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COMMUNICATION

Polysaccharide–Polynucleotide Complexes (IV): Antihydrolysis Effect of the Schizophyllan/Poly(C) Complex and the Complex Dissociation Induced by Amines

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Polymer complexes formed by schizophyllan and poly(C) showed a unique antihydrolysis effect when poly(C) was subjected to hydrolysis under basic conditions. The complexation reduced the hydrolysis rate to 80% of the control (*i.e.*, poly(C) itself). However, when we added oligoamines with the intention of catalyzing the hydrolysis, the oligoamines induced dissociation of the complex instead of acceleration of the hydrolysis.

INTRODUCTION

Schizophyllan (SPG) is a natural polysaccharide produced by 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 adopts a triple helix (t-SPG) in nature, which can be dissociated into a single chain (s-SPG) by dissolving in dimethylsulfoxide (DMSO) (*3*,*4*). The s-SPG chain can retrieve the original triple helix by exchanging DMSO for water (*5*). Recently, we found that when this renaturing process is carried out in the presence of polynucleotides, the resultant triple helix consists of two s-SPG chains and one nucleotide chain (*6*). This macromolecular complex is very novel and, in fact, none has ever found that such a specific interaction can take place between a polysaccharide and polynucleotides. This finding suggests that the s-SPG/polynucleotide complex can be applicable to a DNA or RNA delivery system. One of the key issues in this application, which has to be addressed, is how strongly the complex resists hydrolysis of the nucleotides.

In this report we focus on the s-SPG/poly(C) complex and examine the antihydrolysis capability (*1*) in a basic aqueous solution and (*2*) in various aqueous oligoamine

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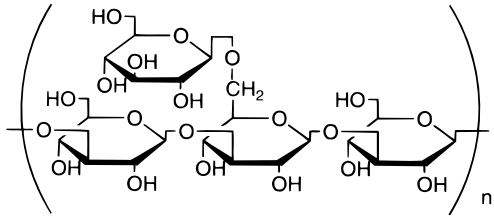

FIG. 1. Repeating unit of schizophyllan.

solutions, using the circular dichromic (CD) and ultraviolet (UV) spectroscopic methods. The latter study is specially important, because it is well known that oligoamines specifically interact with the phosphate anion of polynucleotides and thus accelerating hydrolytic cleavage of the phosphorate ester (*7,8*). Our interest lies in how the s-SPG/polynucleotide complex behaves in the presence of amines, when hydrolysis has to compete with ion pair formation of the polynucleotides and amines.

EXPERIMENTAL PROCEDURE

Materials. t-SPG was kindly supplied by Taito Co., Japan. The molecular weight and the number of repeating units were evaluated to be 1.5×10^5 and 231, respectively (*9*). Poly(C) purchased from Pharmacia, RNase free distilled water from Nippon Gene and spectroscopic grade DMSO from Kishida were used for all measurements. Propylamine (PA), ethylenediamine (EDA), and diethylenetriamine (DETA) were purchased from Wako.

Complex formation. We prepared the s-SPG/poly(C) complex by mixing a s-SPG/DMSO and a poly(C)/water solution, according to a reported method (*6*). The thus prepared mixture was left at room temperature for 1 h to allow the complexation to complete. The UV or CD measurement was started after the sample solution was heated up to the hydrolysis temperature in a UV or CD cell and an amine or base solution was added to the sample solution. The final concentrations (after mixing with base or amine) were usually controlled as follows: [poly(C) (monomer unit)] = 2.5×10^{-4} mol dm^{-3}, [s-SPG (monomer unit for main chain)] = 3.4×10^{-3} mol dm^{-3}, [amine] = 0–0.2 mol dm^{-3}, [Tris] = 8.3×10^{-2} mol dm^{-3} (pH = 10.), and [EDTA] = 1.0×10^{-3} mol dm^{-3}. Here, Tris was used as a buffer, knowing that this buffer does not affect the complex nature.

Spectroscopic measurement. The CD and UV spectra in 200–400 nm region were measured on a Jasco J-720WI spectropolarimeter and a Jasco V-570UV/VIS/NIR spectrometer respectively with in a 1-cm cell. We carried out the reaction appropriately at 45°C for convenience of rate measurements. Higher temperatures than this enhance the hydrolysis and induce complex dissociation, on the other hand, lower ones suppress the reaction rate not enough to be detected.

RESULTS AND DISCUSSION

Figure 2 compares the UV spectral changes between poly(C) in Fig. 2A and the poly(C)/s-SPG complex in Figure 2B. As mentioned in the experimental section, we

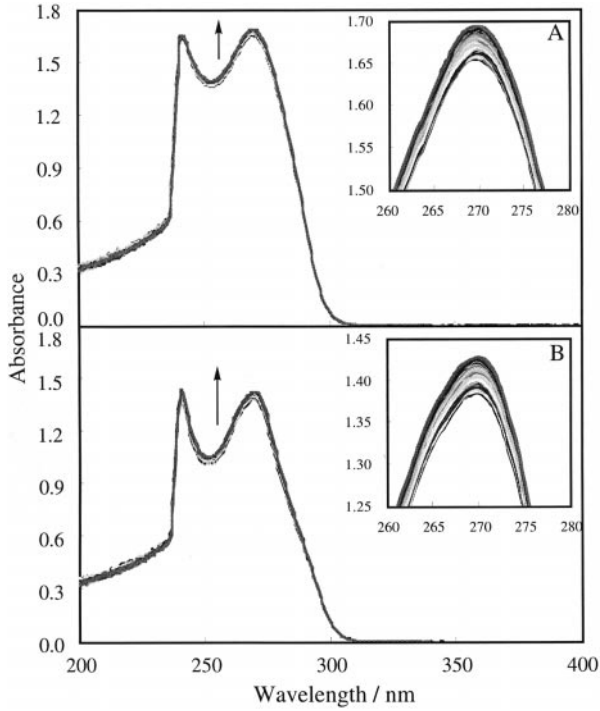

FIG. 2. Changes in the UV spectra during hydrolysis by base for poly(C) in A and for poly(C)/s-SPG in B. Inset: magnification of the 260- to 280-nm region.

