

## Synthesis of the Polysaccharide, (1→5)- $\alpha$ -D-Ribofuranan and Its Catalytic Activities for the Hydrolysis of Phosphates and the Cleavage of Nucleic Acids

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**Abstract:** The polysaccharide, (1→5)- $\alpha$ -D-ribofuranan, was synthesized by a cationic ring-opening polymerization of 1,4-anhydro-2,3-di-*O*-benzyl- $\alpha$ -D-ribofuranose with the aid of boron trifluoride etherate and subsequent debenzoylation. This polysaccharide catalyzed the hydrolysis of ethyl *p*-nitrophenyl phosphate, uridylyl(3'→5')uridine ammonium salt, and 4-*tert*-butylcatechol cyclic phosphate *N*-methyl pyridinium. The polymer also catalyzed the cleavage of nucleic acids (DNA and RNA). The hydrolysis of ethyl *p*-nitrophenyl phosphate in the presence of the polymer was accelerated by  $1.5 \times 10^3$  times relative to the uncatalyzed reaction. The catalytic activity was attributable to the *vic-cis*-diols of the riboses being located inside the active center that is formed by polymer chain folding; these diols form hydrogen bonds with two phosphoryl oxygen atoms of the phosphates so as to activate the phosphorus atoms to be attacked by nucleophile (H<sub>2</sub>O).

**Keywords:** polysaccharide, catalytic activity, ribose, nucleic acid.

### Introduction

Synthesis and activity tests of the enzyme models and mimics draw much attention to the elucidation of the enzyme structures and mechanisms.<sup>1-4</sup> Especially, after the discovery of catalytic nucleic acids such as ribozyme and deoxyribozyme,<sup>5,6</sup> the design and synthesis of nuclease mimics, catalyzing the cleavage of DNA or RNA, continue to be an attractive goal in nucleic acid research. In contrast to the enormous study on the activities of natural nucleases, however, only a few nuclease models were proposed.<sup>7</sup>

Recently we reported on a nuclease model, 5-( $\beta$ -cyclo-dextrinylamin)-5-deoxy- $\alpha$ -D-ribose,<sup>8</sup> which catalyzed the hydrolysis of phosphodiester, and on nuclease mimics, the ribose-containing polymers, which accelerated the cleavage of DNA.<sup>9,10</sup> We proposed that the *vic-cis*-diol groups of riboses form hydrogen bonds with the two oxygen atoms of the phosphate so as to activate the phosphorus atoms to be attacked by water, resulting in the acceleration of the hydrolysis. In this work, we prepared the polysaccharide, (1→5)- $\alpha$ -D-ribofuranan, which consists of ribose rings and

has a structure very similar to those of naturally occurring polysaccharides. Surprisingly, this synthetic polysaccharide also catalyzed the cleavage of nucleic acids (DNA and RNA) and the hydrolysis of phosphates. Here we report on the synthesis and activity tests of the polysaccharide as well as the action mechanism of the catalysis.

### Experimental

**Materials and Instrumentation.** Chemicals were purchased from Sigma-Aldrich. Supercoiled ds DNA (pBR 322 Vector), BMV RNA, Lambda DNA/Hind III Markers, RNA Markers (0.28-6.58 kb) and Ethidium Bromide Solution were purchased from Promega. Uridylyl(3'→5')uridine ammonium salt (**35UpU**), uridine 2'-monophosphate lithium salt (**2MPU**), uridine 3'-monophosphate sodium salt (**3MPU**), and uridine 5'-monophosphate (**5MPU**) were used as received. Tris-buffer materials (TRIZMA<sup>®</sup> Base and TRIZMA<sup>®</sup> HCl) were recrystallized from deionized water and ethanol three times to exclude metal ions. The substrates, ethyl *p*-nitrophenyl phosphate (ENPP),<sup>10</sup> 4-*tert*-butylcatechol cyclic phosphate *N*-methyl pyridinium (CP),<sup>11</sup> and 1-phosphate (1P)<sup>8</sup> and 2-phosphate (2P)<sup>8</sup> of 4-*tert*-butylcatechol were prepared according to the literatures. <sup>1</sup>H and <sup>13</sup>C

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NMR spectra were recorded on Varian Gemini 200 spectrometer. The reaction rates were determined with a Hitachi (Model 200-20) spectrophotometer thermostated ( $\pm 0.1$  °C). Measurement of molecular weights was carried out by Waters 150-Cplus GPC with a RI detector.

**2,3-di-*O*-benzyl-(1 $\rightarrow$ 5)- $\alpha$ -D-ribofuranan (2).** The monomer, 1,4-anhydro-2,3-di-*O*-benzyl- $\alpha$ -D-ribofuranose (**1**), was prepared according to the literatures.<sup>12,13</sup> Monomer **1** (1.13 g, 1.2 mmol) was dissolved in methylene chloride and cooled to -78 °C. After 15 min, boron trifluoride etherate (8 mol%) as a catalyst was transferred into the mixture and stirred for 12 h, and then precipitated in petroleum ether to give **2** (yield 46.9%,  $[\alpha]_D = 145^\circ$ , lit.<sup>12</sup>: 147-153°), <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  3.3-3.5 (d, 1H, H<sup>5</sup>), 3.6-3.75 (q, 1H, H<sup>5</sup>), 3.75-3.95 (q, 1H, H<sup>2</sup>), 3.95-4.15 (q, 1H, H<sup>3</sup>), 4.15-4.35 (d, 1H, H<sup>4</sup>), 4.35-4.5 (d, 2H, -CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.55-4.7 (d, 2H, -CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 4.9 (d, 1H, H<sup>1</sup>), 7.1-7.5 (br, 10H, -CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>).

