Polysaccharide–Polynucleotide Complexes (III): A Novel Interaction between the β-1,3-Glucan Family and the Single-Stranded RNA Poly(C)

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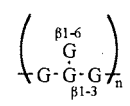
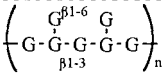
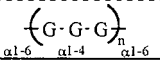
We measured circular dichroism spectra to examine the complex-formation capability of various kinds of polysaccharides with poly(C). The results show that schizophyllan and lentinan can interact with poly(C), on the other hand, amylose, pullulan, dextran, and curdlan cannot. This difference clearly indicates that the water soluble β-1,3-glucan can bind with polynucleotides. The molecular weight dependence of the complex formation has established that at least 30 glucose sequence length is necessary to incorporate the most bases into the complex.

Our previous work[1] presents the first clear evidence to prove that some polysaccharide can bind with polynucleotides.[2] The polysaccharide we used in the previous work was schizophyllan (SPG), whose main chain consists of β-1,3-glucans and one β-(1→6) glucosyl side chain links to the main chain at every three glucose units.[3] We demonstrated that the single chain of SPG (s-SPG) with M_w = 150000 can interact with poly(C) or poly(A) to form a novel macromolecular complex; where M_w is the weight-average molecular weight. These findings intrigue us whether other β-1,3-glucans such as lentinan and curdlan can interact with polynucleotides. This report presents our preliminary examination of what is the key factor to induce the novel polysaccharide–polynucleotide complexation.

Table 1 presents the polysaccharide samples used in this study as well as their molecular characters. Lentinan has two β-(1→6) glucosyl side chains in the five glucose units of the main chain and curdlan has no side chain (see Table 1). The side chains in SPG and lentinan make them water soluble. The chemical structures of dextran, pullulan, and amylose are also presented in the Table. All saccharides were first dissolved in DMSO to remove (or denature) the inherent superstructures, then the saccharide-DMSO solutions were diluted with a poly(C) aqueous solution so that the water volume fraction (V_w) was 0.95. The concentrations of poly(C) ($C_{poly(C)}$) and saccharide ($C_{saccharide}$) were 2.50×10^{-4} mol dm^{-3} / unit and 3.38×10^{-3} mol dm^{-3} / (glucose unit in the main chain), respectively. All the samples thus prepared were kept at least 3 days at 5 °C before the measurement. Circular dichroism (CD) spectra in 240–320 nm region were measured on a Jasco J-720W I spectrometer with a 1-cm cell. The molecular ellipticity ([θ]) was evaluated by the conventional way[4] and the [θ] value at the peak top of the positive band (λ_{max}) was denoted as $[\theta]_{max}$.

Table 1 Molecular characters of the polysaccharides used and their complex formation capability

Sample	M_W	Chemical structure[a]	Complex formation capability
schizophyllan	1.5×10^5		yes
schizophyllan	6500		yes
schizophyllan	3500		yes
schizophyllan	1980		yes
lentinan	7.5×10^5~ 1.0×10^5		yes
curdlan	>1.5×10^5 [b]	β1-3	no
amylose	1.6×10^5	 α1-4	no
dextran	4.0×10^4	α1-6[c]	no
pullulan	-		no

[a] G represents glucose residue. [b] Estimated from the intrinsic viscosity in DMSO. [c] There is some α1-3 and α1-4 branch points.

Figure 1 (a) compares the CD spectra at 10 °C between poly(C) and its mixtures with six difference polysaccharides listed in Table 1. Poly(C) gives the CD spectrum with λ_{max} = 276 nm and $[\theta]_{276} = 5.33 \times 10^4$ deg cm^{-2} dmol^{-1}. Mixing with dextran, pullulan, or amylose does not induce any change in the poly(C) spectrum. On the other hand, schizophyllan and lentinan drastically change the spectra. The 4th column in Table 1 summarizes the presence or absence of the complex. These results elucidate that the β-1,3-glucan backbone is essential to form the complex. We could not obtain the CD spectra for the curdlan + poly(C) system, because curdlan was precipitated when we added water to the curdlan-DMSO solution. Curdlan shows poor solubility in water, because it has no glucosyl side chain that is necessary to water solubility. Figure 1 (b) presents the M_w dependence of the CD spectra at 10 °C for s-SPG +

