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Bioorganic Chemistry 31 (2003) 163–171
BIOORGANIC CHEMISTRY
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Polysaccharide–polynucleotide complexes (15): thermal stability of schizophyllan (SPG)/poly(C) triple strands is controllable by α-amino acid modification

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Received 9 September 2002

Abstract

Schizophyllan (SPG), a β-1,3-glucan polysaccharide which is known to form macromolecular complexes with certain polynucleotides, was modified by a reductive amination method with α-amino acids (Arg, Lys, and Ser). The thermal stability of the complexes as estimated by T_m was enhanced in SPG–Arg and SPG–Lys conjugates which have p*I* values higher than the pH of the medium (8.0). The T_m shift increased with the increase in the percentage of α-amino acid introduced and the highest T_m values attained were 64 °C for SPG–Arg conjugate and 62 °C for SPG–Lys conjugate, which are higher by 13 and 11 °C, respectively, than those of the unmodified SPG + poly(C) complex. In the SPG–Ser conjugate with a p*I* lower than the medium pH (8.0), the T_m values decreased with an increase in the percentage of Ser. Formation of the macromolecular complex was no longer detected above 13.2% Ser. The findings indicate that the T_m values are easily controllable by the type and percentage of the introduced α-amino acids. We believe, therefore, that the present conjugates, consisting of naturally originated SPG and α-amino acids, provide an important lead for developing nontoxic artificial vectors and to control the affinity with polynucleotides in response to medium pH and temperature.

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doi:10.1016/S0045-2068(03)00020-8

1. Introduction

Schizophyllan (SPG) is a natural polysaccharide produced by the fungus *Schizophyllum commune* and its repeating unit consists of three β-(1 → 3) glucoses and one β-(1 → 6) glucose side-chain linked at every third main-chain glucose (Fig. 1) [1,2]. SPG forms a triple helix in water and dissociates into a single chain (s-SPG) in dimethylsulfoxide (DMSO) [3–5]. The s-SPG chain can regain the original triple helix structure by exchanging DMSO for water [5–7]. Recently, we found that when this solvent-exchange process is carried out in the presence of polynucleotides, the resultant triple helix consists of two s-SPG chains and one nucleotide chain, indicating that the SPG–polynucleotide interaction is energetically more favored than the SPG–SPG interaction under some specific conditions [8,9]. Subsequent study revealed that this complexation is characteristic of water-soluble β-1,3-glucans and not observed for other polysaccharides [10]. As far as we know, this is the first clear evidence that a neutral polysaccharide can form a macromolecular complex with polynucleotides. We believe that s-SPG/polynucleotide complexes may provide a new methodology for gene technology, such as gene carriers, affinity separation columns, biosensors, etc. [11]. In view of these applications, it would be advantageous to be able to control the thermal stability of the complexes by chemical modification of SPG. In this paper, we introduced an amino group into the side-chain of SPG by combining periodate oxidation with subsequent reductive amination (Scheme 1).

To enhance the affinity between SPG and polynucleotides, it seemed most effective and expeditious to introduce cationic groups into either the main-chain or the side-chain glucose units. It is known, however, that selective modification of OH groups in sugar derivatives is fairly difficult. Previously, we found one convenient method, by which one can introduce functional groups only into the side-chain glucose units. This method involves oxidation cleavage by periodate anion (IO_4^-) of the 1,2-diol group [12], which in SPG exists only in the side-chain glucose units. Thus, one can "selectively" oxidize the 1,2-diol groups into aldehyde groups, which are useful to introduce various functional groups (Scheme 1). We thus introduced 2-aminoethanol into these aldehyde groups by Schiff base formation followed by $NaBH_4$ reduction [13]. As expected, introduction of only 2.4% of the 2-aminoethanol group (per single side-chain glucose unit) significantly improved the thermal stability of the complex. Compared with the melting temperature of the complex (T_m), the 2-aminoethanol-modified SPG + poly(C) complex had a T_m value higher by 8 °C than the unmodified SPG + poly(C) complex [13].

