Polysaccharide–polynucleotide complexes. Part 12.† Enhanced affinity for various polynucleotide chains by site-specific chemical modification of schizophyllan‡

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Recently, we found that schizophyllan (SPG), which is a natural and neutral polysaccharide, can form macromolecular complexes with certain polynucleotides. In order to increase the stability and the affinity of the complexes, we synthesized a 2-aminoethanol-appended SPG (N-s-SPG) by utilizing periodate oxidation and reductive amination. Although the modification level was only 2.4 ± 0.3%, the melting temperature of the poly(C)–N-s-SPG complex was increased by 8 °C comparing with the poly(C)–s-SPG complex. In addition, the conformation of the complex was scarcely perturbed by the introduction of amino groups. When we formed complexes of N-s-SPG and other polynucleotides, the melting temperature of their complexes increased without exception. Moreover, the amino modification induced complexation with poly(U), which does not form a complex with SPG. The affinity for the short chain nucleotide was also enhanced. These results indicate that the present modification method is quite useful to improve complex stability and we believe, therefore, that this strategy will make it possible to apply SPG to a novel gene carrier.

Introduction

Nonviral gene delivery systems are of great interest because these systems are safer and less immunogenic than viral systems.[1] Cationic polymers and amphiphiles are generally used as gene vectors because they can easily form polyion complexes with anionic polynucleotides and the resultant complexes can permeate through cell membranes. Although the efficiency of transfection has been increased by introduction of membrane active peptides,[2] proteins,[3] sugar moieties[4] and other biorelated materials,[5] the efficiency of transgene expression is still low. The reason for this could be related to the inherent property of the complexes formed by the polyion interaction. It is well known that polyion complexes become unstable in the presence of serum that includes a lot of biorelated molecules such as proteins, fatty acids, *etc.*[6] It is considered, however, that so far they are the most effective vectors in nonviral systems.

Recently, we found that schizophyllan (SPG) can specifically form a macromolecular complex with certain polynucleotides.[7] SPG is a natural and neutral polysaccharide produced by fungus *Schizophyllum commune*. The main chain consists of β-1,3-glucan and every third glucose unit has a β-1,6-glucoside side chain as shown in Fig. 1a.[8] The three chains are twisted together to form a triple helix (t-SPG) in water, in which strong hydrogen bonds between hydroxy groups in the 2-positions are

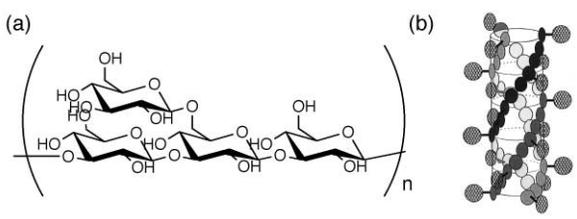

Fig. 1 Repeating unit of SPG (a) and a representative model of the triple-helix (b). In the panel (b), the plain circles represent the main chain glucose residues and the meshed ones, the side chains.

formed in the main chain.[9] The t-SPG chain is dissociated into a single chain of SPG (s-SPG) when it is dissolved in dimethyl sulfoxide (DMSO).[10] The s-SPG chain can regain the original triple helix by exchanging DMSO for water.[11,12] We found that when this solvent-exchange process is carried out in the presence of certain polynucleotides, the process creates a novel macromolecular complex.[7] Both the hydrophobic and the hydrogen bonding interactions play important roles in the thermal stabilization of the complexes.[13] Stoichiometric analysis revealed that two repeating units of SPG form the complex with three nucleic acids, in another words, when the length of each polymer is equal, the complex consists of two s-SPG chains and one polynucleotide chain.[7,13] Moreover, this complex has characteristics inherent to macromolecular complexes; these are (1) the polynucleotide chain incorporated into the complex can be protected from enzymatic degradation[14,15] and (2) the polynucleotide chain can be immediately released from the complex and form a DNA duplex when it meets the

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complementary nucleotide chain.[16] These characteristics are essential for gene vectors, in fact, when we examined the antisense-DNA–s-SPG complex in an *in vitro* transcription–translation assay, the complex suppressed the expression.[15] This fact indicates that s-SPG can be a new type of gene vector in a nonviral system. From the viewpoint of applying this complex to *in vivo* systems, it would be advantageous to improve the affinity of s-SPG for various nucleotide sequences by chemical modification.

According to our previous studies,[13,17–19] the β-1,3-glucan main chain of SPG is essential for complex formation, on the other hand, the side chain only provides solubility. In order to maintain the DNA-binding ability, therefore, our modification should be limited to the side chain. There are a lot of reports on the chemical modification for polysaccharides.[20] Among them, we chose periodate oxidation followed by the reductive amination as a method for modifying SPG. Periodate oxidation is a highly specific reaction to convert 1,2-diol groups to a pair of aldehyde groups without any significant side reaction; therefore, it has been widely applied to various biomolecules.[21–23] The advantage of applying this oxidation to SPG is that the reaction takes place only at the side chain because the main chain of SPG does not include any 1,2-diol groups, whereas the side chain does. In addition, the subsequent reductive amination also takes place at the aldehyde groups produced by the oxidation.[24,25] In our previous communication, we introduced a few percentages of 2-aminoethanol groups into the side chain of SPG and found that the resultant amino-modified SPG (N-s-SPG) increases the melting temperature of the poly(C)–N-s-SPG complex without any significant conformational changes.[26] In this full paper, we extend the complexation of N-s-SPG to other polynucleotides in order to compare the stability and the affinity differences between the N-s-SPG and s-SPG (unmodified one) complexes.

