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Bioorganic Chemistry 31 (2003) 216–226
BIOORGANIC CHEMISTRY
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Polysaccharide–polynucleotide complexes. Part 7. Hydrogen-ion and salt concentration dependence of complexation between schizophyllan and single-stranded homo RNAs

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Received 2 April 2002

Abstract

Schizophyllan belongs to a β-1,3-D-glucan family, which exists as a random coil in dimethyl sulfoxide (DMSO) and as a triple helix in water, respectively. The schizophyllan single chain forms a complex with single-stranded homo RNAs in water/DMSO mixed solvents. Using circular dichroism, we studied the complexation and its stability as a function of apparent pH (pH*) in a mixed solvent system and as a function of the salt concentration. The complex is formed in the pH* range 6.5–10, and dissociated in the pH* range 4–6. Both poly(A) and poly(C) adopt a double strand in the pH* range 4–6 and a single strand in the pH* range 6.5–10. Therefore, the conformational change of each polynucleotide is responsible for dissociation/association of the complex, i.e., the single strand of the polynucleotides can form complexes, whereas the double one cannot. This result indicates that hydrogen bonding and similarity of the helix parameters are essential for the complex formation. The melting temperature of the complex reaches the maximum around 0.05 M of NaCl and KCl, and the value of the maximum temperature depends on the cation species.

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0045-2068/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0045-2068(03)00034-8

Keywords: Schizophyllan; β-1,3-Glucans; Conformational change of polynucleotide; RNA; Polysaccharide–polynucleotide complex

1. Introduction

Schizophyllan is a cell wall polysaccharide produced by the fungus *Schizophyllan commune*, where the main chain consists of β-1,3-D-glucans and the one β-(1→6)-D-glucosyl side chain links to the main chain at every three glucose residues [1], as shown in Fig. 1. Yanaki et al. [2] and Young and Jacobs [3] showed that schizophyllan exhibits antitumor activity against *Sarcoma 180* as well as lectin activity against *Limulus amebocyte* lysate. Norisuye et al. [4–7] extensively studied the dilute solution properties of schizophyllan and determined that schizophyllan adopts a triple helix conformation [8] (see (b) in Fig. 1) in water and a random coil in dimethyl sulfoxide (DMSO) [4,5]. In addition, when DMSO is added to the schizophyllan aqueous

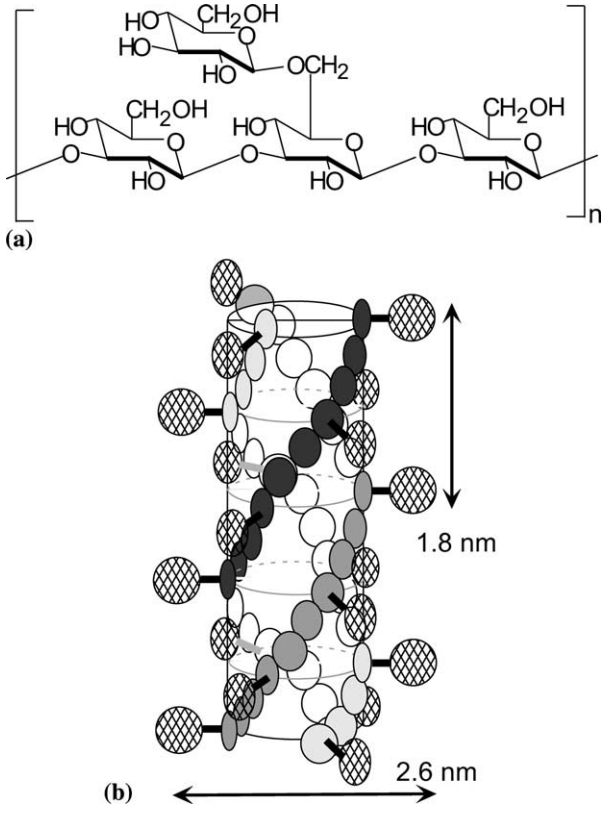

Fig. 1. Repeating unit of schizophyllan (a) and its representative model of the triple-helix (b). In (b), the plain circles represent the main chain glucose residues and the meshed ones, the side chains.

