Polysaccharide-polynucleotide Interaction (XI); Novel Separation System of RNAs by Using Schizophyllan-appended Column

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Schizophyllan belongs to the β-1,3-glucan family and can form a complex with particular polynucleotides.[1] We applied this complexation to specifically separate polynucleotides by constructing a schizophyllan-appended column. The column trapped poly(C) and poly(A), while it did not show any affinity to poly(G), which is consistent with the experimental results obtained in the homogenous solution. The present results indicate that the s-SPG column can be a new separation device for biomolecules.

Nature of polysaccharides can be determined by two factors, the monomer chemical structure related to the spatial arrangement of the hydroxyl group and the linkage between the glucans related to the chain conformation. By using these two factors, polysaccharides can bind to other biomaterials. This binding ability of polysaccharides has been utilized to separate lectins,[2] proteins,[3] and some chiral molecules.[4] Among others, a pioneering column separation work was reported by Okamoto et al. using cellulose and amylose phenylcarbamate derivatives.[4]

Schizophyllan (SPG) is a water-soluble β-1,3-glucan produced by *Schizophyllum commune* of *Basidiomycota*. The main chain consists of β-(1 $\rightarrow$ 3)-D-glucan and one β-(1 $\rightarrow$ 6)-D-glucosyl side chain links to the main chain at every three glucose residues (see Figure 1).[5] Norisuye et al. have shown that SPG adopts a triple helix conformation in water and a single stranded chain in dimethylsulfoxide (DMSO).[6,7] When water is added to the DMSO solution, the single stranded SPG (s-SPG) collapses owing to the formation of the hydrogen bonds (renaturation).[8] Recently, we found that s-SPG forms a complex with single stranded polynucleotides.[1,9,10] Furthermore, the complexation showed an interesting molecular specificity. The complexation occurs with poly(C), poly(A), poly(U), poly(dA), and poly(T), but not with poly(G) and poly(dG). This recognition capability implies that we can compose a new column separation device for polynucleotides. Our idea is following. We prepare an s-SPG supported gel and load the gel in a glass column. When the polynucleotide solution is flowed into the column, the s-SPG chain can trap the specific polynucleotides. After this process, the trapped compound can be eluted by changing the solvent condition.

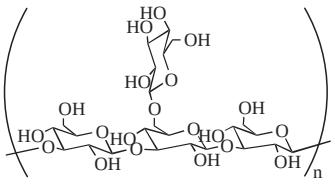

Figure 1. The repeating unit of schizophyllan (SPG).

Taito Co. kindly supplied a SPG sample and we denoted it as s-SPG1 (see Table 1). The weight-average molecular weight (M_w) in DMSO was determined to be 1.5×10^5 by viscometry.[7] We hydrolyzed the main chain of s-SPG1 according to the acid hydrolysis method[11] and obtained three low molecular weight samples. The s-SPG1 sample was heated at 80–90 °C for 3 h in a DMSO/90% formic acid mixed solvent (1/9), and after the reaction, formic acid was vacuum-evaporated. The reaction mixture was dissolved in a large amount of distilled water and then acetone was added dropwise to the solution until it became turbid. The precipitants were collected by centrifugation and the supernatant was served for further precipitation. By repeating this process, three samples (s-SPG2, 3, and 4) were obtained. The molecular weights were evaluated by gel permeation chromatography (GPC) for each sample. The GPC measurement was carried out at 40 °C on a HLC-8020 system (Hitachi) equipped with two α-4000 columns using 2.0×10^{-2} mol dm^{-3} LiBr *N*,*N*-dimethylformamide as the eluting solvent. Table 1 presents the number average molecular weight (M_n), M_w, the molecular weight distribution (M_w/M_n), and the sample codes.

Table 1. Molecular characteristics of the s-SPG samples

Sample	$M_w/10^{3a}$	$M_n/10^{3a}$	M_w/M_n[a]
s-SPG1	216.0	81.0	2.7
s-SPG2	25.3	15.0	1.7
s-SPG3	14.6	9.7	1.5
s-SPG4	3.9	3.0	1.3

[a]The instrument was calibrated by Tosoh's standard polystyrenes.