Experimental

Materials

t-SPG (M_w = 450 000, degree of polymerization 230) was kindly supplied by Taito Co., Japan. Poly(A), poly(C), poly(dA) and poly(dT) were purchased from Amersham Pharmacia, Japan, poly(G) from Sigma and poly(U) from Yamasa, Japan. The degree of polymerizations were calculated from the reported sedimentation velocities[27] to be about 570 (poly(A)), 320 (poly(C)), 300 (poly(dA)), 260 (poly(dT)), 300 (poly(G)) and 400 (poly(U)). RNase free, deionized and distilled water was purchased from Nippon Gene, dimethyl sulfoxide, of spectroscopic grade, from Kishida Chemicals, sodium periodate and acetic acid from Wako Chemicals and 2-aminoethanol from Tokyo Chemical Industry, Japan. All chemicals were used without further purification. The present 2-aminoethanol-appended SPG was synthesized according to the established method (see Scheme 1).[26] The content of the introduced amino group was evaluated as the amount of nitrogen% determined by elemental analysis[28] and the conversion ratio was estimated to be 2.4 ± 0.3%.

Measurement and instruments

All samples were prepared by mixing a polynucleotide–water solution with a s-SPG–DMSO solution, according to the reported method.[13] This prepared mixture was left at 4 °C for 3–10 days to allow the complexation to complete.[29] The final concentrations were usually controlled as follows: [poly(A)] = 2.4×10^{-4} mol dm^{-3}/monomer unit, [poly(C)] = 2.5×10^{-4} mol dm^{-3}/monomer unit, [poly(G)] = 2.3×10^{-4} mol dm^{-3}/monomer unit, [poly(U)] = 2.5×10^{-4} mol dm^{-3}/monomer unit, [poly(dA)] = 0.7×10^{-4} mol dm^{-3}/monomer unit, [poly(dT)] = 0.6×10^{-4} mol dm^{-3}/monomer unit, [s-SPG or N-s-SPG] = 8.5×10^{-4} mol dm^{-3}/repeating unit, [Tris] = 0–8.0×10^{-2} mol dm^{-3} (pH = 8.0). The circular dichroism (CD) spectra were measured on a Jasco J-720WI spectropolarimeter in the 240–340 nm region using a 1 cm cell.

Results and discussion

Comparison of the CD spectra of poly(C) + s-SPG (+s-SPG) and poly(C) + N-s-SPG (+N-s-SPG)

Fig. 2 compares the CD spectra of poly(C) itself, a mixture of

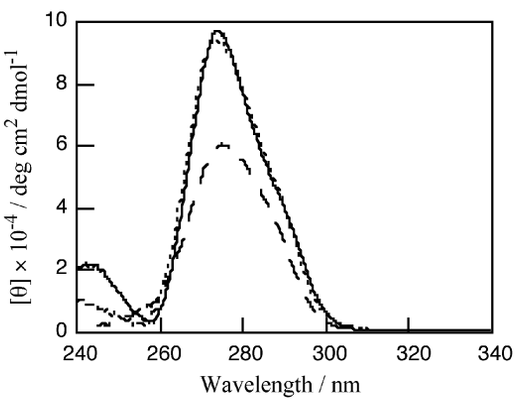
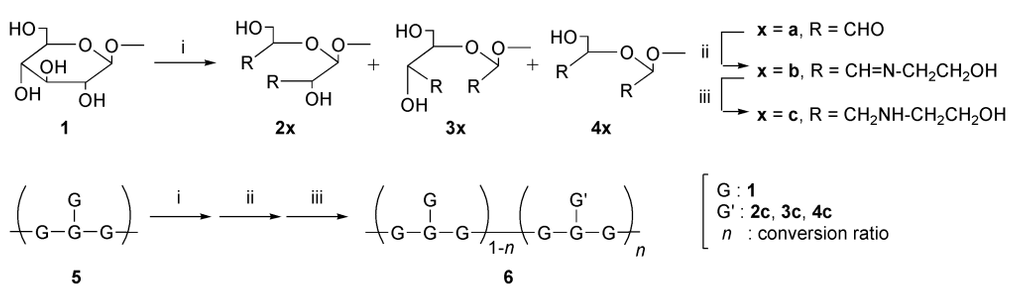


Fig. 2 Comparison of the CD spectra of poly(C) (broken line), +s-SPG (dotted line) and +N-s-SPG (solid line) measured at 15 °C in neutral and non-salt solution: [poly(C)] = 2.5×10^{-4} mol dm^{-3}/monomer unit.

poly(C) and s-SPG (+s-SPG) and a mixture of poly(C) and N-s-SPG (+N-s-SPG) at 15 °C, where [θ] is the molecular ellipticity. Neither s-SPG nor N-s-SPG has any absorbance in the wavelengths presented in the figure. Therefore, all CD intensities are ascribed to the conformational asymmetry of the poly(C) chain, in particular, the spectrum in this wavelength region sensitively reflects how the cytosine bases are stacked in the helix.[30]

As shown in Fig. 2, the spectrum of +s-SPG is different from that of poly(C) itself; the 275 nm band increases by 50% and a new band arises at around 242 nm. According to our previous study,[7,13] these spectral changes can be ascribed to the complexation between s-SPG and poly(C). The spectrum of +N-s-SPG also has these two characteristic bands and the intensity at



Scheme 1 Schematic illustration of the chemical modification of SPG. *Reagents and conditions*: (i) $NaIO_4$, H_2O, 4 °C, 2 days; (ii) 2-aminoethanol, DMSO, rt, 2 days; (iii) $NaBH_4$, DMSO, rt, 1 day.