**(1 $\rightarrow$ 5)- $\alpha$ -D-Ribofuranan (RF).** **2** (0.53 g, 0.72 mmol) was dissolved in 6.8 mL of 1,2-dimethoxyethane and the solution was added to a sodium (0.14 g) - liquid ammonia solution (20 mL) at -78 °C. After 2.5 h, ammonium chloride (1.5 mmol) was added to the mixture until a dark color disappeared and then a small amount of MeOH (5 mL) was added. After evaporation of ammonia, 50 mL of water was added and then the aqueous solution was washed with methylene chloride. The polymer was separated by dialysis through a cellulose membrane tube of a molecular weight cutoff of 3500. Number-average molecular weight (*M<sub>n</sub>*) of the polymer remained in the membrane was measured to be 3800 (polydispersity, PD = 1.29) by gel permeation chromatography in water with poly (ethylene glycol) standards (yield 11 %). <sup>13</sup>C NMR (50 MHz, D<sub>2</sub>O):  $\delta$  102.93 (C<sup>1</sup>), 83.56 (C<sup>4</sup>), 71.47 (C<sup>2</sup>), 70.37 (C<sup>3</sup>), 68.59 (C<sup>5</sup>), same as the data reported.<sup>12</sup>

**Kinetic Measurements for Ethyl *p*-Nitrophenyl Phosphate (ENPP).** A solution of **RF** ( $1.6 \times 10^{-5}$  M) and a solution of ethyl *p*-nitrophenyl phosphate ( $7.0 \times 10^{-3}$  M) were prepared in water buffered with 0.02 M Tris (pH 7.4) and the ionic strength was adjusted to 0.02 (KCl). The definite portions of the catalyst and the substrate solutions were mixed in a measuring cell. A solution of the same substrate concentration buffered with Tris (pH 7.4) at the ionic strength of 0.02 (KCl) was filled in the reference cell. The reaction rates were determined by measuring the absorption (*A<sub>t</sub>*) of *p*-nitrophenol (400 nm) as a function of time (*t*) with a Hitachi (Model 200-20) spectrophotometer thermostated ( $\pm 0.1$  °C).

**Kinetic Measurements for Uridine Compounds.** The polymeric catalyst ( $1.2 \times 10^{-5}$  M) and phosphates ( $3.5 \times 10^{-4}$  M) were dissolved in Tris-buffer solution (pH 7.4) with ionic strength of 0.02 (KCl) at 50 °C. A reaction solution (80  $\mu$ L) was taken by a microsyringe at definite time intervals and injected into a Waters LC (conditions for **35UpU**: Waters Untrahydrogel 120 GPC column, UV detector  $\lambda = 265$  nm, eluent = 0.02 M KCl, flow rate = 1.0 mL/min and

conditions for **2MPU**, **3MPU**, and **5MPU**: Cosmosil C18 reverse phase column, UV detector  $\lambda = 265$  nm, eluent = 0.01M pH 7.4 phosphate buffer, flow rate = 0.7 mL/min). The concentrations of the reactants and products were determined by peak areas, which were corrected by the extinction coefficients of the compounds. ( $\epsilon = 8780$  for Uridine, 5629 for **35UpU**, 7860 for **2MPU**, 7170 for **3MPU**, 4430 for **5MPU** at 285nm and pH 7.4).

**Kinetic Measurements for 4-*tert*-Butylcatechol Cyclic Phosphate *N*-Methyl Pyridinium (CP).** The polymeric catalyst and phosphates were dissolved in Tris-buffer solution (pH 7.4) with ionic strength of 0.02 (KCl) at 25 °C. A reaction solution (80  $\mu$ L) was taken by a microsyringe at definite time intervals and injected into a Waters LC (conditions: Waters Untrahydrogel 120 GPC column, UV detector  $\lambda = 285$  nm, eluent = 0.025 M KCl, flow rate = 0.8 mL/min). The concentrations of the reactants and products were evaluated by peak areas, which were corrected by the extinction coefficients of the compounds ( $\epsilon = 2460$  for **CP**, 3500 for **1P**, 3490 for **2P**, 2310 for **BC** at 285 nm and pH 7.4).

