Pergamon

Tetrahedron Letters 43 (2002) 5597–5600

TETRAHEDRON LETTERS

The catalytic activity of ribose-containing polymers for the hydrolysis of phosphodiester and the cleavage of nucleic acid

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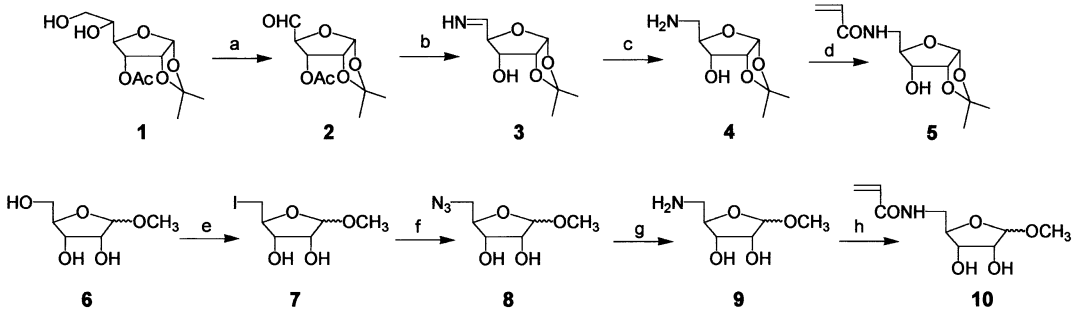
Received 10 June 2002; accepted 12 June 2002

Abstract—In order to investigate the catalytic activity for the hydrolysis of phosphodiester and the cleavage of nucleic acids, ribose-containing polymers **11**, **12** and **13** were synthesized. While polymer **11** showed no catalytic activity, polymers **12** and **13** catalyzed the cleavage of nucleic acids and the hydrolysis of a phosphodiester substrate. The catalytic activity was attributable to the *vic-cis* diols of riboses on polymer chains, which formed hydrogen bonds with two phosphoryl oxygen atoms of phosphates so as to activate the phosphorous atoms to be attacked by nucleophiles (H_2O).

In the biological system there are plenty of biopolymers containing riboses. One of the typical biopolymers containing riboses with *vic-cis*-diols is poly (ADP-ribose) formed from NAD^+ in chromatin. Since its discovery in 1966,[1] the polymer has been suggested to be involved in numerous biological reactions,[2] although its functions are not clear so far. Poly (ADP-ribose) forms during apoptosis[3] and DNA repair,[4] where nuclease will be required. Recently we reported that the alternating copolymers of ribose derivatives and maleic anhydride catalyzed cleavage of ss oligonucleotides.[5,6] The polymers, however, failed to cleave ds DNA of high molecular weights, which may be in part due to the stiffness of the polymer backbones, preventing them from binding DNA effectively. Herein, we described the synthesis for the flexible vinyl polymers containing riboses and the cleavage of a dinucleotide, ds DNA, and RNA as well as the hydrolysis of a phosphodiester substrate.

Synthesis of monomer **5** was accomplished in four steps from 3′-*O*-acetyl-1′,2′-*O*-isopropylidenene-5′,6′-diol-α-D-allofuranose (**1**), which was synthesized by deprotection of 5′,6′-acetonide groups of 1′,2′:5′,6′-di-*O*-isopropylidene-α-D-allofuranose according to the lit-



Scheme 1. Synthesis of monomer **5** and **10**. *Reagents and conditions*: (a) $NaIO_4$, silica gel, 25°C, 40 min; (b) NH_3 in MeOH, 25°C, 24 h; (c) Pd/C, 4 atm H_2, 25°C, 48 h; (d) acryloyl chloride, 25°C, 2 h; (e) PPh_3, I_2, Py., 25°C, 24 h; (f) NaN_3, 110°C, 24 h; (g) PPh_3, H_2O, 25°C, 24 h; (h) acryloyl chloride, 0°C, 11 h.

