Synthetic ribofuranose-containing polymers show catalytic activity in the hydrolysis of phosphodiesters

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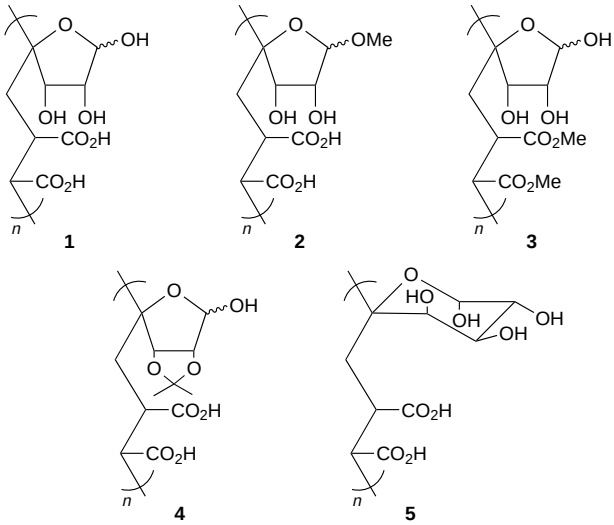
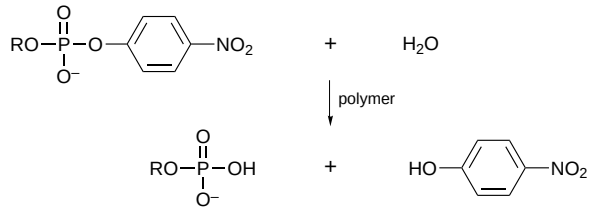
Synthetic polymers containing ribofuranose rings as pendent groups catalyse the cleavage of DNA and the hydrolysis of phosphodiesters with a rate acceleration of 10^3 compared with that of the uncatalysed reactions.

In the past three decades there has been great interest in the synthesis of enzyme-like polymers.[1,2] A few synthetic polymers have been found to show catalytic activity; for example, imidazole-containing polymers are reported to catalyse the hydrolysis of phenyl esters.[3,4] While investigating polynucleotide analogues,[5–8] we have found that synthetic ribofuranose-containing polymers show catalytic activity for the hydrolysis of phosphodiesters and the cleavage of oligodeoxyribonucleotides. This is the first example of synthetic polymer catalysis of phosphodiester hydrolysis.

The catalytic activity seemed to be caused by the hydroxy groups on the furanose rings of the polymers. In order to elucidate the structural requirements for the catalytic activity, five polymers were synthesized.† Polymers **1**–**4** contained ribofuranose derivatives, while polymer **5** had pyranose rings in their polymer chains. The number-average molecular weights of polymers **1**–**5** were measured to be 8.4, 13.0, 9.3, 9.5 and 17.0 $\times$ 10^3 by gel permeation chromatography, respectively. The *vic*-OH groups at C-2′ and C-3′ on the furanose rings of **1**–**3** were in the *cis*-configuration, while the OH groups at C-1′, C-2′, C-3′ and C-4′ on the pyranose ring of **5** were in either the *aeea* or *eaae* conformations. Approximately 30% of the 1′-OH groups of **1** and **3** were in the *cis*-configuration with respect to 2′-OH on the same furanose rings.‡





Hydrolysis rates of ethyl *p*-nitrophenyl phosphate (substrate) were measured in Tris buffers (pH = 7.4, ionic strength = 0.02, KCl) *via* the UV absorption (400 nm) of the *p*-nitrophenol evolved. To check whether any side reaction other than the hydrolysis of the substrate occurred (Scheme 1), the reaction mixtures in the presence of **1** or **3** ([substrate] = 4.67×10^{-4} m, [**1**] = [**3**] = 1.62×10^{-5} m, pH = 7.4 at 50 °C, 24 h) were examined by ^{31}P NMR. In both cases only two peaks, at δ 2.69 for ethyl phosphate and δ −5.97 for the substrate (relative to phosphoric acid at δ 0), were observed. The same result was also obtained by liquid chromatography analysis, indicating that the hydrolysis occurred exclusively.