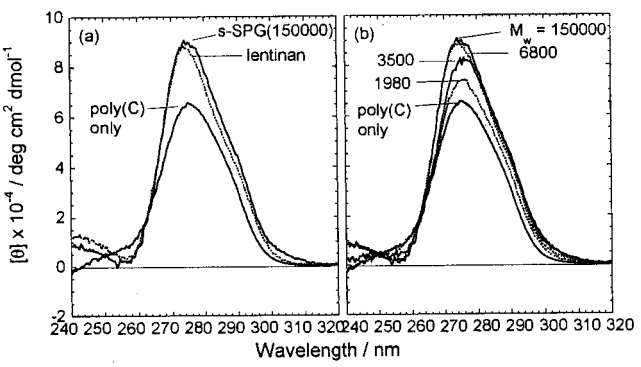


Figure 1. CD spectra of poly(C) in the presence or absence of β-1,3-glucans; V_w = 0.95, [poly(C)] = 2.50×10^{-4} mol dm^{-3} / unit, [saccharide] = 3.38×10^{-3} mol dm^{-3} / (glucose unit in the main chain), 10 °C. (a) Relationship between the chemical structure of the saccharides and the complex formation. (b) M_w dependence of SPG.

poly(C) system. The spectra for M_w = 150000 and 6800 are exactly same, indicating that there is no molecular weight effect on the complex formation for the case of $M_w \geq 6800$. On the other hand, with decreasing M_w from 6800 to 3500, then 1980, $[\theta]_{276}$ decreases systematically. Since we already know that all bases are incorporated into the complex formation for M_w = 150000,[1] the ratio of the base free from the complex can be estimated to be 49% for M_w = 3500, and 76% for M_w = 1980. Based on these results, we can conclude that the lower limit of the glucose sequence length is around 30 units (M_w = 6500) to incorporate the most bases of poly(C) into the complex.

Figure 2 shows the temperature dependence of $[\theta]_{max}$ for poly(C) and its mixtures with lentinan and the four s-SPG samples with different M_w. For the case of M_w = 150000, $[\theta]_{max}$ of poly(C) + s-SPG system drastically decreases at 50 °C and above that temperature the $[\theta]_{max}$ values for the mixture merge into those of poly(C). This feature indicates that the complex is dissociated at 50 °C.[1,5] The "melting" temperature is presented as clear evidence that the dissociation takes place in a cooperative manner (or auto-accelerative cleavage) such as the dissociation of double helix DNA.[6] The complex with lentinan is dissociated in the same manner as that of s-SPG (M_w = 150000). This feature is consistent with their M_w values (see Table 1).

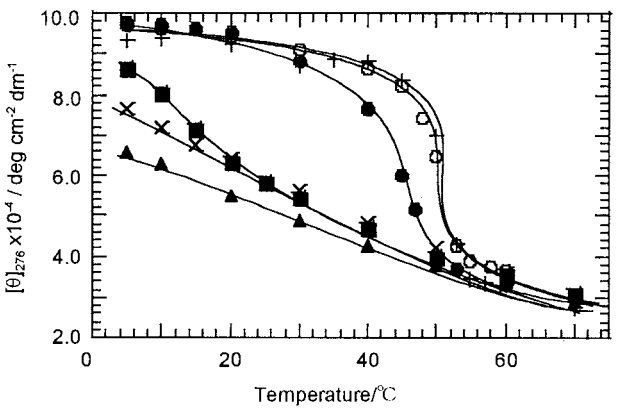


Figure 2. Temperature dependence of $[\theta]_{max}$ of poly(C) in the absence(▲) or presence of β-1,3-glucans; s-SPG (M_w = 150000) (○), s-SPG (M_w = 6800) (●), s-SPG (M_w = 3500) (■), s-SPG (M_w = 1980) (×), lentinan (+).

With decreasing M_w for s-SPG, the dissociation tends to occur at lower temperature (i.e., the transition temperature-region becomes wider). This temperature dependence indicates that the affinity for the complexation decreases with decreasing M_w. This feature is commonly observed as the low molecular weight effect in the cooperative phenomena of the macromolecular system such as helix-coil transition of polypeptides.[7]

In this work, we have successfully proven that only the β-1,3-glucan sequence is essential for the complex formation and the lower limit of the glucose sequence length is around 30 units. The local polymer conformation frequently adopts the most stable structure in solvents.[8] Assuming that this principle is applicable to our system, we compare the most stable structure as follows. According to conformational energy calculation[9] and X-ray crystallography,[10,11] the most stable structure of the β-1,3-glucan sequence is presumed to be the right handed (6_1) helix with 17.4 Å pitch. Amylose takes the left handed (6_1) helix with 8 Å pitch and dextran is expected to behave like a flexible chain.[11] The most stable structure of poly(C) is known as the right handed (6_1) helix with 18.6 Å pitch.[6] It is surprising that there is remarkable coincidence in the helix parameter between poly(C) and the β-1,3-glucans. This coincidence suggests that the pre-organized conformation [i.e., the similar helix to that of poly(C)] of s-SPG can makes it easy to form the complex with poly(C). Curdlan does not interact with poly(C), although it has a β-1,3-glucan sequence. Curdlan shows poor solubility in water, because it has no glucosyl side chain. Curdlan should self-aggregate with inter- or intra-molecular interactions in water before it interacts with poly(C). It shows that the glucosyl side chains of SPG and lentinan are important to make the polysaccharides soluble.

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References and Notes

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