275 nm is identical with that of +s-SPG. These features indicate that N-s-SPG forms a macromolecular complex with poly(C) and the complex structure is almost the same as that with the s-SPG system. A decrease or deformation in the CD spectrum is common when ion-pairs induce complexation. This is because ion-pair formation is so kinetically favourable that overall helical conformation is perturbed. However, we do not observe such an influence in the CD spectrum of +N-s-SPG. It is particularly worthy of mention that although N-s-SPG has cationic charges in its structure, the $[\theta]_{275}$ value is scarcely changed. These facts indicate that the amino group introduced into SPG may enjoy attractive electrostatic interactions with phosphate anions but do not perturb the original cytidine stacking.

According to our molecular model for the s-SPG–poly(C) complex,[17] the cytosine moieties stack inside the complex to avoid unfavourable contact with water, while the phosphate anions and the glucose side chains stick outwards from the complex due to hydrophilic interactions. As mentioned above, the amino group is introduced into the side chain, therefore, it should be possible for the amino and phosphate moieties to form ion-pairs outside the complex without perturbing the cytosine stacking.

Although the overall feature of the CD spectrum of +N-s-SPG is similar to that of +s-SPG, the intensity of $[\theta]_{242}$ due to +N-s-SPG is almost twice as large as that due to +s-SPG. The origin of the 242 nm band is not yet clear, however, this enhancement should be related to the ion-pair formation. In fact, the intensity of $[\theta]_{242}$ is independent of the Tris concentration for +s-SPG, but on the other hand, it is dependent on the Tris concentration for +N-s-SPG (see Fig. 3). As shown in

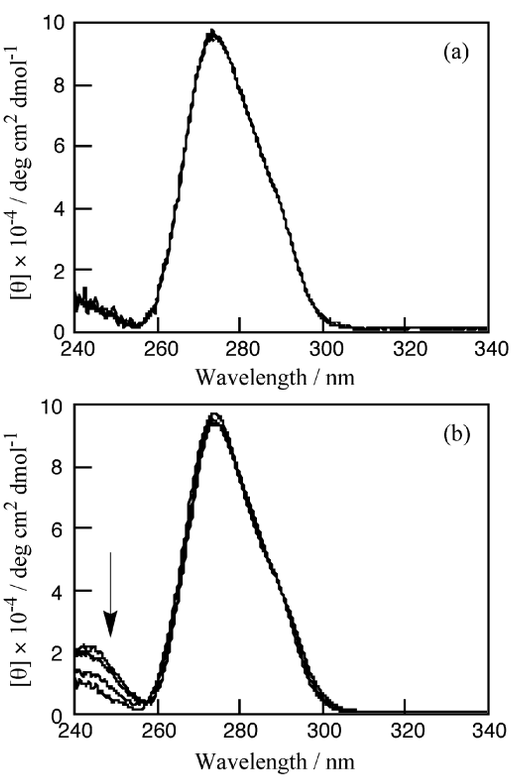


Fig. 3 CD spectral changes in poly(C) ([poly(C)] = 2.5 × 10^{-4} mol dm^{-3}/monomer unit) with an increase in the Tris concentration (0–80 mmol dm^{-3}): (a) +s-SPG and (b) +N-s-SPG.

Fig. 4, which plots $[\theta]_{242}$ *vs.* Tris concentration, $[\theta]_{242}$ for +N-s-SPG is larger than that of +s-SPG at low Tris concentrations. However, the $[\theta]_{242}$ value for +N-s-SPG gradually decreases with increasing Tris concentration and finally merges to that for +s-SPG above 80 mmol dm^{-3}. Since Tris can generate a

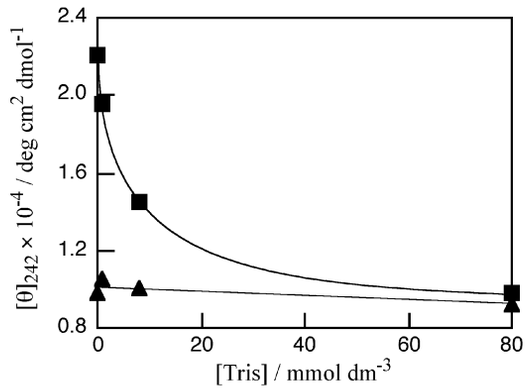


Fig. 4 Tris concentration dependence of $[\theta]_{242}$ for +s-SPG (▲) and +N-s-SPG (■).

cationic charge at pH = 8.0, the cation presumably binds to the phosphate anions in poly(C). Therefore, Tris and N-s-SPG cations compete with each other for binding to the phosphate anions, and the increment in Tris concentration is favourable for Tris to bind to poly(C). This ion competition effect can explain why the increment in Tris concentration reduces the intensity of $[\theta]_{242}$ for +N-s-SPG. These results suggest that a greater $[\theta]_{242}$ value is a characteristic feature of the ion-pair formation in the N-s-SPG–poly(C) complex.