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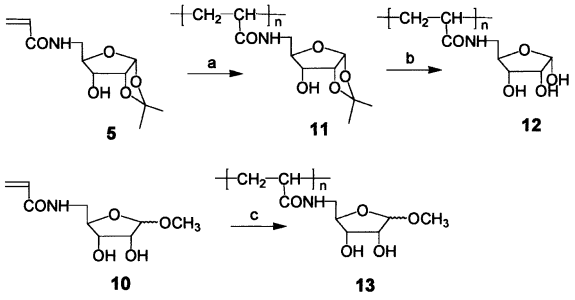
0040-4039/02/$ - see front matter
PII: S0040-4039(02)01123-1

erature (Scheme 1).[7] Oxidation of **1** with the aid of $NaIO_4$ in the suspension of silica gel in CH_2Cl_2 afforded (97.5%) **2**, which was confirmed by the appearance of aldehyde carbonyl peak at 197.3 ppm in the ^{13}C NMR spectrum. Reaction of **2** with ammonia saturated in MeOH at 25°C afforded (95.7%) **3**, whose structure was supported by the appearance of the peak for NH=*C*H at 139.8 ppm in the ^{13}C NMR spectrum. Hydrogenation of the imine moiety of **3** with the aid of Pd on charcoal in MeOH (25°C, 24 h, 60 psi H_2) yielded the corresponding amine **4** (70.6%), identified by the chemical shift change of the peak for *C* from 139.8 to 49.1 ppm. Reaction of **4** with acryloyl chloride in anhydrous THF at 25°C gave the corresponding monomer **5** (54.3%), supported by the appearance of signals for the acrylic amide carbon (*C*ONH) at 165.1 ppm and double bond carbon (CH_2=*C*H) at 125.9 and 131.2 (CH_2=*C*H) ppm.

Monomer **10** was synthesized with starting from 1′-*O*-methyl-D-riobse (**6**) (Scheme 1), which was obtained by methylation of α-D-ribose according to the literature.[8] Reaction of **6** in a dioxane solution containing PPh_3, I_2 and pyridine yielded (60.9%) 5′-deoxy-5′-iodo-1′-*O*-methyl-D-ribose (**7**), which was reacted with sodium azide in DMF at 60°C to give (97.8%) azido moiety **8**. The structure of **8** was supported by the signal of the carbon next to azide (CH_2N_3) at 51.1 ppm and the appearance of the stretching band at 2119 cm^{-1} in the IR spectrum. Reaction of **8** with PPh_3 in THF/H_2O afforded (64.4%) the corresponding amine **9**, identified by the chemical shift change of the peak for *C* from 51.1 to 39.5 ppm. Monomer **10** was obtained (47.1%) by the reaction of **9** with acryloyl chloride in THF and the structure was confirmed by the appearance of new peaks for acrylamido groups at 124.7 ppm (CH_2=*C*H), 130.4 ppm (CH_2=*C*H), and 164.9 ppm (*C*=O) in the ^{13}C NMR spectrum. The elemental analysis results were satisfactory for all the compounds.

The polymerization of monomers **5** (2.47 mM) and **10** (1.15 mM) was carried out in water with the initiator $K_2S_2O_8$ (2 mol%) at 80°C to yield poly(5′-acrylamido-5′-deoxy-1′,2′-*O*-isopropylidene-α-D-ribose) (**11**) (83.7%) and poly(5′-acrylamido-5′-deoxy-1′-*O*-methyl-D-ribose) (**13**) (68.1%), respectively. The polymers were isolated by precipitation in acetone. The structures of polymers **11** and **13** were identified by ^1H NMR spectroscopy. The acryl amide proton signals at 6.3–6.8 ppm completely disappeared and a broad peak at 2.0–2.6 ppm of ethylene groups on the polymer chain showed up. Polymer **11** was hydrolyzed by stirring for 24 h at 25°C in 1N HCl solution (Scheme 2). After neutralization with a dilute NaOH solution, poly(5′-acrylamido-5′-deoxy-α-D-ribose) (**12**) was isolated (64.6%) by dialysis through a cellulose membrane of molecular weight cut off of 1000 and then by freeze-drying. After hydrolysis, acetonide proton signals at $\delta = 1.34$ and 1.59 ppm disappeared in the ^1H NMR spectrum of polymer **12**. The polymerization data are summarized in Table 1.

