Molecular Recognition of Adenine, Cytosine, and Uracil in a Single-Stranded RNA by a Natural Polysaccharide: Schizophyllan

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Hydrogen-bonding interactions play a central role in molecular recognition of neutral and ionic species in aprotic solvents;[1] however, the ones in water are usually ineffective in the specific recognition. This situation makes difficult to recognize saccharides, because the sugars are practically soluble only in water. Some proteins, such as concanavalin A, utilize hydrogen-bonding interactions for saccharide-binding after excluding most (but not all) water molecules which may hamper the hydrogen-bond-based protein-saccharide interactions.[2−5] These binding modes are mimicked in aprotic solvents to some extent.[1,5−8] It is generally accepted that the "ionic" hydrogen-bonding interaction may be a better choice than the "neutral" hydrogen-bonding interaction to bind saccharides. It thus occurred to us to that RNA- and DNA-containing anionic phosphate groups in the main chain might interact with oligosaccharides and polysaccharides even in aqueous media.

Reagents that can recognize a specific sequence of RNA or DNA are of significant importance in the genetic engineering. This is usually achieved either by the formation of complimentary hydrogen bonds as in peptide-sustained nucleic acids[10] and amide-liked *N*-pyrrole oligomers[11] or by the electrostatic interaction as in polyethyleneimine, polypyridine, and aminoglycoside.[12−15] According to the motif mentioned above, we examined a specific interaction between a single-stranded RNA and a β-(1→3)-glucan which is called schizophyllan. We have found that the interaction is so peculiar to the combination with the base as to be assumed applicable to specific molecular recognition. In this contribution we report a preliminary result for the base recognition capability by use of this new class of specific interactions.

Schizophyllan is a β-(1→3)-glucan with the chemical structure shown in Figure 1 and is known to act as an effective antitumor medicine.[16,17] Norisuye et al.[18−20] extensively studied the dilute solution properties of schizophyllan and already found that it dissolves in water as a triple helix (see (b) in Figure 1) and in DMSO as a single chain (s-SPG). It is known that when water is added to the DMSO solution, s-SPG collapses owing to the hydrophobic interaction and forms both intra- and intermolecular hydrogen bondings.[20]

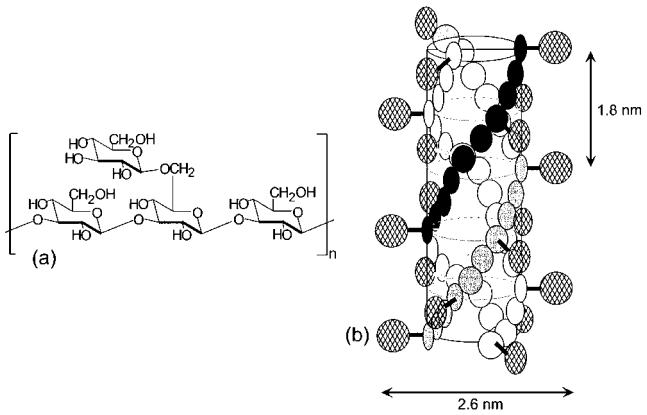

Figure 1. Repeating unit of schizophyllan (a) and its representative model of the triple-helix (b). In the panel (b), the plain circles represent the main chain glucose residues and the meshed ones, the side chains.

The triple helix of schizophyllan was kindly supplied from Taito Co. in Japan. The molecular weight and the number of the repeating unit were found to be 1.5×10^5 and 231, respectively.[18] Poly(A), poly(C), and poly(U) were purchased from Pharmacia, and RNase free neutral water and spectroscopic grade DMSO (both from Wako) were used for all measurements. Mixtures of the polynucleotide and s-SPG were prepared by adding a s-SPG/DMSO solution to a polynucleotide/water solution. The concentrations of the s-SPG/DMSO and polynucleotide/water solutions were chosen so that, after mixing, the polynucleotide concentration ($C_{\mathrm{poly(X)}}$, where X can be A, C, or U) was $7.1-7.8 \times 10^{-3}$ g/dL, the s-SPG concentration ($C_{\mathrm{s-SPG}}$), $6-7 \times 10^{-2}$ g/dL, and the volume fraction of water in the mixture (V_{w}), 0.93. The apparent pH of the DMSO/water solutions was found to be 8.0−8.5. Circular dichroism (CD) and absorbance spectroscopy in 230−320 nm region were measured in the temperature range of 5−70 °C on a Jasco J-720WI spectropolarimeter and a Jasco V-570 UV/VIS/NIR spectrometer, respectively.