Comparison of the melting behaviours of +s-SPG and +N-s-SPG

Fig. 5 compares the temperature dependence of $[\theta]_{275}$ of

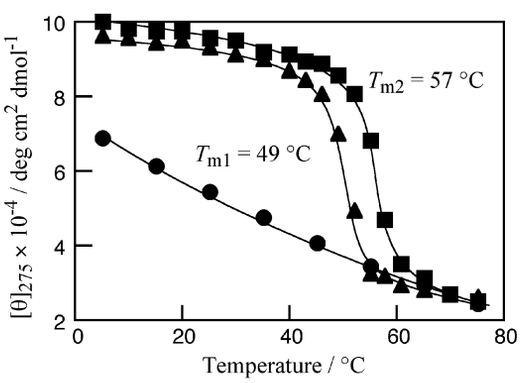


Fig. 5 Comparison of the melting behaviours of +s-SPG and +N-s-SPG. ●: poly(C) itself, ▲: +s-SPG and ■: +N-s-SPG. T_{m1} is the melting temperature of the polynucleotide–s-SPG complex and T_{m2} is that of polynucleotide–N-s-SPG.

poly(C), +s-SPG and +N-s-SPG. The melting temperature of the complexes (T_m) was determined by a conventional method[31,32] and the resultant values are presented in the figure. The complex made from poly(C) and N-s-SPG dissociates at a higher temperature by 8 °C than that of poly(C) and s-SPG. In addition, dispersion of the melting process (sharpness of the curvature), which is a measure of the cooperativity in the complex dissociation, is almost similar for both cases.

Fig. 6 plots the Tris concentration as a function of T_m for +s-SPG and +N-s-SPG. For +s-SPG, T_m seems independent of the Tris concentration. On the other hand, T_m for +N-s-SPG decreases with increasing Tris concentration. This concentration dependence of T_m can be explained by the ion-competition effect mentioned above. These facts indicate that the increment of T_m is ascribed to the ion-pair formation between the polymer chains. It is surprising that only 2.4 ± 0.3 mol% of amino group introduced into SPG can induce such a high enhancement of T_m.

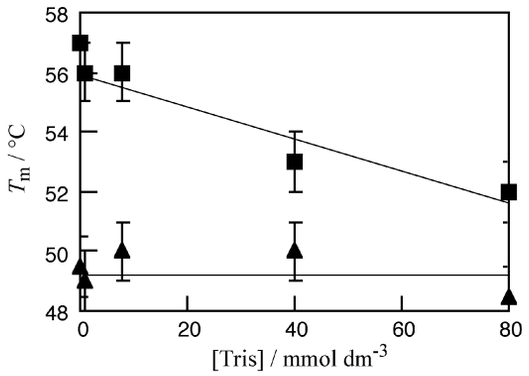


Fig. 6 Tris concentration dependence of T_m for +s-SPG and N-s-SPG. ▲: +s-SPG and ■: +N-s-SPG.

Stoichiometric analysis of the complex formed from poly(C) and N-s-SPG

Our previous studies showed that the specific interaction in the poly(C)–s-SPG complex is due to hydrogen-bonding and hydrophobic interactions and these interactions synergistically work to provide the stoichiometric number.[13] Since the ion-pair formation is stronger than the above specific interactions, N-s-SPG may randomly bind to poly(C). If this occurs, the maximum of the Job plot for +N-s-SPG should be affected by the presence of such a non-specific electrostatic interaction.

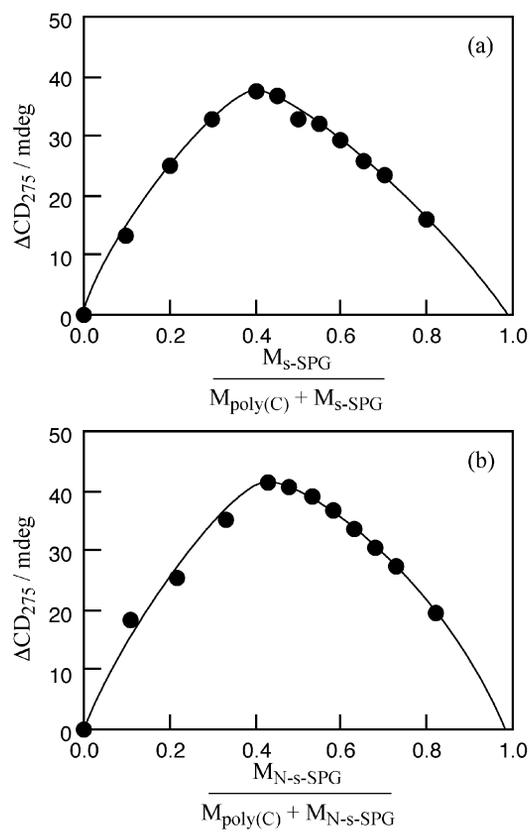


Fig. 7 Job plots: the sum (poly(C)+s-SPG) was kept constant (2.80×10^{-4} mol dm^{-3}). (a): +s-SPG, (b): +N-s-SPG.