Figure 2 shows the comparison of the temperature dependence of $[\theta]_{max}$, where $[\theta]_{max}$ is the molecular ellipticity at the peak top of the positive band.[12] As we already reported, s-SPG and poly(C) form a complex at low temperature region and provide a larger $[\theta]_{max}$ value than poly(C) itself.[1,10] Upon heating, the complex is

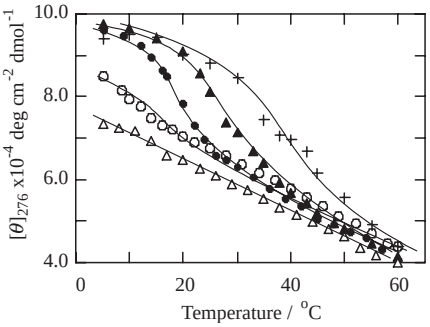

Figure 2. Temperature dependence of $[\theta]_{max}$ of poly(C) in the absence (△) or the presence of SPG; s-SPG1 (+), s-SPG2 (▲), s-SPG3 (●), s-SPG4 (◯): [poly(C)] = 2.5×10^{-4} mol dm^{-3}/nucleotide, [SPG] = 1.1×10^{-3} mol dm^{-3}/repeating unit, pH 7.5 (5.0×10^{-2} mol dm^{-3} phosphate buffer).

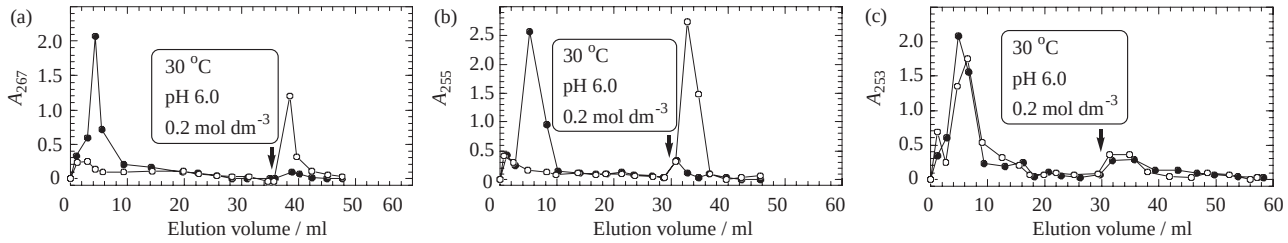

Figure 4. Comparison of the elution curves between the reference (●) and s-SPG (○) columns. (a), poly(C); (b), poly(A); (c), poly(G). Binding the RNA molecules was carried out in the retaining solvent; 5 °C, pH 7.5, and 5.0×10^{-2} mol dm^{-3} phosphate buffer, and the column was left at 5 °C for 12 h, then the column was rinsed with the same solvent. After flowing 30 ml of the rinsing solvent, the solvent was changed to the eluting one; 30 °C, pH 6.0, and 0.2 mol dm^{-3} phosphate buffer.

dissociated and the $[\theta]_{max}$ eventually merges in that of poly(C). Figure 2 confirms our results; furthermore it clearly shows the molecular weight dependence of the complex stability, i.e., with decreasing M_w the dissociation occurs at lower temperature.[10] The melting temperature (T_m) of each complex was determined to be 42, 29, 21 and <5 °C for s-SPG1, s-SPG2, s-SPG3, and s-SPG4, respectively.

In order to construct an s-SPG-appended column, we decided to use s-SPG3. The reasons are as follows. Both s-SPG1 and s-SPG2 need a relatively higher temperature to release the poly(C). Furthermore, the higher molecular glucan makes it more difficult to chemically modify the reducing terminal. On the other hand, s-SPG4 seemed to show only the weak interaction.

The reducing terminal of s-SPG3 was covalently bound to the amino group on a gel support surface by the reductive amination (see Figure 3).[13] We used AF-Amino TOYOPEARL 650M (Tosoh) as the support and approximately 1.0×10^{-4} mol/1 ml-gel of amino groups are attached on the gel support. The residual amino groups were reacted with acetic anhydride. By elemental analysis, the amount of immobilized s-SPG3 on the gel support was evaluated to be 16 mg/1g-gel. The gel support was packed in a glass column with 1.0 cm in diameter and 11 cm in length. As a reference, we prepared an acetylated amino gel support.

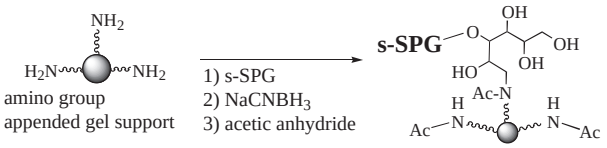

Figure 3. Synthesis of s-SPG-appended gel support.

Dissociation of the s-SPG/poly(C) complex can be carried out by increasing temperature above T_m and changing pH below 6.0.[10] It is known that the pyrimidine base in poly(C) is protonated in the acidic pH region and forms a double stranded helix.[14] This conformational change leads the dissociation of the complex. Therefore, we used an aqueous solution (pH 6.0) as an elute solvent at 30 °C.

