one week and longer than one week did not provide an appreciable change in the CD spectrum. As shown in the figure, the spectra obtained in the temperature range of 0–60 °C (upper panel) have isosbestic points at 257 and 280 nm, and those at 65–90 °C (lower panel) have isosbestic points at 254 and 270 nm. The upper panel is totally different from the lower panel. These isosbestic

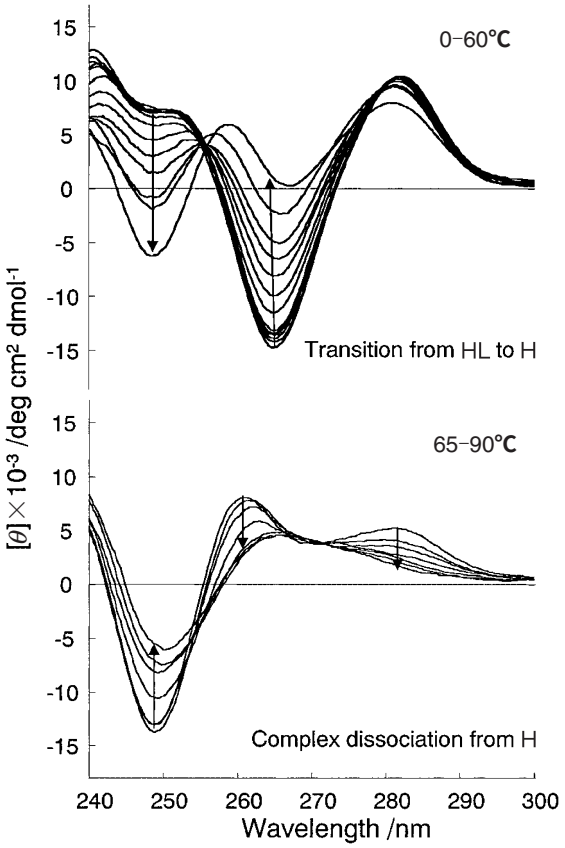


Figure 8. Temperature dependence of the CD spectra for poly(dA)+s-SPG in non-salt and neutral solution. Upper panel shows the changes between 0–60 °C and the lower panel, 65–90 °C.

points indicate that the CD spectrum involves two competing conformations in equilibrium and one conformation becomes dominant on heating. Figure 8 thus confirms the poly(dA) chain in the complex undergoes a conformational transition on heating below T_m of the complex, and the low and high temperature forms are designated as HL and H, respectively.

Acknowledgment. This work is finically supported by the "Organization and Function", PRESTO, and SORST programs in Japan Science and Technology Corporation (JST).

REFERENCES AND NOTES

1. W. Saenger, in "Principles of Nucleic Acid Structure", Springer-Verlag, New York, N.Y., 1984.
2. J. W. Chase and K. R. Williams, *Ann. Rev. Biochem.*, **55**, 103 (1986).
3. M. D. Powell and D. M. Gray, *Biochemistry*, **34**, 5635 (1995).
4. K. Sakurai and S. Shinkai, *J. Am. Chem. Soc.*, **122**, 4520 (2000).
5. K. Tabata, W. Ito, T. Kojima, S. Kawabata, and A. Misaki, *Carbohydr. Res.*, **89**, 121 (1981).
6. T. Norisuye, K. Yanaki, and H. Fujita, *J. Polym. Sci.*, **18**, 547 (1980).
7. K. Yanaki, T. Norisuye, and H. Fujita, *Macromolecules*, **13**, 1462 (1980).
8. T. M. McIntire and D. A. Brant, *J. Am. Chem. Soc.*, **120**, 6909 (1998).
9. M. Mizu, T. Kimura, K. Koumoto, K. Sakurai, and S. Shinkai, *Chem. Commun.*, 429 (2001).
10. K. Sakurai, M. Mizu, and S. Shinkai, *Biomacromolecules*, **2**, 641 (2001).
11. N. Berova, K. Nakanishi, and R. W. Woody, Eds., "Circular Dichroism: Principles and Application", 2nd ed, Wiley-VCH, Canada, 2000.
12. N. Stephen, in "Nucleic Acid Structure and Recognition", Oxford University Press Inc., London, 2002.
13. C. S. M. Olsthoorn, L. J. Bostelaar, J. F. M. Rooij, J. H. van Boom, and C. Altona, *Eur. J. Biochem.*, **115**, 309 (1981).
14. C. S. M. Olsthoorn, L. J. Bostelaar, J. H. van Boom, and C. Altona, *Eur. J. Biochem.*, **112**, 95 (1980).
15. S_{dA} and N_{dA} specifically stand for the *C2′-endo* and *anti* and *C3′-endo* and *anti* conformations for poly(dA) chain.
16. V. V. Filimonov and P. L. Privalov, *J. Mol. Biol.*, **122**, 645 (1978).
17. B. Pullman and A. Pullman, *Progr. Nucl. Acid Res. Mol. Biol.*, **9**, 327 (1969).
18. D. Porschke, *Mol. Biol. Biochem. Biophys.*, **24**, 191 (1977).
19. M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature*, **365**, 566 (1993).
20. S. Tomac, M. Sarkar, T. Ratilainen, P. Wittung, P. E. Nielsen, B. Norden, and A. Graslund, *J. Am. Chem. Soc.*, **118**, 5544 (1996).
21. P. E. Nielsen and G. Haaima, *Chem. Soc. Rev.*, **26**, 73 (1997).