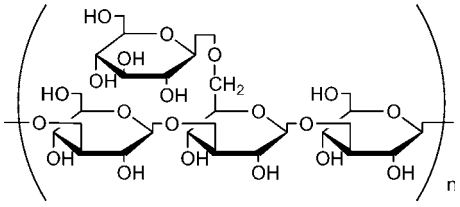
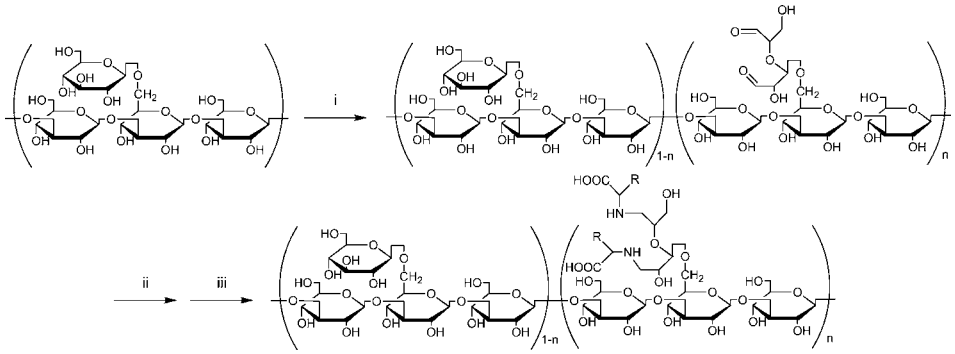


Fig. 1. Repeating unit of schizophyllan [1–4].



Scheme 1. Schematic illustration for the introduction of α-amino acid groups into SPG: n indicates α-amino acid modification percentage. Reagents and conditions: (i) $NaIO_4$, H_2O, 4 °C, 2 days, (ii) α-amino acid methyl ester, K_2CO_3, DMSO, r.t., 2 days, (iii) $NaBH_4$, DMSO, r.t., 1 day.

When considering the application of modified SPGs as candidates for artificial vectors, one should introduce, instead of the 2-aminoethanol group, some nontoxic functional group. α-Amino acids satisfy these prerequisites, because they are natural compounds, and have an amino group amenable to the modification method shown in Scheme 1. Furthermore, one can obtain insight into the charge effect of introduced cationic arginine (pI = 10.76), lysine (pI = 9.74), or anionic serine (pI = 5.68) on the thermal stability of the complexes [14]. In this paper, we report not only this charge effect, but also the effect of the percentage of α -amino acid introduced on the thermal stability of the complexes with poly(C).

2. Experimental procedure

2.1. Materials

SPG was kindly supplied by Taito, Japan. The molecular weight and the number of repeating units were evaluated to be 1.5×10^5 and 231, respectively [8]. Poly(C) was purchased from Pharmacia, RNase-free distilled water was obtained from Nippon Gene, and spectroscopic grade DMSO was obtained from Kishida and used for all measurements. L-Arginine methyl ester dihydrochloride, L-lysine methyl ester dihydrochloride, and L-serine methyl ester hydrochloride were purchased from Watanabe Chemical Industries.

2.2. Methods

The selective oxidation of the 1,2-diol group was carried out as described [13]. The modification ratios of the aldehyde groups introduced were controlled by the amount of sodium periodate ($NaIO_4$). After freezing-and-pumping followed by dialysis (fractionated molecular weight = 12,000; Viskase Companies), SPG with the

aldehyde side-chain was obtained. The general procedure for introduction of α-amino acid to SPG was as follows. K_2CO_3 (1.0 g) and oxidized SPG (0.1 g; 0.030 mmol/calculated aldehyde units) were mixed in dry DMSO. To the DMSO solution were added α-amino acid methyl ester hydrochloride (3.0 mmol) and the resultant DMSO mixture was stirred under nitrogen at room temperature. After 2 days, an excess of $NaBH_4$ (0.3 g) was added and the mixture was stirred another 1 day. After quenching unreacted $NaBH_4$ with acetic acid, DMSO and inorganic materials were removed by dialysis under basic conditions. The ester group of the α-amino acid introduced was hydrolyzed to the corresponding acid during this process, because the ester IR peak at 1747 cm^{-1} (nujol) completely disappeared. Freezing-and-pumping of the resulting solution gave the α-amino acid-modified SPG.

The percentage of α-amino acid introduced was determined by elemental analysis. The s-SPG-amino acid/poly(C) complex was prepared by mixing s-SPG-amino acid in DMSO and poly(C) in water, as described [9]. The thermal stability of the complexes was estimated by the CD spectral method, using a J-720WI spectropolarimeter with a 1.0 cm cell.