The extent of active center formation will depend on the polymer chain length, i.e. molecular weight. To obtain the polymers having different molecular weights with narrow distribution, polymer **12** was successively dialyzed through a series of cellulose membranes of molecular weight cut off of 3500, 8000, 15 000, and 25 000. The four filtrates obtained by dialyzing through



Scheme 2. Synthesis of polymers **11**, **12** and **13**. *Reagents and conditions*: (a) $K_2S_2O_8$, H_2O, 10 h, 80°C; (b) HCl, 18 h; (c) $K_2S_2O_8$, H_2O, 15 h, 70°C.

Table 1. Polymerization data

Polym. no.	Monomer (mmol/L)	$K_2S_2O_8$ (μmol/L)	Yield (%)	Mn[a]	PD[b]
11	**5** (2.47)	49.4	83.7	19 300	1.55
12	–	–	64.6	18 000	1.67
13	**10** (1.15)	23.0	68.1	18 500	1.53

[a] Number-average molecular weights of the polymers were measured by GPC (Waters Co.) on Ultrahydrogel 250 column with poly(ethylene glycol) standards (eluent: aqueous 0.1N $NaNO_3$ solution).

[b] Polydispersity.

the series of the membranes and the polymer solution remained in the last tube were freeze-dried to give five polymer portions having different number-average molecular weights of 2500, 6400, 9200, 17 600, and 27 100, respectively. We have employed ethyl *p*-nitrophenyl phosphate (ENPP), as a phosphodiester substrate model, to evaluate the catalytic activity of the polymers. Hydrolysis rates were determined by the time dependent release of *p*-nitrophenol ($\varepsilon_{400nm} = 10\,268$) in Tris-buffer (pH 7.4, ionic strength = 0.02, KCl) at 50°C. The hydrolysis of phosphate substrates and nucleic acids was often catalyzed by metal ions with various ligands. To exclude this possibility, we used the deionized water (resistivity >18 MΩ cm^{-1}) and crystallized Tris-buffer materials. The initial hydrolysis rates of the substrate (ENPP) in the presence of polymer **12** were measured[6] and plotted against the number-average molecular weights of the polymers (Fig. 1). The initial hydrolysis rate was jumped above Mn of 17 800 and therefore we used the polymer with Mn of 17 800 as a catalyst for the hydrolysis of natural nucleic acids.

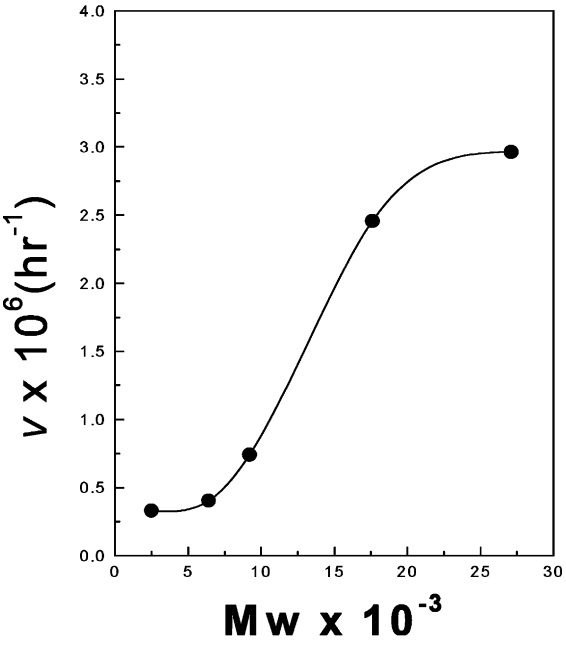


Figure 1. The initial rates as a function of number-average molecular weights at pH 7.4 (Tris-buffer), 50°C, $\mu = 0.02$ (KCl). [substrate] = 1.87×10^{-3} M, [polymer **12**]† = 5.26×10^{-6} M.