Figure 2 compares the CD spectra between the polynucleotides and their mixtures with s-SPG at 5 °C, where $[\theta]$ is the molecular ellipticity. For the mixture of poly(A) and s-SPG (poly(A) + s-SPG), the addition of s-SPG increases the CD intensity at around 260 nm by more than double, for poly(C) + s-SPG the addition increases the peak at 275 nm by about 1.5 times and generates a new peak around 245 nm. However, poly(U) + s-SPG does not show any difference from poly(U). Since s-SPG itself has no absorption in the 230−320 nm region, the change in CD can be ascribed to a conformational change of the polynucleotides. Furthermore, it is interesting that the difference in the base molecule shows the distinctive difference in the CD change. On the other hand, when poly(C) or poly(A) was mixed with a triple helix of schizophyllan, amylose, and dextran, CD did not change at all (not shown herein). As a summary of the foregoing finding,

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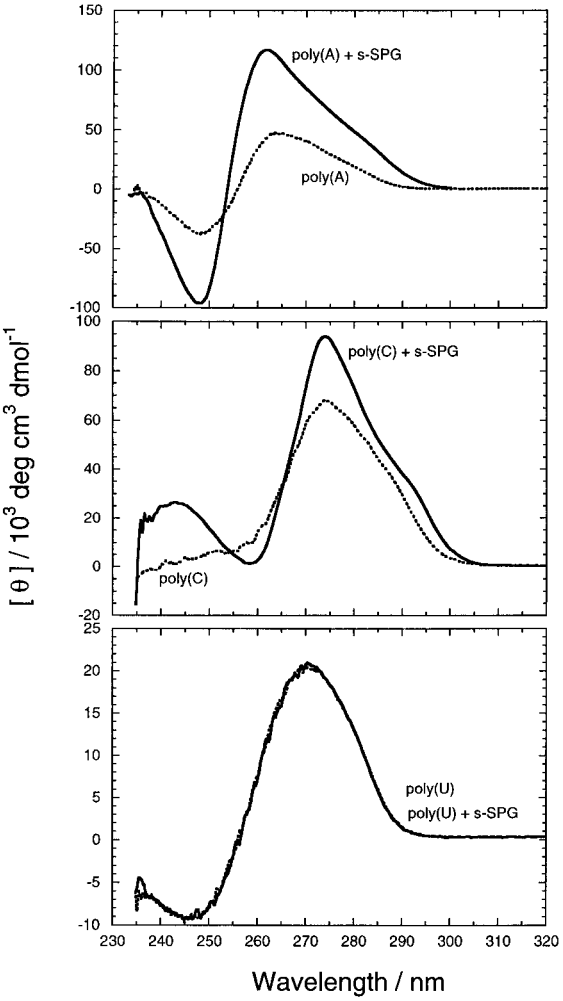

Figure 2. Comparison of the CD spectra at 5 °C between the single-stranded polynucleotides and their mixtures with s-SPG. $V_w = 0.92$, $C_{poly(X)} = 0.007-0.008$ g/dL, and $C_{s-SPG} = 0.06-0.07$ g/dL. The dotted and solid lines indicate the CD spectra for the polynucleotides and their mixtures with s-SPG, respectively.

Table 1. Results of the UV and CD Measurements[a]

sample code	λ_{max} of UV nm	$\epsilon \times 10^{-3}$ L mol cm^{-1}	λ_{max} of CD nm	$[\theta]_{max} \times 10^{-3}$ deg cm^2 dmol^{-1}
poly(C) in water	270	6.51	274	69.0
poly(C) in $V_w = 0.93$	270	6.41	275	67.2
poly(C) + s-SPG in $V_w = 0.93$	269	5.68	274	93.1
poly(A) in $V_w = 0.93$	257	7.98	263	47.4
poly(A) + s-SPG in $V_w = 0.93$	255	7.22	261	117.7

[a] Measurements were carried out at 5 °C. ϵ: molar absorption coefficient. $[\theta]$: molar ellipticity. V_w = water/(water + DMSO) in volume.

one can conclude that the CD spectral change is ascribed to a specific interaction of the glucose unit in s-SPG with the nucleic base unit in the polynucleotides but not due to "ionic" hydrogen bonding with the phosphate unit.