solution (denaturation), the helix remains intact up to the water fraction in the DMSO–water mixture (V_w) = 0.14 [5]. However, they found that, when water is added to the DMSO solution (renaturation), the single chain of schizophyllan (s-SPG) collapses at $V_w = 0.14$ owing to hydrophobic interactions [7]. Brant and coworkers [9,10] and Jacobs et al. [11] independently showed that the local structure of the renatured product can return to the original triple helix although the entire structure is not the same as the original rod like molecules. Recently, McCormick et al. [12] found an interesting interaction between schizophyllan and an amphipathic protein called SC3p [12], which plays an important role in fungal attachment to a host organism. These pioneering studies have encouraged efforts to understand the nature of this polysaccharide. The mechanism of its antitumor activity is not well understood at the molecular level and remains a challenging issue in the field of saccharide biochemistry.

Sakurai and Shinkai [13] were the first to demonstrate that s-SPG forms a macromolecular complex with poly(C) and poly(A) during renaturation. This work may provide a new clue to understand the mysterious bioactivity of this polysaccharide. Their subsequent studies [14,15] revealed that (1) two schizophyllan chains and one poly(C) chain form a triple helix and (2) one glucose in each s-SPG, (i.e., two glucose in total) form hydrogen bonds with one cytosine residue (see chemical structure (C) in Fig. 5). Their X-ray crystallography showed that the helix parameters of the complex are close to that of triple-helix schizophyllan: a right-handed 6_1 triple helix with a 17.4 Å pitch [15]. According to the literature [16], the helix parameter of the single-stranded poly(C) is a right-handed 6_1 helix with a 18.6 Å pitch. These two parameters are surprisingly close so that we can assume that there should be little loss in the conformational entropy after the complexation. This explains why poly(C) and s-SPG form the complex.[1]

The above discussion indicates that conformational similarity between two chains is important for the complex formation. Polynucleotides also undergo conformational transitions upon changing the hydrogen-ion concentration and the ionic strength [16]. Therefore, complexation presumably depends on these solution properties. In this paper, we explore the relationship between the complexation and the hydrogen-ion and salt concentrations.

2. Materials and methods

The triple helix of schizophyllan (t-SPG) was kindly supplied by Taito (Tokyo, Japan). The intrinsic viscosities ([η]) of t-SPG and s-SPG in water and DMSO at 25 °C were determined to be 6.1 and 0.92 dL g^{-1}, respectively, and from those [η] values the molecular weights were calculated to be 4.5×10^5 and 1.5×10^5 (690 and 230

[1] Poly(A) forms a right-handed 9_1 helix with a 25.4 Å pitch. This parameter is apparently different from that of the complex. However, since the axial rise per residue is close: 2.9 Å for SPG and 3.1 Å for poly(A), we can consider that it is easy for poly(A) to fit the conformation to the complex without major entropy loss.

in the degree of polymerization), respectively. Poly(A) and poly(C) were purchased from Amersham Pharmacia (Buckinghamshire, UK) and the degree of polymerization, calculated from the reported sedimentation velocities [17], are 320 and 570, respectively. RNase free, deionized, and distilled water, spectroscopic grade DMSO, NaCl, and KCl were purchased from Wako Chemical (Osaka, Japan). A polynucleotide sample was mixed with s-SPG by adding a s-SPG/DMSO solution to a polynucleotide/water solution. Hereinafter, we denote the mixture as poly(X) + s-SPG, where X can be A or C. The water volume fraction in the water/DMSO mixture (V_w) was fixed at 0.92 for all measurements. The molar concentrations of s-SPG ($M_{s\text{-}SPG}$) and the polynucleotide ($M_{poly(X)}$) in the DMSO/water mixture were 1.0×10^{-3} and 2.6×10^{-4} M, respectively. The apparent pH (pH*) of the DMSO/water solutions was controlled by adding NaOH and HCl aqueous solutions, and monitored with a Horiba D-22 pH meter at room temperature. All the samples were kept at 5 °C for at least 3 days before the measurement.