Fig. 7 plots ΔCD_{275} against the molar ratio for the repeating unit of +s-SPG (a) and +N-s-SPG (b), respectively, where ΔCD_{275} is defined by eqn. (1),

$$\Delta CD_{275} = CD_{275} - \frac{M_{s\text{-}SPG}}{M_{poly(C)} + M_{s\text{-}SPG}} CD_{275,poly(C)} \qquad (1)$$

where CD_{275} and $CD_{275,poly(C)}$ are the observed CD intensities at 275 nm for a mixture of poly(C) and s-SPG (N-s-SPG) and poly(C) itself, respectively, and $M_{poly(C)}$ and M_{SPG} are the molar concentrations of cytidine and the repeating unit of s-SPG (N-s-SPG), respectively.

As shown in Fig. 7 (b), the +N-s-SPG system has a maximum at around 0.4, which is identical to that of s-SPG. Coincidence in the stoichiometric number indicates that the complex formed from N-s-SPG and poly(C) consists of two N-s-SPG chains and one poly(C) chain, which is the same as for the s-SPG system. In other words, the non-specific electrostatic interaction does not affect the stoichiometry in the complex.

Stabilization model of the complex between N-s-SPG and poly(C)

N-s-SPG can form a macromolecular complex consisting of two N-s-SPG chains and one nucleotide chain. Furthermore, the complex is dissociated in a cooperative manner similar to that of the s-SPG system. Although the ion-pairs are formed between the polymer chains, the stacked conformation of nucleobases is maintained. In contrast, the melting temperature for +N-s-SPG drastically increased. Taking these results into consideration, one may propose that this stability enhancement can be explained by the schematic models as shown in Fig. 8.

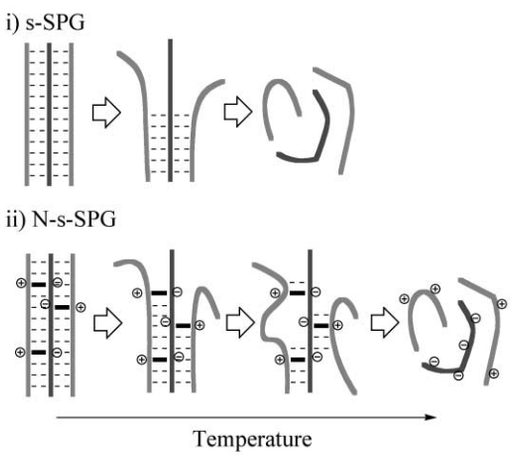


Fig. 8 Schematic illustration of "pinning effect"

In the polynucleotide–s-SPG complex, the number of the hydrogen bonds between the polymer chains decreases with increasing temperature and suddenly the three strands become dissociated above T_m because there are not enough hydrogen bonds to bind the polymer chains together. On the other hand, ion-pairs as well as hydrogen bonds can form between the polymer strands of the polynucleotide–N-s-SPG complex as shown in (ii). In general, the electrostatic interactions are relatively strong and are classified as long-range interactions (longer than the hydrogen-bonding interactions), so that the ion-pair formation between the polymer chains tends to suppress the cooperative dissociation of the multivalent hydrogen bonding. Namely, the ion-pair has an ability to pin the polymer chains together to prevent them from dissociation. It is undoubted that this 'pinning effect' contributes to the enhancement of the melting temperature.

Affinity enhancement for other polynucleotides

According to our previous study,[13] s-SPG can form complexes with poly(A), poly(C), poly(dA) and poly(dT) in neutral and non-salt conditions. Considering that N-s-SPG has a cationic charge and can pin the polysaccharide and polynucleotide chains together through ion-pair formation, it may increase the affinity for other polynucleotides. Since a few polynucleotides are very sensitive to pH changes, we used Tris buffer

at a concentration of 0.8 mmol dm^{-3} (pH = 8.0) to avoid conformational transition of polynucleotide chains:[33,34] this concentration does not have any influence on either the thermal stability or the conformation (*vide supra*).

Fig. 9 compares the temperature dependence of $[\theta]_{max}$ of +s-SPG and +N-s-SPG for poly(A) and poly(dT). Although the data are not presented, neither a different peak nor a shoulder band is observed for +N-s-SPG. As can be seen, T_m of +N-s-SPG is higher than that of +s-SPG in both cases. These results are ascribed to ion-pair formation and are in good agreement with those in the poly(C) system.