The dinucleotide, 2′-deoxy-adenylyl (3′→5′)-2′-deoxyadenosine (dApdA), was incubated in the presence of **12** at pH 7.4 (Tris-buffer), $\mu = 0.02$ (KCl), and 37°C for 24 h and the reaction mixture was investigated by HPLC (Fig. 2). 92% of dApdA was hydrolyzed to give 2′-deoxyadenosine (dA), 2′-deoxy-adenosine-3′-phosphate (dAp), and 2′-deoxyadenosine-5′-phosphate (pdA). The mole ratio of dA to dAp to pdA was found to be 5:4:1.

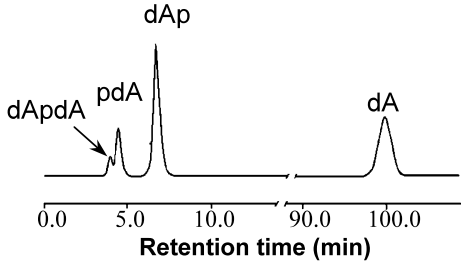


Figure 2. Liquid chromatogram of the hydrolysis products of dinucleotide (dApdA). Conditions: C18 μ-Bondapak reverse phase column, UV detector $\lambda = 254$nm, eluent; 0.25 M pH 7.4 phosphate buffer, flow rate; 0.8 mL/min, [dApdA] = 4×10^{-4} M, [polymer]† = 9.6×10^{-6} M.

Supercoiled ds DNA (pBR 322 DNA Plasmid) and RNA (BMV RNA) were incubated at 37°C in the presence of polymers **11** and **12** at pH 7.4 (Tris-buffer) as well as in a buffer solution alone for 6 h, and visualized by EtBr-agarose gel electrophoresis. The cleavage of pBR 322 DNA Plasmid in the presence of polymer **12** (lane 1) is clearly shown in Fig. 3A. The circular supercoiled DNA (form I) was converted to circular relaxed DNA (form II) via single-strand cleavage. Fig. 3B shows that BMV RNA was cleaved in the presence of polymer **12** to yield lower base pair segments (lane 1). Only partial

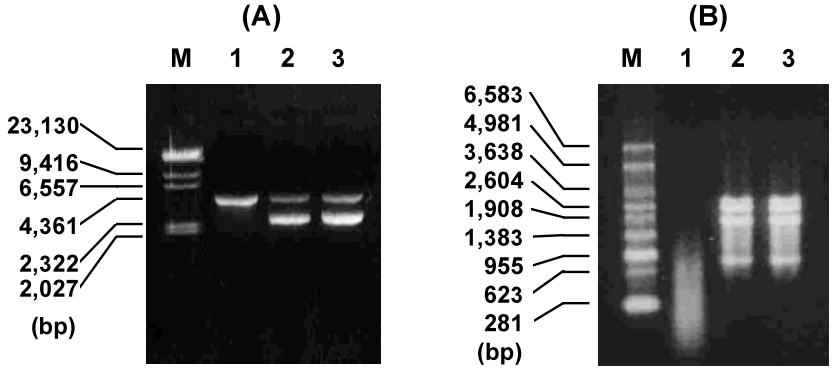


Figure 3. Electrophoresis diagram of the reaction mixtures on the agarose gel: (A) supercoiled ds DNA (pBR 322) and (B) RNA (BMV); lane 1: in the presence of polymer **12**, lane 2: in the presence of polymer **11**, and lane 3: in buffer solution. [polymer]† = 2.63×10^{-7} M, [nucleic acid] = 50 mg/L.

† Concentration of repeating unit.

cleavage was observed in a buffer solution alone (lane 3) and in the presence of polymer **11** (lane 2) under the same conditions.

In conclusion, we showed that the flexible vinyl polymers containing *vic*-*cis* diols of riboses catalyzed the cleavage of a dinucleotide and nucleic acids (DNA and RNA) as well as the hydrolysis of phosphodiester substrate. This catalytic activity was attributable to the *vic*-*cis* diols of ribose on polymer chains, which formed hydrogen bonds with two phosphoryl oxygen atoms of phosphates so as to activate the phosphorous atoms to be attacked by nucleophiles (H_2O).[9]

Acknowledgements

This work was supported by a grant from the Ministry of Education and Korean Science and Engineering Foundation.

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