Circular dichroism (CD) in 230–320 nm region was measured on a Jasco J-720WI spectropolarimeter with a 1.0 cm cell equipped with a water-jacket to control the cell temperature. The measurements were carried out over the pH* range 4–10, the salt concentration range 0–0.3 M, and the temperature range 5–70 °C. The molecular ellipticity ([θ]) was determined by conventional methods [14] and the [θ] value at the peak top or bottom position was denoted as $[\theta]_{max}$.

3. Results and discussion

3.1. The apparent pH dependence of complexation

Fig. 2 compares the CD spectra between poly(C) and poly(C) + s-SPG at pH* 4, 7, and 10 (from top to bottom). The CD spectra of two solutions (i.e., polynucleotide itself and its mixture with s-SPG) are identical at pH* 4, but significantly different from each other at pH* 10 and 7. Furthermore, the poly(C) spectrum at pH* 4 is different from those of poly(C) at pH* 10 and 7, suggesting the presence of a transition between pH* 4 and 7. Fig. 3 presents the CD spectra of poly(A) and poly(A) + s-SPG at pH* 4, 7, and 10. Similar to the poly(C) system, the spectra of poly(A) and poly(A) + s-SPG are identical at pH* 4 and different at pH* 7 and 10, and the poly(A) spectrum changes with increasing pH* from 4 to 7.

The s-SPG sample has no absorption in the 230–300 nm region where the CD measurements were carried out. Therefore, the CD spectrum in this region is related to chirality of the polynucleotide chains, particularly to how the base moieties stack in the helix [16]. Previously, we examined the CD spectra for the poly(A) and poly(C) systems at pH* 7 and found that the difference between polynucleotide itself and its mixture can be ascribed to the fact that the polynucleotide and s-SPG form a macromolecular complex [13–15]. Since each spectrum at pH* 10 for the mixture is identical to that of the corresponding sample at pH* 7, the complex is maintained at pH 10. At pH* 4, there is no difference between the polynucleotide itself and the mixture, indicating that s-SPG cannot form a complex with the polynucleotides at pH* 4.

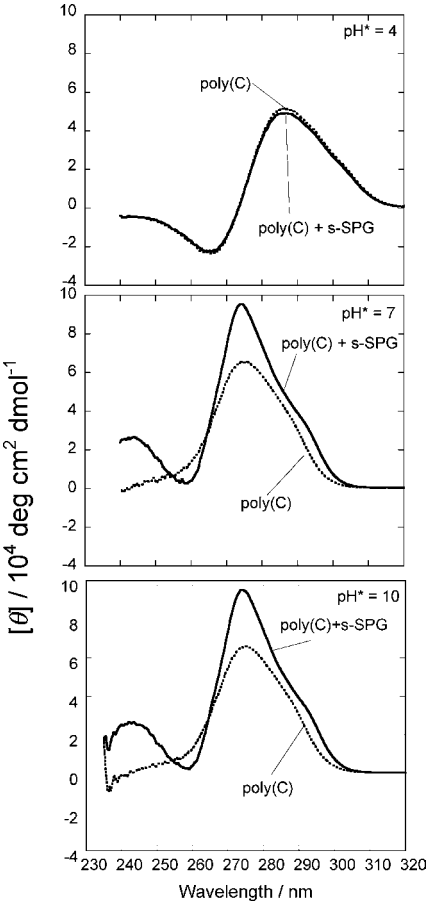

Fig. 2. The apparent pH dependence of the CD spectra for poly(C) (dotted lines) and its mixture with s-SPG (solid lines) measured at 10 °C.

