Chemical Modification of Curdlan to Induce an Interaction with Poly(C)[1]

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The molecular weight of the commercially available curdlan is over 10^6, which makes it difficult to bind to polynucleotides. This paper presents a chain-scission reaction of curdlan and the molecular weight dependence of the binding ability. We found that curdlan can bind to poly(C) when the molecular weight is less than about 10^5. However, the complex stability is still less than that of the single chain of schizophyllan.

Exploration of reagents that can bind to RNA or DNA is of significant importance in the genetic engineering. Most investigations have been done on cationic compounds such as polylysine and polyethylenimine that can form the ion pair with DNA. However, the ion pair is such a strong attractive force that the complexation may disturb the original DNA conformation. This sometimes causes to eliminate functions of DNA (DNA compaction).[2]

We are first to find that a neutral polysaccharide, schizophyllan, can form a macromolecular complex with some polynucleotides.[3] This interaction is relatively moderate enough not to induce the DNA compaction.[3–8] This novel interaction occurs only for water-soluble β-1,3-glucans (such as schizophyllan (SPG) and lentinan).[4] These glucans take a triple helix in water and a random coil in dimethyl sulfoxide (DMSO). The complexation undergoes between the single chain of schizophyllan (s-SPG) and some polynucleotides such as poly(C), poly(A), poly(dA), and poly(T). The stoichiometric study suggests that two saccharide chains and one nucleotide chain form the complex, i.e., s-SPG:poly(C) = 2:3 in the molar ratio.[7]

We claimed that the commercially available curdlan can not bind to any polynucleotides.[4] This exception to the β-1,3-glucan families is due to its poor water-solubility. However, the other aspects of curdlan completely satisfy the requirements for the complexation. The poor water-solubility of curdlan, especially renatured one, can be ascribed to its large molecular weight. Therefore, we made an attempt to reduce the molecular weight by hydrolysis of its main chain and to examine the binding ability. This communication presents our preliminary results for the attempt.

Curdlan and poly(C) were purchased from Wako Chemical and Pharmacia, respectively. The number of the base in the poly(C) was 570. A purified schizophyllan sample with M_w = 1.5×10^5 in DMSO was kindly supplied from Taito Co.

Chain scission of curdlan was carried out by an acetal cleavage reaction in *N*,*N*-dimethylformamide (DMF) with *p*-toluenesulfonic acid as an acid catalyst.[9] After the chain scission, the curdlan/DMF solution was poured into methanol for precipitation and this procedure was repeated twice. The molecular weights of the curdlan samples were evaluated by gel permeation chromatography (GPC) using a HLC-8020; two α-4000 columns are connected in series, LiBr (20 mM)/DMF was used as an elute solvent, and the instrument was calibrated by TOSOH's standard polystyrenes.[10] The reaction time, M_w, the molecular weight distribution (M_w/M_n), and the sample codes are presented in Table 1, where M_n is the number average molecular weight. Complexation was carried out by mixing a curdlan/DMSO solution and a poly(C) aqueous solution according to the known method.[3,7] The water volume fraction was fixed at 0.93, the molar ratio of curdlan to poly(C) was in the range of 4–6 (excess amount of curdlan for the stoichiometry, assuming that curdlan form a similar complex with polynucleotide as s-SPG[8]), and all mixtures were stored for aging for at least 3 days before the measurement. Circular dichroism (CD) in 240–320 nm region was measured on a Jasco J-720WI spectropolarimeter in the temperature range 5–80 °C.

Table1. Molecular weight of curdlan and SPG and their sample code

Sample code	Reaction time / h	$M_w/10^4$	M_w/M_n
L-1	0 (received)	140	1.4
L-2	6	10	2.0
L-3	18	4.0	1.4
L-4	48	2.9	3.0
s-SPG		40[a]	2.5

[a]SPG takes the triple helix in DMF, M_w for this sample is 4.5×10^5 in water.[3]

As shown in Table 1, with increasing the reaction time, M_w decreases drastically from 1.4×10^6 to 2.9×10^4 and then the decrement of M_w is saturated. It will be related to the content of water in the reaction solvent. In another words, we can control the molucular weight of curdlan by changing the water volume in the solvent. Furthermore, the value of M_w/M_n is relatively smaller than that of SPG,[10] indicating that our fractionation procedure is effective for curdlan.