By measuring the hydrolysis rates in the buffer solution alone and in the presence of the monomers (succinic acid and ribofuranose) and the polymers **1**–**5** at 50 °C, it was found that polymers **4** and **5** and the monomers showed no acceleration of the hydrolysis, while **1**, **2** and **3** with *vic*-*cis*-diols on the furanose rings exhibited catalytic activity. These results suggested that the *vic*-*cis*-diols of furanose rings were responsible for the catalytic activity. The *vic*-dicarboxyl groups of the polymers were of little importance for the catalytic activity, as seen in the results of the reactions of **3**, **4** and **5**.

In the hydrolysis, the measured rate (v_m) is the sum of the rate of the catalysed reaction (v_c) and the uncatalysed reaction (v_b) [eqn. (1)]. The value of v_c was therefore obtained from eqn. (2).

$$v_m = v_c + v_b \quad (1)$$

$$v_c = v_m - v_b \quad (2)$$

The initial v_c values were obtained at constant concentrations of **1**, **2** and **3** by changing the substrate concentrations. Michaelis–Menten kinetics for **1**, **2** and **3** were confirmed by performing the double reciprocal plot of Lineweaver and Burk $[v]^{-1}$ (*vs.* $[\text{substrate}]^{-1}$) (Fig. 1), which gave K_m and V_{max}. The k_{cat} was obtained from eqn. (3). We assumed that each polymer

$$k_{cat} = V_{max}/[E]_o \quad (3)$$

chain formed one active site, since no significant catalytic activity for the polymers with *M*n's lower than 4000 (PD = 4) was observed. The k_{cat} values were found to be 4.22×10^{-1} h^{-1} for polymer **1**, 1.65×10^{-1} h^{-1} for **2**, and 9.04×10^{-1} h^{-1} for **3**. The k_{cat} of **3** was about 10^3 higher than that of the uncatalysed reaction (9.12×10^{-4} h^{-1}). Polymers **1** and **3** showed higher catalytic activities than **2**, probably because the former polymers contained *ca*. 30% more *vic*-*cis*-diol groups than the latter.

Noncompetitive inhibition was observed by addition of phenylboronic acid (K_I = 3.33×10^{-4} m) and K_2HPO_4 (K_I = 2.38×10^{-4} m). Competitive inhibition was found in the presence of acetate ions (K_I = 6.02×10^{-4} m). Both forms of inhibition seemed to occur because the *vic*-*cis*-diols were blocked by formation of hydrogen bonds with the inhibitors.

ssDNA of 30 bases was incubated in a buffer solution in the presence of the polymers, and developed on acrylamide gel

(Fig. 2). The extent of ssDNA cleavage was observed in the order of polymer **3** > polymer **1** > polymer **2**, which was coincident with the k_{cat} of the polymers. Polymers **1**, **2** and **3** containing *vic*-*cis*-diol groups of the furanose ring showed clear DNase activity, while no cleavage was observed in the buffer solution or in the presence of **4** or **5**.

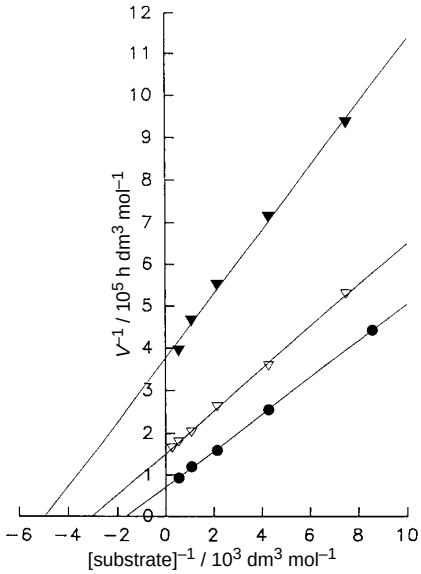


Fig. 1 The reciprocals of the initial rates as a function of the reciprocals of the substrate concentrations for polymers (▽) **1**, (▼) **2** and (●) **3**: [polymer] = 1.62×10^{-5} m in Tris buffer at pH = 7.4, 50 °C, ionic strength = 0.02 (KCl)