Fig. 4 plots the $[\theta]_{max}$ against pH* for the poly(C) and poly(A) systems. The $[\theta]_{max}$ values for the polynucleotides (before mixing, indicated by filled circles in the figure) seem constant between pH* 10 and 7.0. At pH* 6–7, $[\theta]_{max}$ drastically changes and becomes almost constant at pH* values <6 for poly(A) and poly(C). This feature indicates that the polynucleotides themselves (before mixing) undergo a conformational change at pH* 6–7. According to the literature [16,18], poly(C) and poly(A) adopt a single strand in the neutral and basic regions and a double strand in the acidic region. The mixtures show different $[\theta]_{max}$ from those of polynucleotides themselves in the pH* range 6.5–10 and a drastic change in CD at the same pH* where polynucleotides themselves show the transition. In the pH* range 4–6, the polynucleotide itself and the mixture show the same $[\theta]_{max}$ values, indicating no interaction between s-SPG and the polynucleotide. This feature clearly indicates that the conformational change of each polynucleotide is related to the dissociation/association of

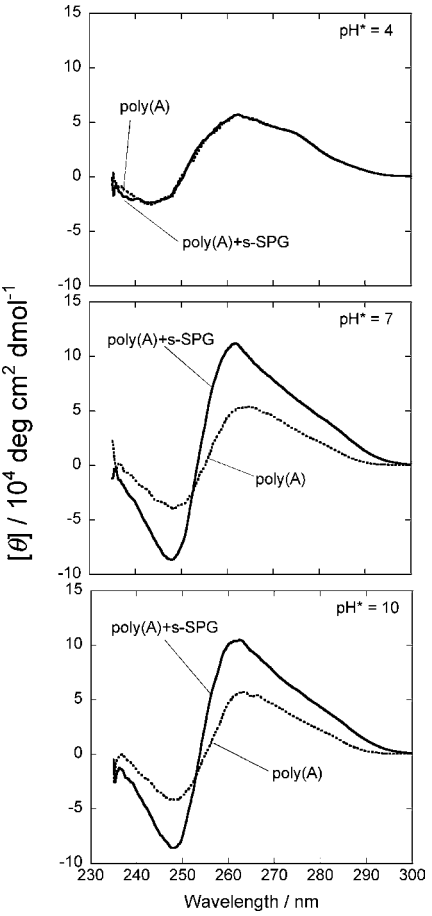

Fig. 3. The apparent pH dependence of the CD spectra for poly(A) (solid lines) and its mixture with s-SPG (dotted lines) measured at 10 °C.

the complex: the single strand of the polynucleotides can form complex, whereas the double one cannot.

Previous work [18] shows that dimerization of the nucleic acids is the origin to form the double stranded conformation at pH* 6–7. Fig. 5 presents the proposed dimer structure for the cytosine and adenine residues [18]. Incremental increases in the hydrogen-ion concentration induces protonation of the base and hydrogen bonds are formed between two bases, which results in the conformational transition of the polynucleotide chain from the single to double strand. It is well known that the pK_a is 4.2–4.6 for N_3 in cytosine and 3.5–3.9 for N_1 in adenosine, respectively [19]. These reported values are lower than those obtained for poly(C) and poly(A). Hartman and Rich [18] studied the titration curve for poly(C) and confirmed that protonation of poly(C) takes place at pH* 6–7. The difference in pK_a between the monomeric and polymeric systems can be explained by a neutralization effect where "the positive