used exactly the same nucleotide concentrations for both the complex and the control, however, the observed absorbance is different; for example, the absolute absorbance at 270 nm (A_{270}) is 1.38 for the complex and 1.58 for the control at the beginning of the reaction. According to previous results (*6,9*) this difference can be ascribed to hypochromism, which occurs in accordance with cytosine stacking in the complex. As time passes, the absorbance increases for both cases. The increment of the absorbance is ascribed to formation of cytidine-monophosphate (CMP), which has a large extinction coefficient than poly(C) and the complex. One can conclude, therefore, that the hydrolysis takes place in the both cases.

In order to convert the A_{270} vs time relation to product concentration (i.e., CMP concentration) vs time relation, we used the following literature values for the extinction coefficient (ε) of the product and the reactant: 6200 cm^{-1} mol^{-1} dm^3 for poly(C), 5420 cm^{-1} mol^{-1} dm^3 for the poly(C)/s-SPG complex, and 9120 cm^{-1} mol^{-1} dm^3 for CMP (*10,11*). The product concentration is plotted against the reaction time in Figure 3. The hydrolysis rate constant is determined from each slope to be 3.6×10^{-8} min^{-1} mol dm^{-3} for poly(C) and 2.8×10^{-8} min^{-1} mol dm^{-3} for the poly(C)/s-SPG complex. Therefore, the reaction rate in the control is 1.25 times as large as that of the complex. This difference clearly indicates that complexation of poly(C) by s-SPG suppresses hydrolysis by base. When we studied the RNase II hydrolysis

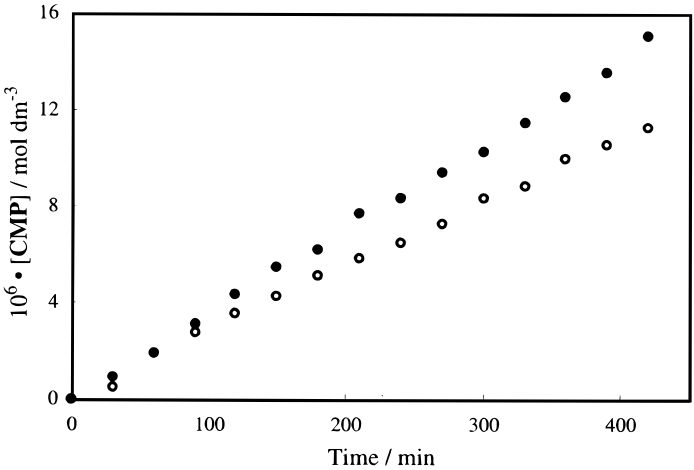

FIG. 3. CMP concentration plotted against reaction time during hydrolysis by base: in the absence of SPG (○), in the presence of SPG (●).

for the poly(C)/s-SPG complex, the complex exhibited a $\frac{1}{6}$ magnitude of the maximum velocity of the control (*12*). When the present result is compared with the enzyme system, the complex works less effectively against hydrolysis in the base hydrolysis system than in the enzyme hydrolysis system. RNases are relatively large molecules and the active site is located inside the enzyme. Therefore, the SPG binding poly(C) creates steric hindrance and can prevent RNases from approaching the nucleotide.

Figure 4 compares the absorbance changes in poly(C) and the complex during the hydrolysis reaction catalyzed by PA, EDA, and DETA. The upper panel shows the controls and the lower one shows the corresponding reactions for the complex. In the control experiments, all data points for the initial stage of the reactions can be fitted by a straight line and the addition of amines increases the slope. This feature demonstrates that the oligoamines accelerate hydrolysis of poly(C) and Table 1 summarizes the reaction rates estimated from these slopes. On the other hand, in panel B, the absorbance of the complex increases in an upward convex manner in the initial stage and then increases linearly. This feature suggests that in order to understand the phenomenon, we have to take an additional factor as well as the catalyzed effect by amines into consideration. The deviation from the linear increment is observed for all samples. Furthermore, with increasing number of amino groups (*i.e.*, from PA to DETA), the deviation is enhanced.

The addition of the amines at pH 10.0 creates an ion-pair between the phosphate anion in poly(C) and the amine cation. Major factors to stabilize the poly(C)/s-SPG complex are the hydrogen-bonding and hydrophobic interactions. Generally speaking, the ion-pair formation is more favorable than both hydrogen-bonding and hydrophobic interactions. Therefore, we can speculate that the added amines bind poly(C) and due to this process the complex is dissociated. Once dissociated, poly(C) should be hydrolyzed in the same rate as that of the control.

Figure 5 demonstrates the CD spectral changes during the initial stage (i.e., the