Figure 1 compares the CD spectra at 5 °C among the mixtures of poly(C) and the curdlans listed in Table 1. The L-1 mixture shows no change as reported by Kimura et al.,[4] evidencing no complexation. The L-2, L-3, and L-4 mixtures show some increment in the intensity at the positive band (both 275 nm and 242 nm), indicating that the complexation takes place between these curdlans and poly(C). Furthermore, the similarity of the CD spectra between the s-SPG and curdlan solutions suggests that curdlan can form the similar type of complex as s-SPG does. Figure 2 presents the results of stoichiometric study, where ΔCD_{275} is $CD_{275(+L\text{-}4)} - CD_{275(poly(C))}$ in each molar fraction. The maximum of the ΔCD_{275} appears

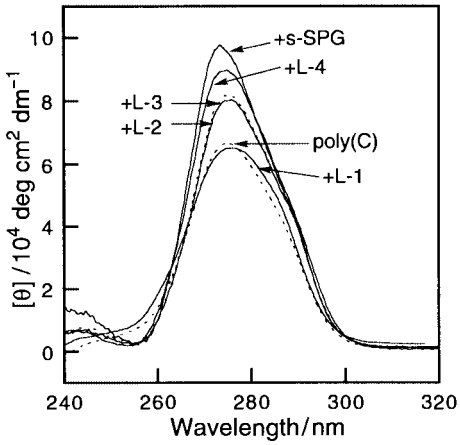

Figure 1. Comparison of the CD spectra for 4 curdlan plus poly(C) solutions, and the poly(C)/s-SPG complex.

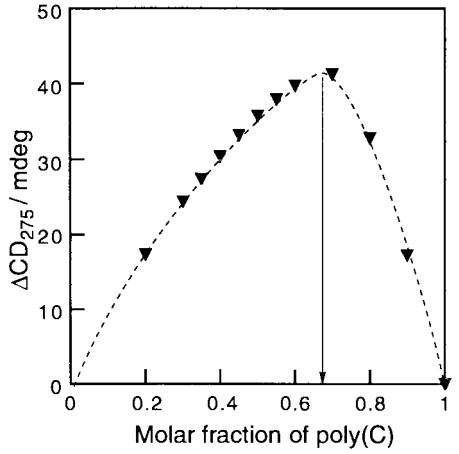

Figure 2. Job plot between poly(C) and L-4: the sum of [poly(C)] + [L-4] was kept constant (2.7×10^{-4} M)

around at 0.6–0.7, indicating that curdlan also form a triple helix consisting of two curdlan chains and one poly(C) chain.[8] By the way, the voltage applied to the CD photodetector was found to be slightly high for the L-1, L-2, and L-3 mixtures. This high voltage is ascribed to the Tyndall phenomenon,[11] indicating the presence of some turbidity.

Figure 3 presents the melting behavior of the complex in the curdlan solutions, comparing with the poly(C)/s-SPG complex and poly(C) itself. The complexes in the curdlan solutions exhibit a broader transition at a lower temperature than the poly(C)/s-SPG complex. This feature suggests that the complex in the curdlan solutions is less stable and less organized than in s-SPG solution. By the way, the intensity of +L-2 is lower than the others even at 60–80 °C, where the complex is considered to be dissociated. This feature suggests that some of poly(C) (ca. 10%) have been co-precipitated with curdlan

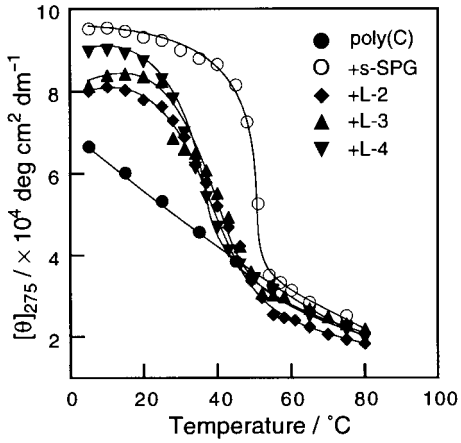

Figure 3. Comparison of the melting curves between the curdlan and schizophyllan complexes.

before the solution was transferred to the CD cell. Although the L-3 and L-1 show some turbidity, decrease of the poly(C) concentration was not observed.

In conclusion, curdlan can bind to poly(C) by reducing its molecular weight. However, owing to its poor water-solubility, the complex stability is less than that of s-SPG.

References and Notes

1 This is the 10th paper in the series of "Polysaccharide–polynucleotide complexes"
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10 Using polystyrene as the standard may not be appropriate for polysaccharides because there is no similarity in conformation and chemical structure. However, there is no other standard available for our system. Therefore, there may be some ambiguity in the value of the molecular weight distribution.
11 JASCO Technical Manual for CD, 1998.