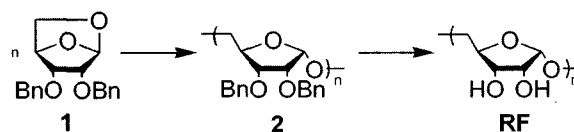
**Hydrolysis of dApdA.** **RF** ( $4.53 \times 10^{-6}$  M) and dApdA ( $9.6 \times 10^{-6}$  M) were incubated at pH 7.4 (Tris-buffer), ionic strength of 0.02 (KCl), and 37 °C for 24 h. The reaction mixture was analyzed by a Waters LC [conditions: C18  $\mu$ -Bondapak column in 0.25 M, eluent = pH 7.4 phosphate buffer, flow rate = 0.8 mL/min. retention times: dApdA (4.22), pdA (4.57), dAp (6.55), and dA (99.28 min), identified by the authentic samples].

**Hydrolysis of of Nucleic Acids.** Supercoiled ds DNA (pBR 322) and RNA (BMV) were incubated in a Tris buffer solution [pH = 7.4,  $\mu = 0.02$  (KCl)] and in the presence of ribose or **RF** at 37 °C ([**RF**] =  $2.82 \times 10^{-8}$  M, [ribose] =  $8.2 \times 10^{-7}$  M, and [nucleic acid] = 50 mg/L). After every incubation time, microtubes were taken and visualized after EtBr-agarose gel electrophoresis with UV light. Lambda DNA/Hind III Markers and RNA Markers (0.28-6.58 kb) were used as markers for DNA and RNA, respectively.

## Results and Discussion

**Polymer Synthesis.** (1 $\rightarrow$ 5)- $\alpha$ -D-Ribofuranan (**RF**) was prepared according to the literature (Scheme I).<sup>12,13</sup> The cationic polymerization of 1,4-anhydro-2,3-di-*O*-benzyl- $\alpha$ -D-ribofuranose (**1**) by boron trifluoride etherate gave 2,3-di-*O*-benzyl-(1 $\rightarrow$ 5)- $\alpha$ -D-ribofuranan (**2**) ( $[\alpha]_D = 145^\circ$ , lit. 147-153°),<sup>12</sup> which was debenzylated with the aid of sodium in liquid ammonia to give **RF**.<sup>12</sup> The polymer was separated by

Scheme I



dialysis through a cellulose membrane tube of a molecular weight cutoff of 3,500. Number-average molecular weight ( $M_n$ ) of the polymer remained in the membrane was measured to be 3,800 (polydispersity, PD = 1.29) by gel permeation chromatography in water with poly(ethylene glycol) standards.

**Catalysis for the Reactions of Phosphates.** Ethyl *p*-nitrophenyl phosphate (ENPP) was used as a substrate. The hydrolysis rate of ethyl *p*-nitrophenyl phosphate was determined in Tris buffers (pH = 7.4, ionic strength = 0.02, KCl) at 50 °C in the presence of **RF** or ribose by measuring the ultraviolet absorption ( $\lambda = 400$  nm) of the *p*-nitrophenol evolved. When the hydrolysis rates were compared with that measured in the buffer solution alone, it was found that ribose showed no catalytic activity while **RF** accelerated the hydrolysis of the substrate.

In the hydrolysis, the measured rate ( $v_m$ ) was the sum of  $v_c$  (catalyzed reaction) and  $v_u$  (uncatalyzed reaction). The  $v_c$  was therefore obtained by subtraction of  $v_u$  from  $v_m$ , i.e.,  $v_c = v_m - v_u$ . The initial  $v_c$  values were obtained at the constant concentration of **RF** by changing the substrate concentrations. Michaelis-Menten kinetics for **RF** was found to be satisfied by plotting the double reciprocal form of Lineweaver and Burk ( $1/v$  vs.  $1/[S]$ ) (Figure 1a), indicating that the polymer built an active center by chain folding, which formed a complex with the substrate.

$K_m$  ( $1.52 \times 10^{-3}$  M) and  $V_{max}$  ( $2.26 \times 10^{-5}$  M h<sup>-1</sup>) were obtained from Figure 1a. We assumed that one **RF** molecule formed one active center. The  $k_{cat}$  could therefore be

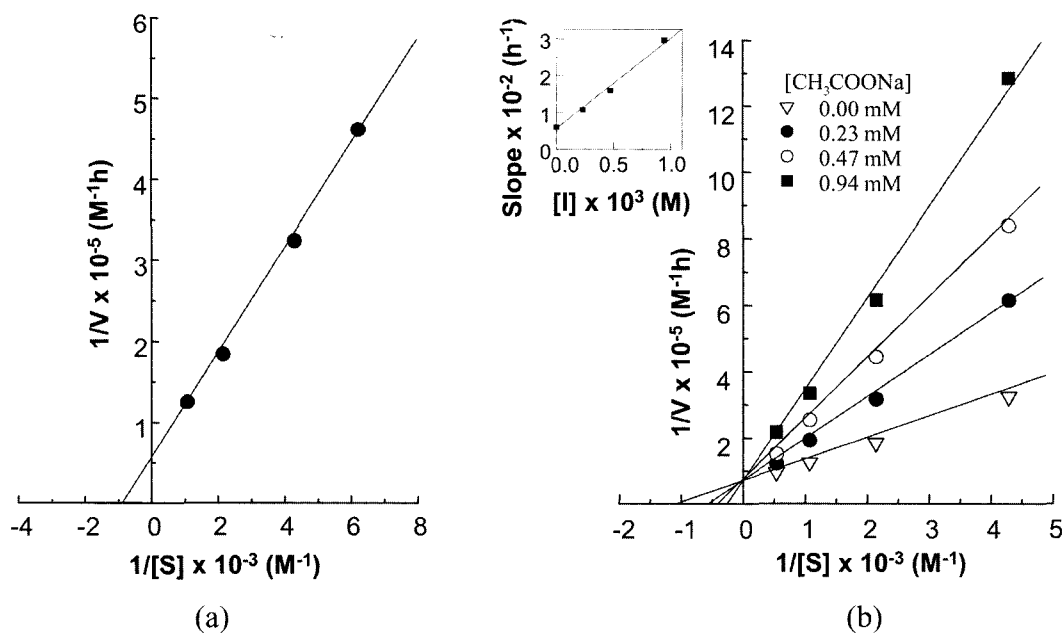
obtained from eq. (1), which was 1.39 h<sup>-1</sup>. This value was about 1500 times larger than that of the uncatalyzed reaction ( $9.12 \times 10^{-4}$  h<sup>-1</sup>).