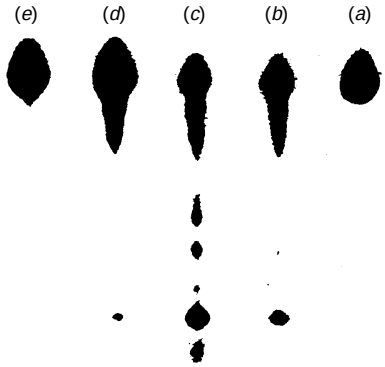


Fig. 2 Autoradiogram of 7 m urea–8% acrylamide gel electrophoretic analysis of the reaction mixtures. ^{32}P-Labelled ssDNA of 30 bases, d(CATGGCAAAGCCAGTATACAAATTGTAATA), corresponding to the human foamy virus proviral DNA in position nt. 3634-3611 (ref. 9), was incubated at 37 °C, ionic strength = 0.02 (KCl) and pH = 7.4 (Tris buffer), for 12 h (*a*) with no additive, in the presence of (*b*) polymer **1**, (*c*) polymer **3**, (*d*) polymer **2** and (*e*) polymer **4**. [DNA] = 7.5×10^{-4} m, [polymer] = 7.3×10^{-5} m. No DNA cleavage was observed in the buffer or in the presence of polymer **4**, while polymers **1**, **2** and **3** catalysed hydrolysis of DNA.

Since no activity was found for the monomer pair, the polymers are likely to form the active sites with a specific conformation. It seems possible that the furanose rings having *vic*-*cis*-diol groups were located inside the active sites, where the phosphodiester substrates were also accommodated. The *vic*-*cis*-diol groups seem to form hydrogen bonds with the two oxygen atoms of the phosphate so as to activate the phosphorus atoms attacked by nucleophiles (H_2O). Further investigation of the mechanism is in progress.

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Footnotes

† Copolymerization of 1-*O*-acetyl-5-deoxy-2,3-isopropylidene-d-*erythro*-pent-4-enofuranose with maleic anhydride (molar ratio 1 : 2) in bulk at 90 °C for 13 h in the presence of AIBN (2 mol%) resulted in the alternating copolymer, which was hydrolysed to give **1** using aqueous HCl–dioxane at 97 °C for 24 h and to give **4** using 0.1 m NaOH at room temperature for 24 h. Polymer **1** was esterified with CH_2N_2 to give **3**. Polymer **2** was obtained by copolymerization of 1-*O*-methyl-5-deoxy-2,3,-*O*-isopropylidene-d-*erythro*-pent-4-enofuranose with maleic anhydride under the same condition as described above and subsequent hydrolysis of the polymer using formic acid at 107 °C for 13 h. Polymer **5** was synthesized by copolymerization of 6-deoxy-1,2 : 3,4-di-*O*-isopropylidene-α-d-*galacto*-hex-5-enopyranose with maleic anhydride under the same conditions described above and subsequent hydrolysis in 68% formic acid at 100 °C for 13 h.

‡ To determine the configuration of the C-1 atoms of the monomers, 1,2,3-tri-*O*-acetyl-5-*O*-trityl-d-ribofuranose was prepared by tritylation and subsequent acetylation of d-ribose, from which the monomers were also prepared. The configuration of the C-1 atom remained intact during polymerization, and the protection and deprotection reactions of the C-1 hydroxy group. In the α form, the C-1 proton is *cis* with respect to the C-2 proton, while in the β form, the C-1 proton is *trans* with respect to the C-2 proton. In the ^{1}H NMR spectrum of 1,2,3-tri-*O*-acetyl-5-*O*-trityl-d-ribofuranose in $CDCl_3$, two peaks appeared at δ 6.54 (d, J = 4 Hz) and 6.22 (s) from the C-1 protons of the α and β forms. The singlet peak at δ 6.22 and the doublet peak at δ 6.54 were assigned to the proton of β form and the proton of α form, respectively, since the angle between the C-1 proton of the α form and the C-2 proton was nearly zero and thus a larger coupling constant was expected. The ratio of the α and β forms was calculated from the peak area ratio.

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