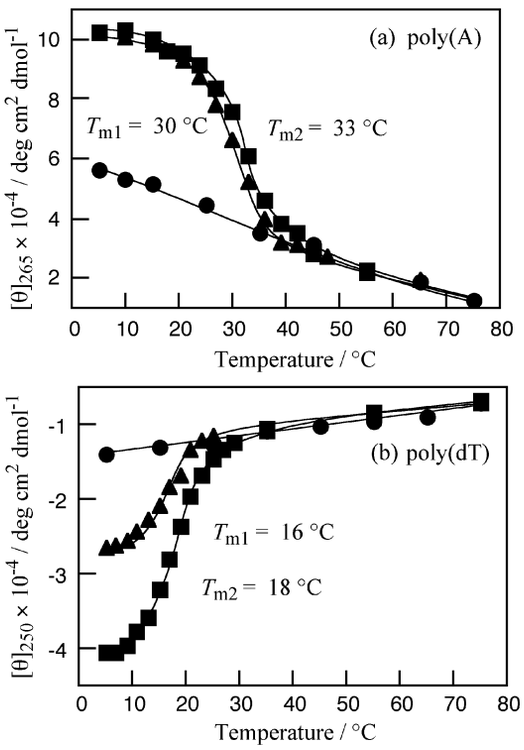


Fig. 9 Comparison of the melting behaviours for poly(A) (a) and poly(T) (b), respectively. ●: poly(A) or poly(dT), ▲: +s-SPG and ■: +N-s-SPG. T_{m1} is the melting temperature of polynucleotide–s-SPG complex and T_{m2} is that of polynucleotide–N-s-SPG.

Fig. 10 presents the temperature effect on the CD spectral change in the poly(dA) system. Comparing (a) with (b), the shape of the spectra seems to be different; the negative 250 nm band of +N-s-SPG is smaller and the positive 262 nm band is larger than those of +s-SPG. Our previous studies revealed that the complex formed from poly(dA) and s-SPG has two different conformations in the complexation state. We defined their state as the *HL* form (low temperature form) and the *H* form (high temperature form), respectively.[35] Judging from the spectral changes, the poly(dA)–N-s-SPG complex takes these two conformations. The *HL* form of +N-s-SPG is identical with that of +s-SPG, whereas the *H* form is different. According to our previous results, in the presence of alkali metal ions such as Na^+ and K^+, the *HL* form easily changes to the *H* form at a much lower temperature and the *H* form is stabilized.[36] We concluded that the poly(dA) chain incorporated into the *H* form complex is similar to the A-type DNA. Generally speaking, A-type DNA is highly stabilized by reducing the electrostatic repulsion. N-s-SPG has a cationic charge and the cations can reduce the electrostatic repulsion to form an ion-pair. Therefore, the *H* form of +N-s-SPG should undergo more effective stabilization than that of +s-SPG.

Fig. 11 shows the temperature dependence of $[\theta]_{252}$ at the isosbestic point wavelength of the two conformations (*HL* and *H*). The melting temperature of +N-s-SPG is increased by 15 °C compared with that of +s-SPG. This enhancement is much higher than that for other polynucleotides. This implies that the stabilization effect shown by the introduced cationic charge is particularly evident in the poly(dA) system.

Table 1 Comparison of the melting temperatures between +s-SPG and +N-s-SPG

	Poly(C)	Poly(A)	Poly(dA)	Poly(dT)
T_{m1}/°C	49	30	63	16
T_{m2}/°C	57	33	78	18
ΔT_m/°C	+8	+3	+15	+2

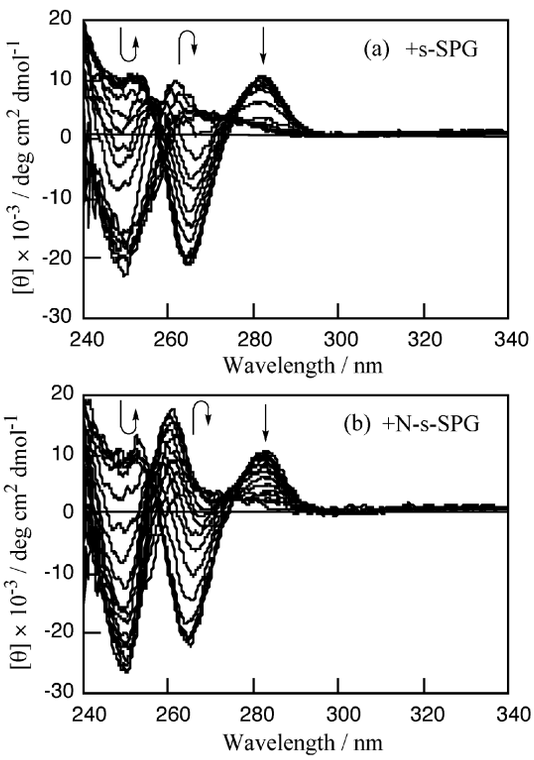


Fig. 10 Comparison of the CD spectra of (a) poly(dA)–s-SPG (+s-SPG) and (b) poly(dA)–N-s-SPG (+N-s-SPG) with an increase in temperature.

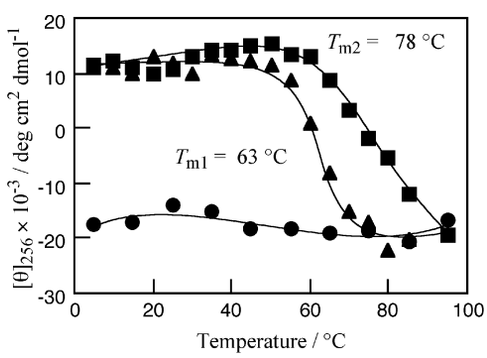


Fig. 11 Comparison of the melting behaviours. ●: poly(dA), ▲: +s-SPG and ■: +N-s-SPG.

The T_m values of both +s-SPG and +N-s-SPG for various polynucleotides are summarized in Table 1. In all cases, the T_m values for +N-s-SPG are larger than those for +s-SPG. This fact confirms that our modification method is very versatile and therefore, of great sucess in improving the thermal stability.