$$k_{cat} = V_{max} / [E]_0 \quad (1)$$

A competitive inhibition (Figure 1b) was found in the presence of acetate ions ( $K_i = 2.69 \times 10^{-4}$  M). The noncompetitive inhibition was also observed by addition of K<sub>2</sub>HPO<sub>4</sub> ( $K_i = 2.38 \times 10^{-4}$  M). Both inhibitions seemed to occur because the *vic-cis*-diols were blocked by formation of the hydrogen bonds with the inhibitors. The acetate ions seemed to compete with the substrate, to form hydrogen bonds with the polymer catalyst. The phosphate ion, as a dianion, could form a stronger hydrogen bond with *vic-cis*-diols of the polymer catalyst than the substrate, leading to a noncompetitive inhibition.

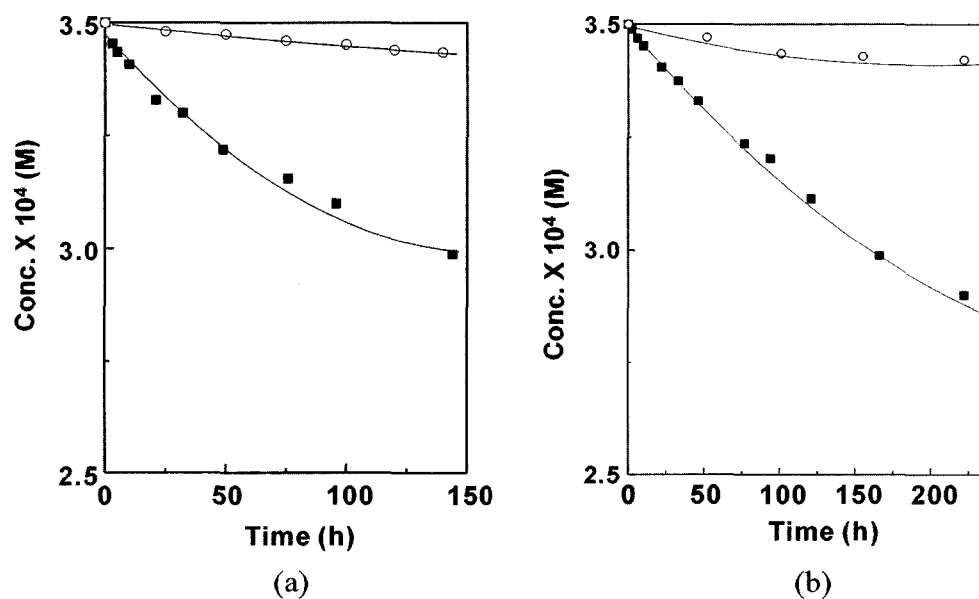
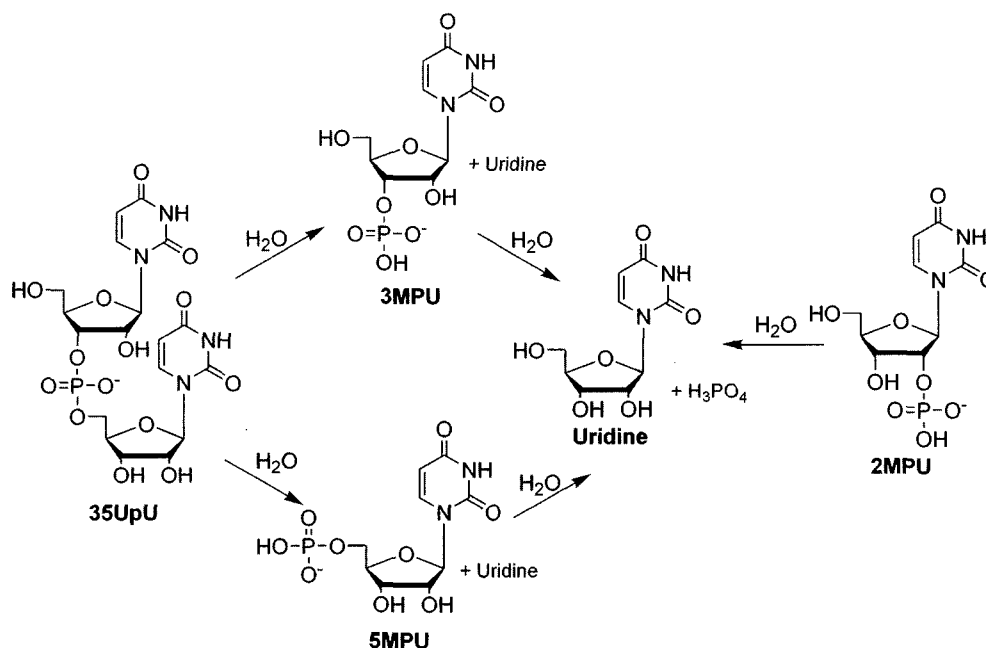
**RF** also catalyzed the hydrolysis reactions of uridylyl (3' → 5')uridine ammonium salt (**35UpU**), uridine 2'-monophosphate lithium salt (**2MPU**), uridine 3'-monophosphate sodium salt (**3MPU**), and uridine 5'-monophosphate (**5MPU**). The hydrolysis rates of the reactions in Scheme II were measured at pH 7.4 (Tris buffer), ionic strength of 0.02 (KCl), and 50 °C by HPLC in the presence of **RF**.