3. Results and discussion

As summarized in Table 1, α-amino acid-modified SPG derivatives were obtained containing 3.6–36.0% Arg [abbreviated as SPG–Arg(3.6)–SPG–Arg(36.0)], 6.6–31.7% Lys [abbreviated as SPG–Lys(6.6)–SPG–Lys(31.7)], and 6.4–39.1% Ser [abbreviated as SPG–Ser(6.4)–SPG–Ser(39.1)]. All SPG–Ser samples were soluble in DMSO. In the SPG–Arg series, SPG–Arg(3.6)–SPG–Arg(18.1) were soluble, although the solution of SPG–Arg(18.1) was somewhat cloudy. In SPG–Lys series, on the other hand, SPG–Lys(31.7) was insoluble in DMSO. Thus, subsequent spectroscopic measurements were carried out only for SPG–Lys(6.6), SPG–Lys(12.2), and SPG–Lys(21.2). Presumably, the p*I* of Lys(9.78) is the closest to the medium pH (8.0), so that the SPG–Lys conjugate polymers partially retain the zwitterionic nature although when the degree of the cationic charge is greater than anionic charge, inter-polymeric aggregation is favored, which results in a cloudy solution or precipitate.

Table 1
Molar ratio (%) in the reaction mixtures and introduced percentages (%) of α-amino acid groups (per side-chain glucose unit)

Molar ratio	Modified SPG		
$NaIO_4$/side-chain glucose unit	SPG–Arg	SPG–Lys	SPG–Ser
10%	3.6 ± 0.1%	6.6 ± 0.3%	6.4 ± 0.3%
40%	9.3 ± 0.2%	12.2 ± 0.2%	13.2 ± 0.4%
70%	—	21.2 ± 0.6%	16.0 ± 1.0%
100%	18.1 ± 0.3%	—	28.0 ± 1.5%
500%	36.0 ± 0.4%	31.7 ± 0.1%	39.1 ± 1.2%

Fig. 2 compares the CD spectra for poly(C), a mixture of s-SPG and poly(C), and a mixture of s-SPG–Arg(3.6) and poly(C). Since neither s-SPG nor s-SPG–Arg(3.6) has any absorbance at the wavelengths presented in Fig. 2, all CD bands can be assigned to conformational changes in the poly(C) chain. It can be seen from Fig. 2 that the spectrum for the s-SPG + poly(C) system is different from that of poly(C) alone in that the 275 nm band increases by 50% and a new band arises near 242 nm [8,9,13]. These spectral changes can be ascribed to complex formation between s-SPG and poly(C), indicating a change into a more ordered helical conformation [8,9,13]. The spectrum of the s-SPG–Arg(3.6) + poly(C) system also has two characteristic bands at 242 and 275 nm. However, the intensity of the CD bands is somewhat weaker. The CD spectra obtained for other SPG–Arg and SPG–Lys samples, when they form the macromolecular complexes with poly(C), were basically similar to that of the SPG–Arg(3.6) + poly(C) system.

In Figs. 3–5, the CD intensity of poly(C) at 275 nm is plotted against temperature. It can be seen from the temperature dependence in Figs. 3 and 4 that the introduction of Arg or Lys enhances T_m values. As shown in Fig. 6, the T_m value tends to increase with the increase in the percentage of α-amino acid introduced. In the SPG–Arg series, the highest T_m value (64 °C) was observed for SPG–Arg(18.1) and in the SPG–Lys series the highest T_m (62 °C) was observed for SPG–Lys(12.2). These values are higher by 13 and 11 °C, respectively, than those of unmodified s-SPG + poly(C) complex (51 °C) [15]. Since the CD spectra of these complexes are basically similar to those of the s-SPG + poly(C) complex (vide supra), one can consider that electrostatic interactions, in addition to hydrogen-bonding and hydrophobic interactions