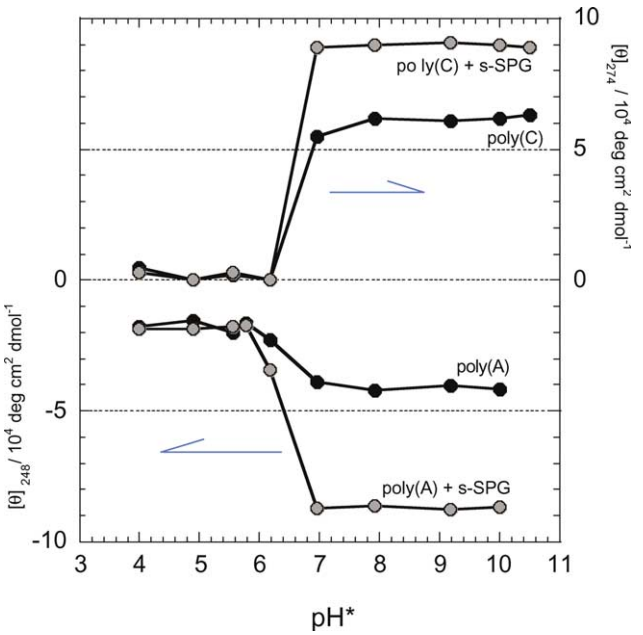

Fig. 4. Comparison of pH* dependence of $[\theta]_{max}$ for the poly(A) system and poly(C) system. For convenience to compare the transition, $[\theta]_{274}$ for the poly(C) is plotted in the upper part and $[\theta]_{248}$ (see Fig. 3, this is the negative band) is plotted in the lower part.

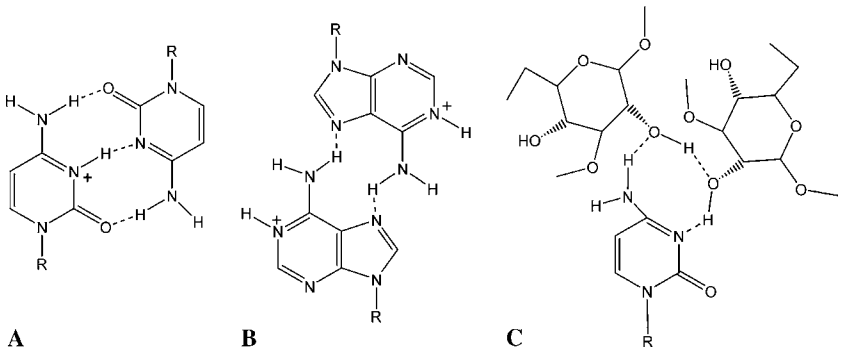

Fig. 5. The hydrogen bonding schemes between cytosine residues in the acidic solution (A), between adenine residues (B), and a proposed model for the complex binding site made of two glucose and one cytosine residues.

charge on the base dimer partially neutralizes the two negative charges on the ribose phosphate chains'' [18]. Since this effect can be generally extended to the poly(A) system, we consider that pK_a of poly(A) also shifts from 3.5–3.9 (monomer) to 6.0. Therefore, we confirm that the protonation and the subsequent conformational change are responsible for the dissociation of the complex.

The preceding discussion suggests that there are two possible causes leading to the dissociation of the s-SPG/polynucleotide complex. One cause is the loss of the hydrogen bonding site for s-SPG. According to our proposed model for the complex, it consists of two s-SPG chains and one polynucleotide chain and these chains interact with each other through the hydrogen bonding as shown in Fig. 5C. Since the dimerization of cytosine uses the same nitrogen atoms (N_3 and N_4) as those involved in the complexation, s-SPG loses a binding site after cytosine undergoes dimerization, resulting in dissociation of the complex.

The second cause for the dissociation is the dissimilarity in the helix parameters between the complex and the polynucleotide duplexes. As mentioned in Section 1, similarity in the helix parameters between the complex and the polynucleotides can explain the complexation. According to the literature [16,18], the helix parameters for the double strand of poly(A) and poly(C) are a right-handed 8_1 helix with a 30.4 Å pitch (3.8 Å for the axial rise per residue) and a right-handed 11_1 helix with a 37.3 Å pitch (3.4 Å for the axial rise per residue), respectively. In both cases, the axial rise per residue is considerably larger than that of the single strand and t-SPG. We suggest that this enlarged residue distance makes it difficult for s-SPG to form a complex with the polynucleotides in the acidic region.