The time-dependent concentration changes of the substrates are shown in Figure 2. As the reactions were pseudo-first order, the rate constants for the hydrolysis were obtained by plotting logarithmic concentrations of the substrates against time and summarized in Table I. **RF** cata-



**Figure 1.** Reciprocals of the initial rates as a function of the reciprocals of the substrate concentrations ( $1/v$  vs.  $1/[S]$ ): (a) in the presence of **RF** and (b) in the presence of **RF** at different concentrations of the inhibitor, sodium acetate.  $K_i$  determination is shown in the upper left plot.  $[RF] = 1.62 \times 10^{-5}$  M at pH = 7.4 (Tris buffer), 50 °C, and  $\mu = 0.02$  (KCl).

## Scheme II



**Figure 2.** Concentration changes vs. time: (a) for the hydrolysis of 35UpU in Tris buffer solution (○) and in the presence of RF (■); (b) the hydrolysis of 2MPU in Tris buffer solution (○) and in the presence of RF (■).

lyzed the hydrolysis reaction of 35UpU with a rate acceleration of 11 compared with the uncatalyzed reaction.

The catalytic activities of RF for the reactions of aromatic phosphates were investigated (Scheme III). The reactions were carried out in Tris-buffer (pH 7.4) at 25 °C and ionic strength of 0.02 (KCl). The starting concentrations of the substrates and the polymer were  $6.0 \times 10^{-4}$  and  $2.14 \times 10^{-5}$  M,