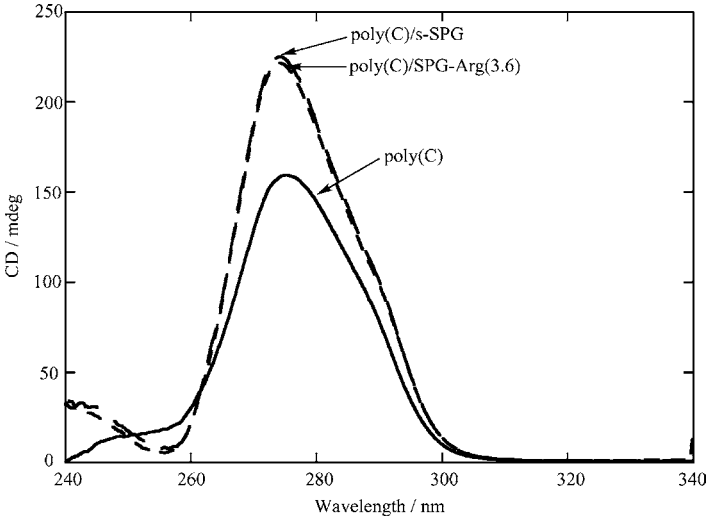


Fig. 2. Comparison of the CD spectra for poly(C), s-SPG + poly(C), and s-SPG–Arg(3.6) + poly(C) measured at 5 °C: [poly(C) (monomer unit)] = 2.5×10^{-4} mol dm^{-3} [s-SPG (monomer unit for main-chain)] = 1.9×10^{-3} mol dm^{-3}, [s-SPG–Arg(3.6) (monomer unit for main-chain)] = 1.9×10^{-3} mol dm^{-3}, [Tris] = 8.3×10^{-4} mol dm^{-3} (pH = 8.0), water:DMSO = 92:8 v/v.

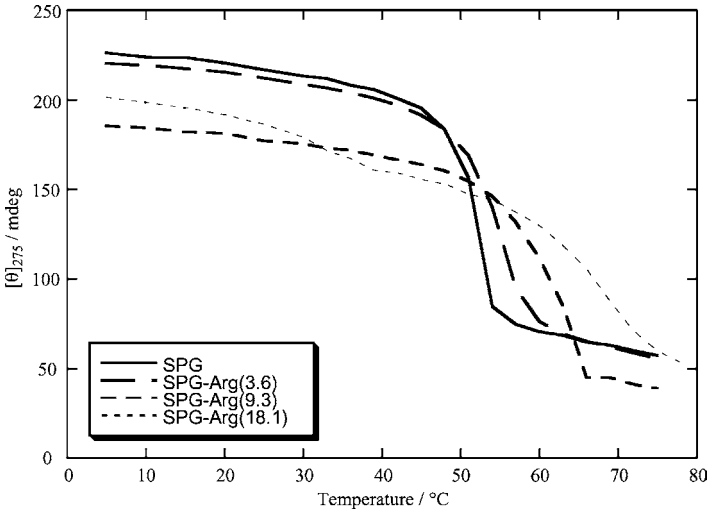


Fig. 3. CD intensity at 275 nm plotted against medium temperature for SPG–Arg + poly(C) systems.

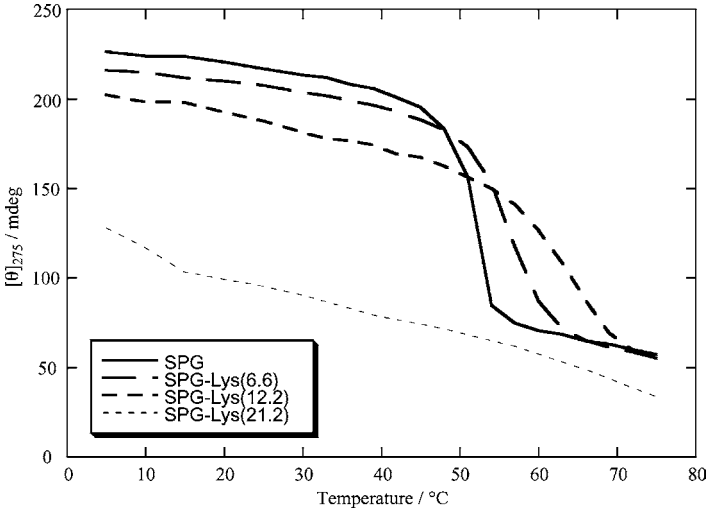


Fig. 4. CD intensity at 275 nm plotted against medium temperature for SPG–Lys + poly(C) systems.

operating in the s-SPG + poly(C) complex, participate effectively in the thermal stabilization of the SPG–Arg and SPG–Lys complexes. As Arg has a guanidinium group capable of acting as a complementary hydrogen-bond donor for a phosphate group, it was expected that the T_m values for the SPG–Arg series might be higher than those for the SPG–Lys series. However, the T_m difference observed in the present system is too small to make this conclusion. Examination of Figs. 3 and 4 also