Table 1 summarizes the results of the CD and UV measurements for poly(A), poly(C), and their mixtures. The addition of s-SPG induces the blue shift and the hypochromic effect in the UV spectroscopy. Both phenomena for polynucleotides can be rationalized in terms of enhancement of the base stacking.[21] The

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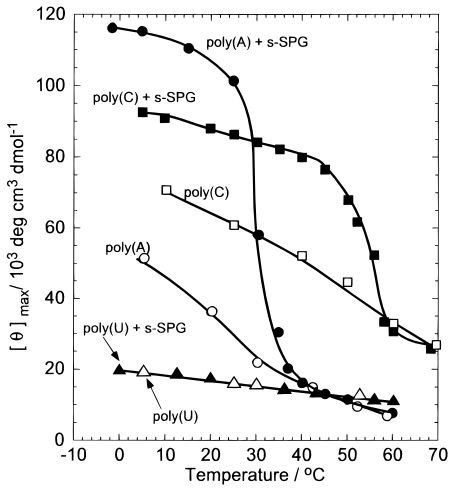

Figure 3. Temperature dependence of $[\theta]_{max}$ for the polynucleotides and their mixtures with s-SPG.

enhanced stacking is also consistent with increment in $[\theta]_{max}$ ($[\theta]$ at the top of the positive band). Therefore, all results consistently indicate that the addition of s-SPG effectively intensifies the base stacking. To enhance the base stacking, polar atmosphere and hydrogen-bonding interactions are necessary.[21] Since the addition of s-SPG to an aqueous solution should not increase the polarity of the solvent, one can conclude that a s-SPG/polynucleotide macromolecular complex is formed through the hydrogen-bonding interaction between the base and the s-SPG unit and that hydrogen bonding stabilizes the base stacking.

Figure 3 presents the temperature dependence of $[\theta]_{max}$ for the three polynucleotides and their mixtures. As shown above, in the lower temperature region poly(A) + s-SPG and poly(C) + s-SPG show larger $[\theta]_{max}$ than poly(A) and poly(C), respectively. As seen in the figure, there is an abrupt decrease in $[\theta]_{max}$ around 32 and 54 °C for poly(A) + s-SPG and poly(C) + s-SPG, respectively, and above the each critical temperature, the mixture's $[\theta]_{max}$ merges in the same plots of the corresponding polynucleotide itself. On the other hand, the $[\theta]_{max}$ for poly(U) + s-SPG shows the same values as that of poly(U) in the entire range. These features confirm the view that s-SPG/polynucleotide macromolecular complexes are formed at lower temperatures in both poly(A) + s-SPG and poly(C) + s-SPG, and no complex is formed in poly(U) + s-SPG. Furthermore, the temperature dependence indicates that the abrupt decrease is associated with the auto-accelerative dissociation of the complexes. It is interesting that this abrupt decrease is similar with the well-known melting behavior of double-helix polynucleotides. The poly(C)/s-SPG complex "melts" at higher temperature than that of the poly(A)/s-SPG complex, which is similar with the fact that the poly(C)/poly(G) complex melts at higher temperature (~110 °C) than that of the poly(A)/poly(U) complex (~65 °C).[21] The difference in the DNA helix melting temperature can be ascribed to the difference in the number of the hydrogen bonds (cytosine has three hydrogen-bonding sites whereas adenosine has two sites). The same explanation should be applicable to the difference in the melting temperature of the s-SPG/polynucleotide complexes.

In this report, we have presented very novel preliminary results for the specific interaction between polynucleotides and s-SPG. This is the first clear finding that polysaccharide can specifically interact with polynucleotides, except for some oligosaccharides.[22] We are in the process of investigating the molecular mechanism of this interaction. We expect that the present findings will provide an important clue to clarify the saccharide-RNA interactions which frequently play a critical role in biological systems.

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