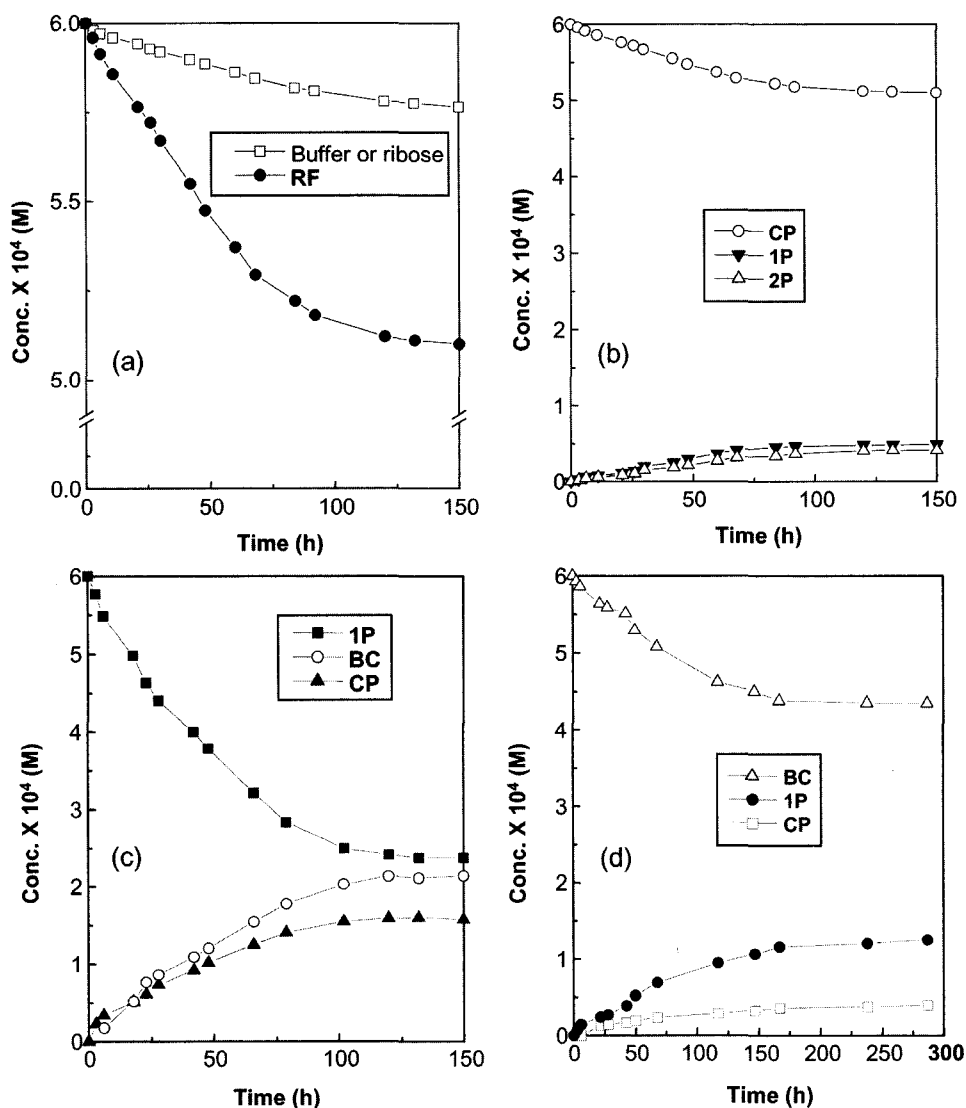
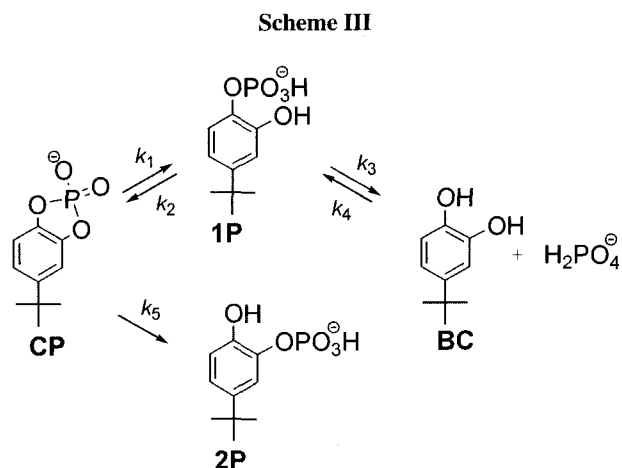
respectively. The concentrations of reactants and products were analyzed by liquid chromatography with a UV detector at 285 nm. Under the reaction conditions, the reaction rates were moderate to be measured and no significant interference of the polymer in LC chromatograms was observed.

The hydrolysis rates of 4-*tert*-butylcatechol cyclic phosphate *N*-methyl pyridinium (CP) were measured in the buffer

**Table I. Hydrolysis Rate Constants of Aliphatic Phosphate Substrates in the Presence or Absence of RF**

	Rate Constant ( $\text{h}^{-1}$ )			
	35UpU	2MPU	3MPU	5MPU
$k_{cat}$	$1.51 \times 10^{-3}$	$8.72 \times 10^{-4}$	$1.64 \times 10^{-3}$	$2.39 \times 10^{-3}$
$k_{blank}$	$1.35 \times 10^{-4}$	$2.26 \times 10^{-5}$	$1.12 \times 10^{-4}$	$2.57 \times 10^{-4}$
$k_{cat}/k_{blank}$	11.2	38.56	14.6	9.3

alone and in the presence of ribose or **RF**. The polymer showed the catalytic activity for the hydrolysis of **CP**, while ribose did not accelerate the reaction. The time-dependent concentration changes of **CP** in the buffer solution alone and in the presence of **RF** or ribose are shown in Figure 3a.



**Figure 3.** Concentration changes vs time: (a) for the hydrolysis of cyclic phosphate (**CP**) in Tris buffer solution (□), in the presence of ribose (○), and **RF** (●); (b) for the reaction of **CP** (○) → **1P** (▼) + **2P** (△) catalyzed by **RF**; (c) for the reaction of **1P** (■) → **CP** (▲) + **BC** (○) catalyzed by **RF**; (d) for the reaction of **BC** (△) +  $\text{H}_2\text{PO}_4^-$  → **1P** (●) → **CP** (□) catalyzed by **RF**.

As the reactions were pseudo-first order, the rate constants for the hydrolysis were obtained by plotting logarithmic concentrations of **CP** against time and found to be  $0.32 \times 10^{-3} \text{ h}^{-1}$  for the uncatalyzed reaction and  $1.51 \times 10^{-3} \text{ h}^{-1}$  in the presence of **RF**, which is nearly 5 times larger than the former. The hydrolysis products of **CP** were **1P** and **2P** as others reported.<sup>7</sup> During the hydrolysis, the concentration of **CP** decreased while **1P** and **2P** simultaneously formed (Figure 3b). After 120 h the reaction reached equilibrium.

When **1P** was mixed with **RF** at pH 7.4, its cyclization to **CP** and hydrolysis to **BC** occurred simultaneously. **1P** was found to be stable in the buffer solution under the same conditions. The concentration changes of the products and the reactant during the cyclization and hydrolysis of **1P** in the presence of **RF** are shown in Figure 3c. The reaction reached equilibrium after 120 h.