Induced complexation in the poly(U) system

Our previous studies revealed that poly(G), poly(dG) and poly(U) cannot form complexes with s-SPG in neutral and

non-salt conditions because the polynucleotides form their own specific conformations.

For poly(G), there are no appreciable differences in the melting behaviours of +s-SPG and +N-s-SPG (Supplementary Information: Fig. S1 ‡), indicating that N-s-SPG does not interact with poly(G) either. It is well-known that poly(G) forms a guanine tetramer (G-quartet) in aqueous solution and the hydrogen-bonding sites of the nucleobase are completely occupied by strong intermolecular hydrogen-bonding interactions to form the tetramer.[37] We have already demonstrated that the presence of an open hydrogen bonding site in the nucleobase is necessary for complex formation. Consequently, it is impossible for the N-s-SPG chain to complex with a poly(G) chain consisting of G-quartet units even though its cationic charge may act as an electrostatic 'trigger' to form macromolecular complexes with polynucleotides.

Fig. 12 compares the temperature dependence of the CD spectra for poly(U), +s-SPG and +N-s-SPG. The figure shows that the spectral changes in +N-s-SPG are different from the others, suggesting that the amino group introduced into SPG induces a novel interaction between poly(U) and N-s-SPG. Recently, we found that the presence of a small amount of alkali metal ion such as Na^+, K^+ and Rb^+ can induce complexation between s-SPG and poly(U).[38] The spectra in Fig. 12(c) are similar to those induced by the alkali metal ions. Furthermore, we measured the CD spectra for a mixture of s-SPG and poly(U) in the presence of 2-aminoethanol and found that nothing happens even when the concentration of 2-aminoethanol increases up to 10 mmol dm^{-3}, which is 320 times higher than that of the amino group in N-s-SPG (see Supplementary Information: Fig. S2 ‡). This result indicates that 2-aminoethanol itself never induces any spectral changes. Furthermore, we constructed the Job plot for the N-s-SPG–poly(U) system (Fig. 13)[39] and found that the stoichiometric number is about 0.50–0.55. This value coincides with the stoichiometric number for the poly(U)–s-SPG complex induced by K^+. Therefore, we concluded that the cations in the N-s-SPG chain break the original hydrogen bonds in poly(U) and allow poly(U) and N-s-SPG to form new hydrogen bonds.

Enhancement of the affinity for short chain polynucleotides

Since the driving force of complexation between polynucleotides and s-SPG is considered to be the sum of many weak interactions such as hydrogen bonds, hydrophobic interactions, *etc.*, the complexation properties should depend on the chain length of each polymer. We have already examined the influence of the chain length in unmodified s-SPG on the complexation ability for deoxyadenosine (dA) and deoxythymidine (dT) oligomers and found that the critical chain length to form a complex was 30-mer and 60-mer, respectively.[35,36] Since the electrostatic interactions are relatively strong, one may expect that N-s-SPG may interact with much shorter nucleotides than s-SPG. In our preliminary study for *in vitro* translation–transcription assay using an antisense DNA, the antisense DNA is required to add 40-mer of an oligo(dA) tail to form a complex with s-SPG.[16] If modification with cationic charge allows s-SPG to interact with much shorter chain nucleotides, one does not need to add such a long tail to the antisense DNA.

Fig. 14 compares the influence of the polymer length of +s-SPG and +N-s-SPG on dA oligomer (a) and dT oligomer (b), respectively. Here, we define $\Delta[\theta]_{283}$ by eqn. (2) and $\Delta[\theta]_{250}$ is also defined by the same equation at 250 nm. Here, $\Delta[\theta]_{283,complex}$ is the observed $[\theta]_{283}$ of +s-SPG or +N-s-SPG and $\Delta[\theta]_{283,nucleotide}$ is the observed $[\theta]_{283}$ of the deoxynucleotide itself.

$$\Delta[\theta]_{283} = \Delta[\theta]_{283,complex} - \Delta[\theta]_{283,nucleotide} \quad (2)$$

As expected, N-s-SPG can form complexes with much shorter chain oligonucleotides than s-SPG. The critical polymer length for dA and dT oligomers is reduced to 25-mer and 30-mer, respectively. This feature indicates that cationic charge plays an important role in increasing complex stability even in much shorter nucleotides.