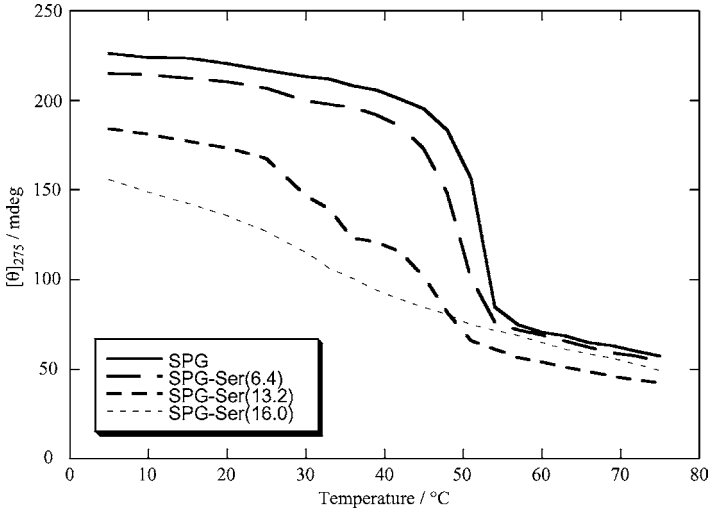


Fig. 5. CD intensity at 275 nm plotted against medium temperature for SPG–Ser + poly(C) systems.

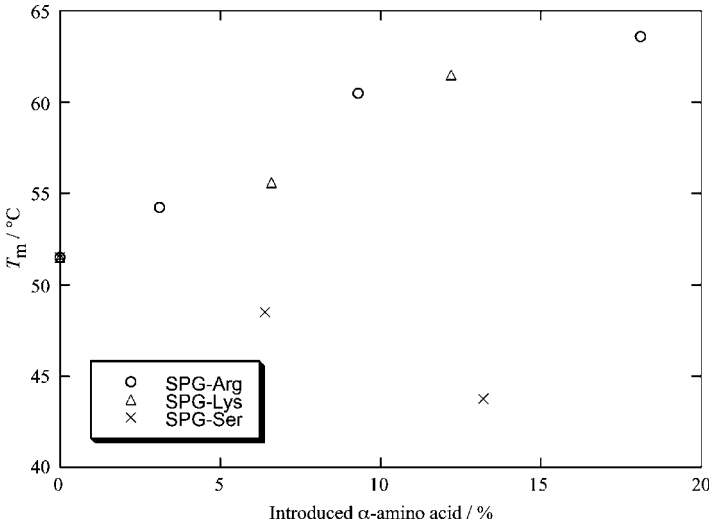


Fig. 6. Plots of α-amino acid percentage introduced (%) vs. T_m.

reveals that with the increase in the percentage of α-amino acid introduced, the $[\theta]_{275}$ values in the low temperature region decrease and the degree of cooperativity in the dissociation process (i.e., the gradient of the anti-sigmoidal curvature) decreases. The results imply that the participation of the electrostatic interaction may intensify the thermal stability of the complexes, but may simultaneously induce some disorder in the hydrogen-bonding complementarity between SPG and poly(C). Interestingly, it can be seen from Fig. 4 that in contrast to the high T_m value (62 °C) of the

SPG–Lys(12.2) + poly(C) system, SPG–Lys(21.2) does not interact with poly(C) at all. As mentioned above, SPG–Lys(21.2) is not soluble in DMSO presumably because of the inter-polymeric aggregation caused by the zwitterionic nature. Thus, the nonbinding properties of SPG–Lys(21.2) are also explained by inter-polymeric interaction among SPG–Lys(21.2) polymers occurring in preference to interaction between SPG–Lys(21.2) and poly(C).

On the other hand, Fig. 5 shows that the T_m values for the SPG–Ser series decrease with the increase in percentage of Ser introduced. Since the Ser residue has a pI = 5.68 and carries an anionic charge at pH 8, the destabilization is ascribed to the electrostatic repulsion between the like charges in SPG–Ser and poly(C). In fact, complex formation is no longer observed for SPG–Ser(16.0), SPG–Ser(28.0), and SPG–Ser(39.1) even though they are soluble in DMSO and the measurement medium (water:DMSO = 92:8 v/v).

4. Concluding remarks

The present paper has demonstrated that the thermal stability of the s-SPG + poly(C) complex can be controlled by the type and the percentage of α-amino acids introduced. The fact that the T_m values can be enhanced above body temperature implies that the present system may be applicable as an artificial vector for an in vivo system. In this context, it is particularly worth emphasizing that the conjugates consist of both nontoxic SPG and α-amino acids. We are now extending this system to the binding of various RNA and DNA species and to their actual transport across biomembranes.

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[15] The T_m for the unmodified s-SPG + poly(C) is slightly affected by the media used. For example, the T_m is 49 °C in absence of buffer [13] and 50 °C in 8.3×10^{-4} mol dm^{-3} Tris buffer (pH = 8.0) (water:DMSO = 95:5 v/v) [10].