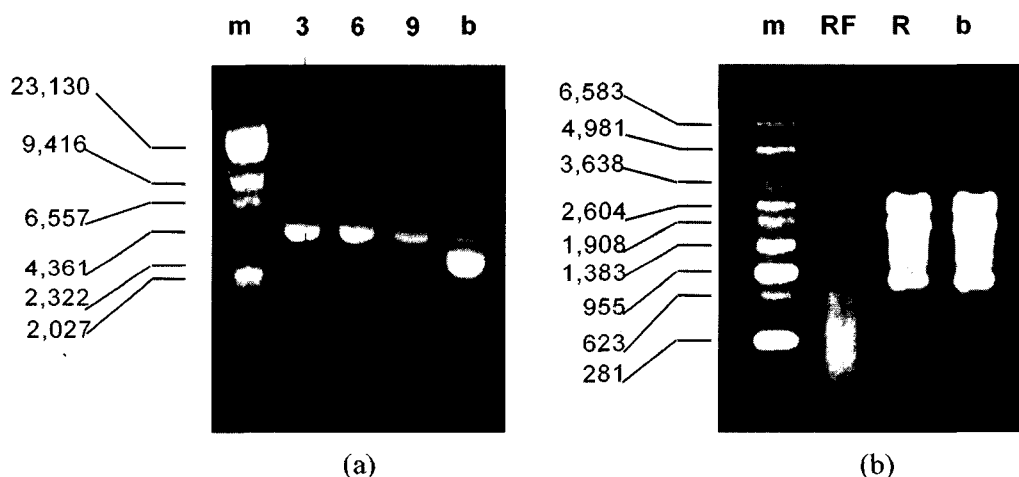
When **RF** ( $2.14 \times 10^{-5} \text{ M}$ ), **BC** ( $6 \times 10^{-4} \text{ M}$ ), and  $\text{KH}_2\text{PO}_4$  ( $6 \times 10^{-3} \text{ M}$ ) were mixed at pH 7.4 (Tris buffer), esterification to **1P** occurred, which was followed by the cyclization to **CP**. In order to lead this reaction to the first order, phosphate was added in excess. This reaction did not occur in the buffer solution alone. The concentration changes vs. time of the reaction are plotted in Figure 3d. At the beginning of the reaction, **1P** formed and thereafter **CP**, and the reaction reached equilibriums after 160 h. The rate constants for the reactions, obtained by plotting logarithmic concentrations against time, were  $k_1 = 0.84$ ,  $k_2 = 3.59$ ,  $k_3 = 5.02$ ,  $k_4 = 2.08$ , and  $k_5 = 0.67 \times 10^{-3} \text{ h}^{-1}$ . The catalytic activities of **RF** for the hydrolysis of phosphodiester to monoester and of phosphomonoester to phosphate, and the esterification of phosphomonoester to diester and of alcohol to phosphomonoester, correspond to those of nuclease, phosphatase, ligase,

and phosphorylase, respectively.

**Catalysis for the Hydrolysis of Nucleic Acids.** Supercoiled ds DNA (pBR 322) and RNA (BMV) were incubated in a Tris buffer solution and in the presence of ribose or **RF** at pH 7.4, 37°C and visualized by EtBr-agarose gel electrophoresis. The electrophoresis diagrams clearly showed that **RF** catalyzed the cleavage of DNA and RNA (Figure 4). In 3 h, a trace of the closed circular supercoiled DNA band still appeared, along with the open circled DNA band of high intensity, but completely disappeared in 6 h. The latter band was fragmented by further reaction for 9 h, showing a smearing tail. No cleavage was observed in a buffer solution alone after 9 h under the same conditions. RNA was hydrolyzed completely to fragments in 8 h in the presence of **RF**, while no cleavage was observed in the buffer solution and in the presence of ribose. As RNA, highly susceptible to hydrolysis, was stable in the presence of ribose with the same mole concentration as the ribose residue concentration of polymer **RF**, obviously a long chain of the polymer was essential for the catalytic activity.