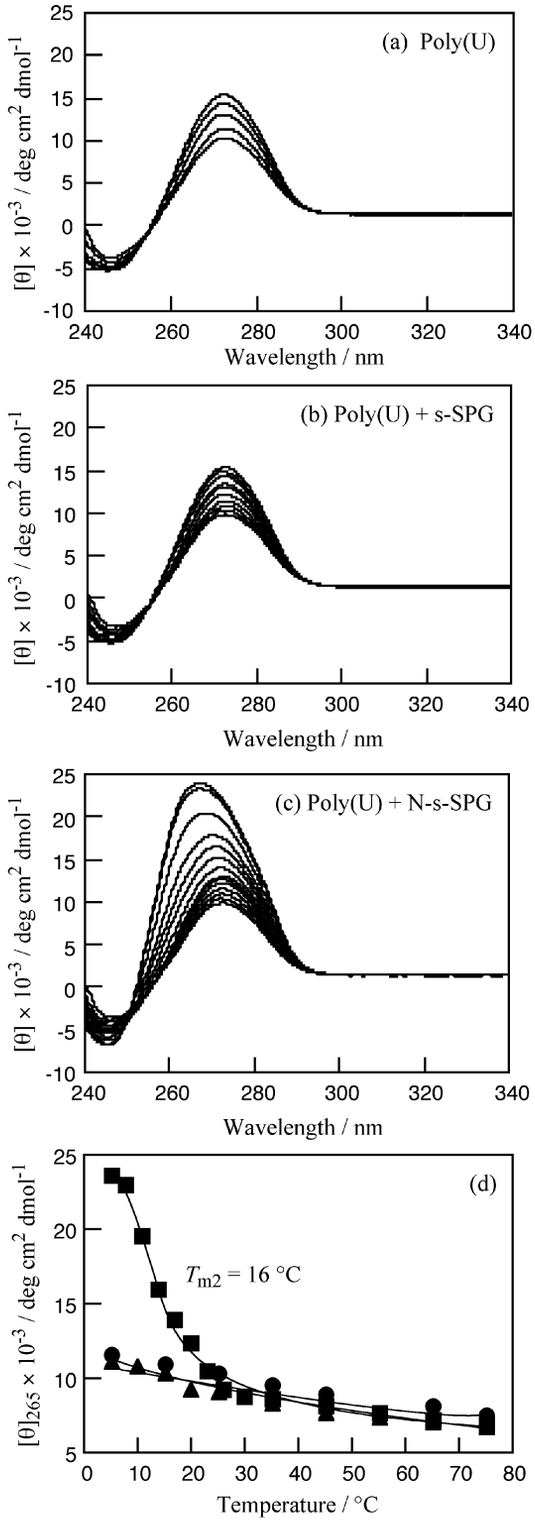


Fig. 12 CD spectral changes with an increase in the temperature. (a) poly(U), (b) +s-SPG and (c) +N-s-SPG. (d) Plots of $[\theta]_{265}$ against temperature for poly(U) (●), +s-SPG (▲) and +N-s-SPG (■).

Conclusion

We have successfully introduced an amino group into the side chain of SPG by utilizing periodate oxidation and reductive amination. This amino-appended SPG can form thermally stable complexes with various polynucleotide chains without any significant conformational changes. In all cases, the T_m

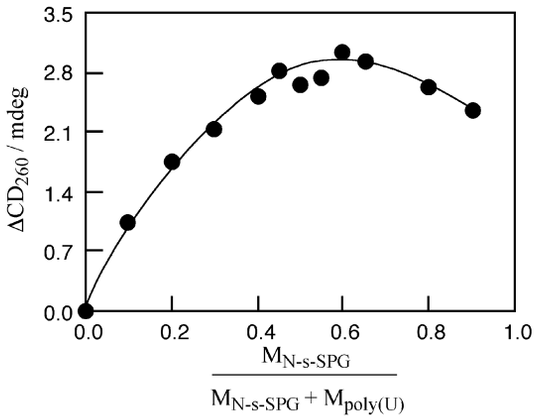


Fig. 13 Job plot: the sum of (poly(U)+N-s-SPG) was kept constant (5.38×10^{-4} mol dm^{-3}).

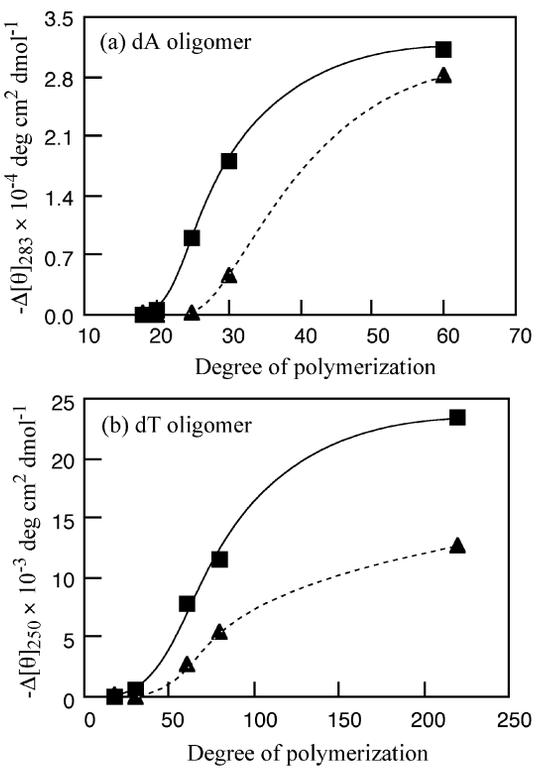


Fig. 14 Polymer length dependence for (a) dA and (b) dT oligomer. ▲: +s-SPG and ■: +N-s-SPG.

values have been enhanced through ion-pair formation between ammonium cations in N-s-SPG and phosphate anions in the polynucleotides. Moreover, it was newly found that poly(U) can form a complex through electrostatic interactions although unmodified s-SPG cannot interact with it under the same conditions. Complexation capability for the short chain nucleotides was also enhanced. These results indicate that our modification method is quite useful for controlling complex stability and therefore we believe that this strategy will make it possible to apply SPG to a novel gene vector both *in vitro* and *in vivo*.

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