Figure 4(a) demonstrates the trapping and eluting behaviors for poly(C) in the s-SPG column, comparing with the reference column. A 100 μl of poly(C) solution (1.3×10^{-3} mol dm^{-3}) was poured into the columns. After left for 12 h at 5 °C, a neutral buffer (pH 7.5, Na_2HPO_4/NaH_2PO_4 5.0×10^{-2} mol dm^{-3}) was flowed into the columns at 5 °C. We know that the poly(C)/s-SPG complex is stable in this solvent. As expected, the elution of poly(C) was not observed in the s-SPG column, on the other hand, most poly(C) was washed away in the reference column. When we flowed an acidic buffer (pH 6.0, Na_2HPO_4/NaH_2PO_4 0.2 mol dm^{-3}) at 30 °C, the bound poly(C) was eluted at more than 90% in the recovery rate. As presented in Figure 4(b), poly(A) shows similar behavior with poly(C). Figure 4(c) shows that most of poly(G) are eluted without binding to both columns in the retaining conditions, although the eluting conditions allow a small amount of poly(G) to be released (see small peaks around 30–40 ml). Both columns show the same behavior, indicating that this small peak is due to poly(G) which has bound in the column with some non-specific interaction, and is released by changing the solvent. The present results are in good agreement with the experimental results obtained in the homogeneous solution. Although the data are not shown here, when we flowed a 1 : 1 mixture of poly(C) and poly(G) through the column, poly(C) retained, whiles poly(G) did not. The detailed result will be discussed more precisely in the corresponding full paper as well as the results for messenger RNA (mRNA).

We prepared an s-SPG-appended gel support by binding the reducing terminal of s-SPG to the amino group on the gel surface. The column made from the gel showed the same specificity to polynucleotides as in the homogeneous solution. The trapped components can be eluted by flowing an acidic solution. It should be mentioned that the present system uses only a neutral saccharide. As far as we know, this is the first to separate polynucleotides without using either cations or complementary bases. Since mRNA from eukaryotic cells has a poly(A) tail with 150–300 bases, the s-SPG column can be used to separate mRNA from total RNA.

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References

1 K. Sakurai and S. Shinkai, *J. Am. Chem. Soc.*, **122**, 4520 (2000).
2 S. Toyoshima, T. Osawa, and A. Tonomura, *Biochim. Biophys. Acta*, **221**, 514 (1970); R. Blosh and M. M. Burger, *Biochem. Biophys. Res. Commun.*, **58**, 13 (1974).
3 C. V. Maina, *Gene*, **74**, 365 (1988).
4 Y. Okamoto, M. Kawashima, and K. Hatada, *J. Am. Chem. Soc.*, **106**, 1004 (1984); B. Chankvetadze, C. Yamamoto, and Y. Okamoto, *J. Chromatogr., A*, **922**, 127 (2001).
5 S. Kikumoto, T. Miyajima, S. Yoshizumi, S. Fujimoto, and K. Kimura, *Nippon Nogei Kagaku Kaishi*, **44**, 337 (1970); S. Kikumoto, T. Miyajima, K. Kimura, S. Okubo, and N. Komatu, *Nippon Nogei Kagaku Kaishi*, **45**, 162 (1970).
6 T. Yanaki, T. Norisuye, and H. Fujita, *Macromolecules*, **13**, 1642 (1980).
7 T. Norisuye, T. Yanaki, and H. Fujita, *J. Polym. Sci., Polym. Phys. Ed.*, **18**, 547 (1980).
8 T. Sato, K. Sakurai, T. Norisuye, and H. Fujita, *Polym. J.*, **16**, 559 (1983).
9 K. Sakurai, M. Mizu, and S. Shinkai, *Biomacromolecules*, **2**, 641 (2001); K. Koumoto, T. Kimura, K. Sakurai, and S. Shinkai, *Bioorg. Chem.*, **29**, 178 (2001).
10 T. Kimura, K. Koumoto, K. Sakurai, and S. Shinkai, *Chem. Lett.*, **2000**, 1242.
11 T. Sasaki, N. Abiko, Y. Sugino, and K. Nitta, *Cancer Res.*, **38**, 379 (1978); Y. Adachi, N. Ohno, and T. Yadomae, *Carbohydr. Res.*, **177**, 91 (1988).
12 K. Nakanishi, N. Berova, and R. W. Woody, "Circular Dichroism Principles and Applications," VCH Publishers, New York (1994), pp 361–365.
13 G. R. Gray, *Arch. Biochem. Biophys.*, **163**, 426 (1976); K. Koumoto, T. Kimura, M. Mizu, K. Sakurai, and S. Shinkai, *Chem. Commun.*, **2001**, 1962.
14 W. Sanger, "Principles of Nucleic Acid Structure," Springer-Verlag, New York (1984), Chap. 6.