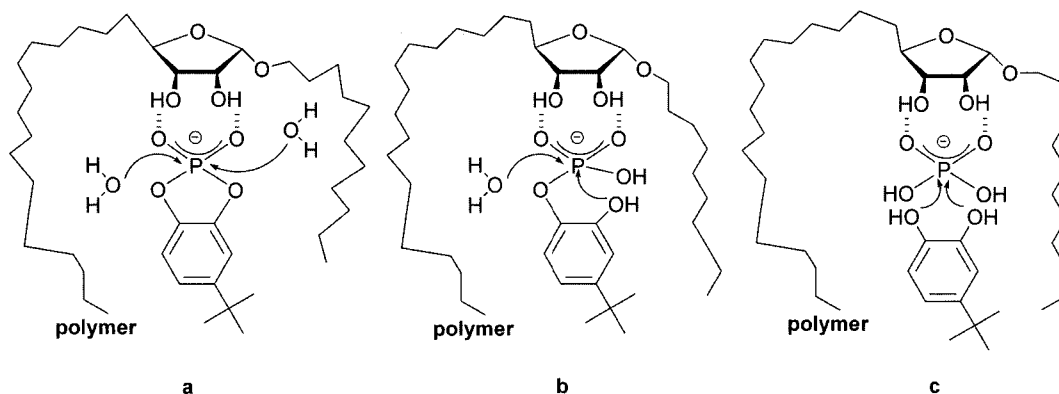
We investigated the hydrolysis sites by using 2'-deoxyadenylyl (3'→5')-2'-deoxyadenosine (dApdA). dApdA was incubated in the presence of **RF** at pH 7.4, 37°C for 24 h and the reaction mixture was analyzed by HPLC. 73% of dApdA was hydrolyzed to give 2'-deoxyadenosine-5'-phosphate (pdA), 2'-deoxyadenosine-3'-phosphate (dAp), and 2-deoxyadenosine (dA). The mole ratio of pdA:dAp:dA was found to be 1:1:2.5. Parts of pdA and dAp were hydrolyzed to dA and phosphate. The peaks were identified by the authentic samples.

**Mechanism of Catalysis.** Based on the experimental results described above and on our previous reports,<sup>8-10</sup> the



**Figure 4.** Electrophoresis diagrams of the reaction mixtures on the agarose gel: Nucleic acids were incubated in the presence of **RF** at 37°C, pH = 7.4 (Tris-buffer),  $\mu = 0.02$  (KCl),  $[\text{RF}] = 2.82 \times 10^{-8} \text{ M}$ , and  $[\text{nucleic acid}] = 50 \text{ mg/L}$ . (a) Supercoiled ds DNA (pBR); lanes m: marker, lanes 3, 6, 9: after 3, 6, and 9 h incubation time in the presence of **RF**, and lane b: in the buffer solution for 9 h. (b) RNA (BMV); lane m: marker, lanes RF and R: incubation for 8 h in the presence of **RF** and ribose, respectively, and lane b: in the buffer solution for 8 h.  $[\text{ribose}] = 8.2 \times 10^{-7} \text{ M}$ .

Scheme IV



mechanism of catalysis can be postulated as follows: since no activity was found for ribose, the polymer backbone was likely to form an active site to hold a substrate therein by chain folding. It seems possible that *vic-cis*-diol groups located inside the active site, where the phosphate substrate was also accommodated. The *vic-cis*-diol groups formed hydrogen bonds with the two oxygen atoms of the phosphate so as to activate the phosphorous atoms to be attacked by nucleophiles. The formation of strong hydrogen bonds was born out by a theoretical study of the interaction between 3,4-dihydroxytetrahydrofuran and  $\text{H}_2\text{PO}_4^-$ .<sup>14</sup>

Scheme IV shows the possible action mechanism for the reaction of aromatic phosphates. Water can attack either side of the activated phosphate to form **1P** or **2P** in the hydrolysis of **CP** (Scheme IVa). The hydrolysis and esterification of **1P** (Scheme IVb) occur when water or the 2-OH group of **1P** attack the phosphorus atom and either break the P-O ester bond or cause the OH group of the phosphate to leave, respectively. The phosphorylation of **BC** (Scheme IVc) occurs by nucleophilic attack of 1-OH or 2-OH of **BC** at the activated phosphorus atom with OH of the phosphate as a leaving group. We examined the possibility of the reactions on the hydroxyl groups of the ribose ring. The polymers used for the reactions were separated by dialysis through a cellulose membrane of a molecular weight cut off of 1000 and were subjected to  $^{31}\text{P}$  NMR analysis. However, P atoms were not detected on the polymers, indicating that the reactions on the hydroxyl groups did not occur.

In the case of hydrolysis of nucleic acids, **RF** polymer seems to form complexes with nucleic acids by hydrogen bonds between hydroxyl groups of the polymer either with phosphate ions or with bases of the nucleic acids in the grooves. Hydrophobic interactions between the polymer backbone and the nucleic acid bases would also contribute to the binding. Once part of the polymer chain was bound to the nucleic acids, free *vic-cis*-diol groups of RF would interact with the two phosphoryl oxygen atoms of nucleic acids to initiate the catalysis.

## Conclusions

It is interesting that the polysaccharide shows nuclease and phosphatase activities. The polysaccharide, (1→5)- $\alpha$ -D-ribofuranan, has not been found in nature so far, but there are plenty of biopolymers containing riboses in the biological system. One of the typical biopolymers containing riboses with *vic-cis*-diols is poly(ADP-ribose) formed from  $\text{NAD}^+$  in chromatin. It was discovered in 1966,<sup>15,16</sup> however, its functions are not yet clear, although it has been suggested to be involved in numerous biological reactions,<sup>17</sup> and especially to have catalytic functions.<sup>18</sup> Poly(ADP-ribose) forms during apoptosis<sup>19</sup> and DNA repair,<sup>20</sup> where nuclease will be required. We hope that this report will contribute to the elucidation of its functions and further investigations are in progress along these lines.

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