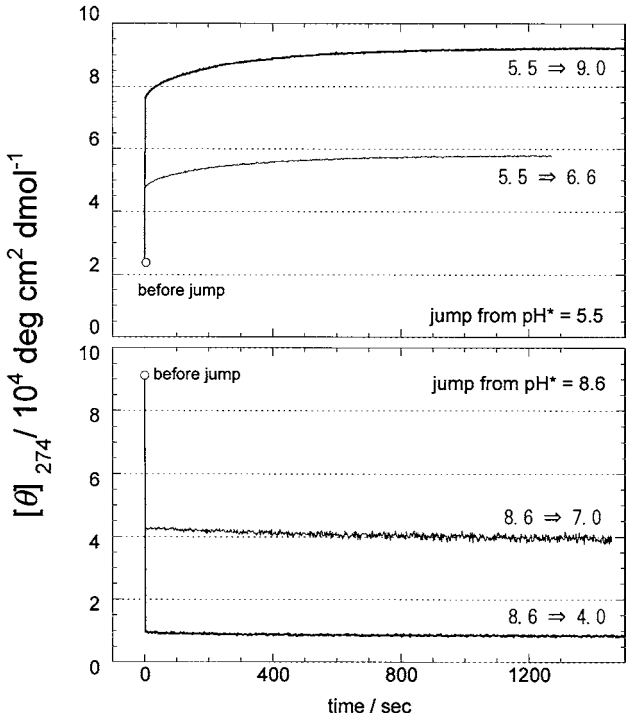

Fig. 6. Time course of $[\theta]_{274}$ after pH^* jumped from the acid to base region (complexation) in the upper panel and form the base to acid region (decomplexation) in the lower panel for the poly(C) + s-SPG system.

Fig. 6 presents the time course of $[\theta]_{274}$ after changing pH* from the basic to the acidic region (complexation) in the upper panel, and from the acidic to the basic region (decomplexation) in the lower panel. The data indicate that the complexation and decomplexation take place reversibly. After changed pH*, $[\theta]_{274}$ reaches the equilibrium value immediately for the decomplexation, while, it takes about 1000 s for complexation. This difference can be explained by the fact that diffusion of both chains is the rate determining step for the complexation.

4. The salt concentration dependence of complexation

Fig. 7 compares the CD spectra between a neutral solution without salt and a neutral solution containing 0.3 M NaCl. Poly(C) shows a substantial increase in the CD intensity with the increasing salt concentration. This observation can be explained by the fact that higher salt concentration enhances the electrostatic shielding, which, in turn, causes an increase in the number of the stacked bases in the helix. On the other hand, the spectrum for the complex seems independent of the salt concentration, confirming that most of the bases are already stacked in the complex [14]. Fig. 8 compares the melting curves when the NaCl concentration is changed from 0 to 0.3 M. The melting temperature (T_m) is determined, using conventional methods ([13,14]), and plotted against the salt concentration for both NaCl and KCl in Fig. 9.

The melting temperature drastically increases at low concentrations, reaches the maximum around 0.07 M, and gradually decreases with further increases in the salt concentration. It is interesting that NaCl and KCl give different T_m, suggesting that a specific interaction between poly(C) and these metal cations is involved. Cations

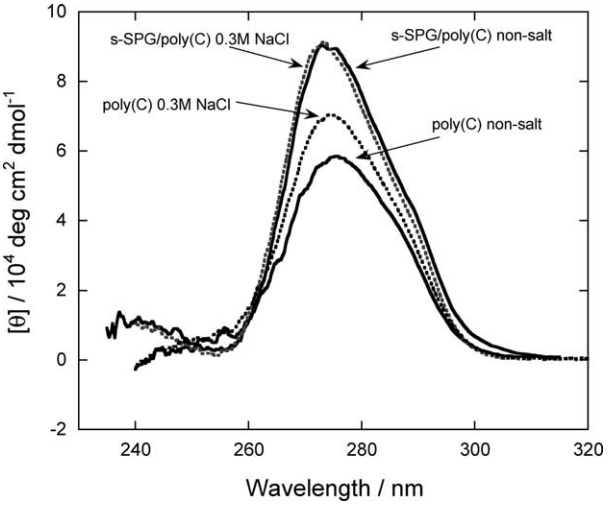

Fig. 7. NaCl concentration dependence of the CD spectra for poly(C) and poly(C) + s-SPG measured at 10 °C. The pH* was controlled by 10 mM Tris buffer.

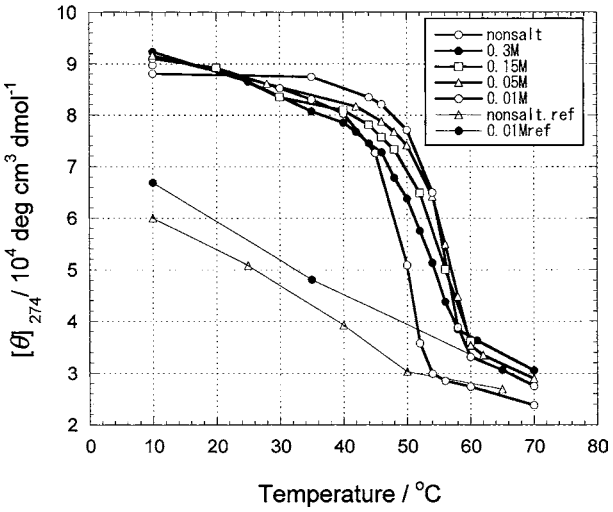

Fig. 8. NaCl concentration dependence of the melting curves of the poly(C) + s-SPG complex. The pH* was controlled by 10 mM Tris buffer. For comparison, the temperature dependence of $[\theta]_{274}$ for poly(C) solutions are presented.

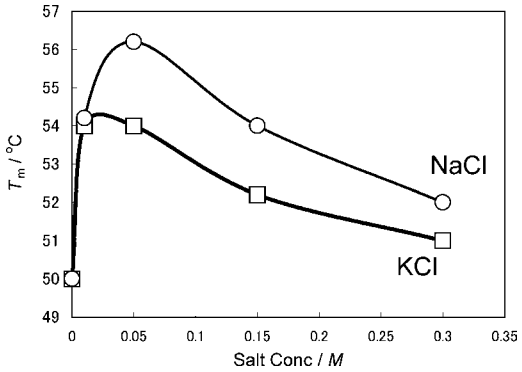

Fig. 9. Comparison of the salt concentration dependence of T_m between NaCl and KCl.

preferably bind to the phosphate anion in polynucleotides and the phosphate anions in polynucleotides interact with the base and ribose through water molecules [16]. Therefore, the increment of T_m at lower salt concentrations may be ascribed to the fact that the cations bound to the phosphate stabilize hydrogen bonding. At higher salt concentrations, the electrostatic shielding effect can stabilize the poly(C) helix as shown in Fig. 9. Hence, the free energy change resulting from decomplexation becomes small, which lowers the T_m. This mechanism might explain the decrease in T_m with increasing the salt concentrations.

5. Conclusions

The present paper demonstrates that formation of the complex between s-SPG and single-stranded RNAs as well as its stability strongly depend on pH and the ionic strength. The complex is formed in the pH^* range 6.5–10 and dissociated in the pH^* range 4–6. Both poly(A) and poly(C) are double stranded in the pH^* range 4–6 and a single strand in the pH^* range 6.5–10. Along with this conformational transition, dissociation and complexation take place reversibly. The T_m reaches the maximum around 0.05 M of NaCl and KCl and the value of T_m depends on the nature of the cation species. This observation suggests that some specific interaction is involved in stabilization of the complex.

Acknowledgments

We thank Taito Co. for kindly providing the schizophyllan sample. This work is financially supported by "Organization and Function," PRESTO, and SORST programs in Japan Science and Technology Corporation (JST).

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