Communications to the Editor

Synthesis of Nucleic Acid Analogues by Alternating Cyclocopolymerization

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There has been considerable interest in the synthesis of polynucleotide analogues to utilize them as model compounds for the natural polymers and their biological activities in polymeric drugs for chemotherapy. Although numerous attempts have been made,[1−3] it is still desirable to synthesize the polynucleotide analogues, whose structures and physicochemical properties have a very close resemblance to those of the natural polymers. We prepared several nucleoside derivatives as monomers (**1** and **5**). To obtain distances between bases of the polymers similar to those in nucleic acids, we copolymerized the monomers with acrylic anhydride. The polymerization proceeded by an alternating cyclocopolymerization (Scheme 1), a newly-attempted radical copolymerization method. Among the synthesized polymers, **4T** is analogous to DNA whereas **7A**, **7U**, and **7H** are analogous to RNA due to the absence or presence of the hydroxy groups on the 2-position of the furanose rings. We report here on the alternating cyclocopolymerization and complex formation of the synthesized polymers with the natural polymers of complementary bases.

(*R*)-(−)-2-Uracil (or thymin)-1-yl-2,3-dihydrofuran (**1U**, mp 142 °C, or **1T**, mp 146−148 °C)[4] and 1-*β*-uracil-1-yl (or hypoxanthin-9-yl)-5-deoxy-2,3-di-*O*-acetyl-D-*erythro*-pent-4-enofuranose (**5U**, mp 158−160 °C, or **5H**, mp 224 °C)[5,6] were synthesized according to the literature. N^6-Ethoxycarbonyladenin-9-yl-5-deoxy-2,3-di-O-acetyl-D-*erythro*-pent-4-enofuranose (**5A**) was synthesized by reaction of adenin-9-yl-5-deoxy-2,3-di-*O*-acetyl-D-*erythro*-pent-4-enofuranose[6] (5 g, 13.6 mmol) with ethyl chloroformate (1.95 mL, 2.04 mmol) in pyridine (0.5 mL) for 12 h at room temperature. The product was purified by recrystallization from chloroform:hexane (yield 65%, mp 143−144 °C).[7]

The copolymerization of the monomers with acrylic anhydride was carried out in benzene or DMF in the presence of AIBN at 90 °C for 24 h (Table 1). The polymers were isolated by precipitating in diethyl ether. The polymers were dissolved in 0.1 N NaOH. The solutions of polymers **3** and **6** were stirred at room temperature for 1 and 24 h, respectively, and dialyzed against water (cellulose membrane, MW cutoff 1000) for 48 h to give poly[{(2*R*)-uracil (or thymin)-1-yltetrahydrofuran-4,5-diyl}-1,3-dicarboxybutylene] (**4U** or **4T**) and poly[{(2*R*)-adenin-9-yl (or uracil-1-yl or hypoxanthin-9-yl)-(3*R*),(4*S*)-dihydroxytetrahydrofuran-5,5-diyl}-2,4-dicarboxypentylene] (**7A**, **7U**, or **7H**).

The copolymerization of the monomers with acrylic anhydride and the hydrolysis of the anhydride polymers were identified with ^{1}H and ^{13}C NMR and IR spectroscopy.[8] In the ^{1}H spectra in DMSO-d_6, the proton peaks of $C_{3'}$−H (δ = 5.16) and $C_{4'}$−H (δ = 6.5) in **1** and of $C_{5'}$−H (δ = 4.5−4.7) in **5** were shifted to δ = 1.4−2.2 and 2.8−3.4 in polymer **3** and to δ = 1.3−2.0 in polymer **6** (*e.g.*, Figure 1 for **3U**). After hydrolysis, the anhydride peaks near 1800 cm^{-1} in the IR spectra of **3** and **6** disappeared and the peaks for the carboxylate anions of **4** and **7** near 1570 and 1400 cm^{-1} emerged. In Figure 2 is shown the ^{13}C NMR spectra of **5U**, **6U**, and **7U**. The signals of $C_{4'}$ (δ = 150.1) and $C_{5'}$ (δ = 87.9) in **5U** were shifted to δ = 84.8 and δ = 35.6 in **6U**, respectively, by polymerization. After hydrolysis, the carbon signal of the anhydride groups (δ = 176.8) in **6U** was shifted to δ = 187.5, corresponding to the carboxy groups in **7U**.

The radical copolymerization of vinyl ethers and dibasic acid anhydride monomers (*e.g.*, maleic anhydride) is known to give alternating copolymers.[9] When the cyclic vinyl ethers (**1** and **5**) were copolymerized with acrylic anhydride in the presence of radical initiators, alternating copolymers **3U**, **3T**, **6A**, **6U**, and **6H** were produced (Scheme 1). During copolymerization the acrylic anhydride would form the glutaric anhydride radical at the growing chain end by cyclization,[10] which would propagate on the vinyl ether monomer. By repetition of the cross-reactions between them, the alternating copolymers would be obtained. Radical homopolymerization was not feasible for monomers of **1** and **5** but for acrylic anhydride. The excess feeding mole ratios of the former monomers at the onset of copolymerization was found to be neccessary for obtaining the alternating copolymers (Table 1).

The alternating structures of the polymers were verified by ^{1}H NMR spectroscopy and a titration method. The anhydride polymers, **3U**, **3T**, **6A**, **6U**, and **6H**, were soluble in polar solvents such as DMSO and DMF, and hence the cross-linking by the diene monomers seemed to not occur. Incomplete cyclization reactions of the acrylic anhydride during the polymerization would result in the polymers having acrylic groups as pendants. However, the proton signals [δ 6.57 (dd), 6.19 (q), and 6.07 (dd) in $CDCl_3$] and the carbon signals (δ 134.5 and 127.1 in $CDCl_3$] for the double bond of the acrylic group were not found in the ^{1}H and ^{13}C NMR spectra of the polymers (Figures 1 and 2). As the nucleic acid bases gave signals downfield apart from the chain protons, their concentrations on the polymer chains could be calculated by the integration values. They coincided with the alternating structures shown by Scheme 1. Titrations[11] of anhydride groups in **3** and **6** also showed that the polymers were composed of an equimolar amount of the comonomers (mol % of acrylic anhydride in the polymers: 50 for **3U**, 52 for **3T**, 49 for **6A**, 51 for **6H** and **6U**).

The structures of polymers **4T**, **4U**, **7A**, **7U**, and **7H** are analogous to those of nucleic acids (**8**), poly(T),[12] poly(dU), poly(rA), poly(rU), and poly(rI), respectively,

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Scheme 1

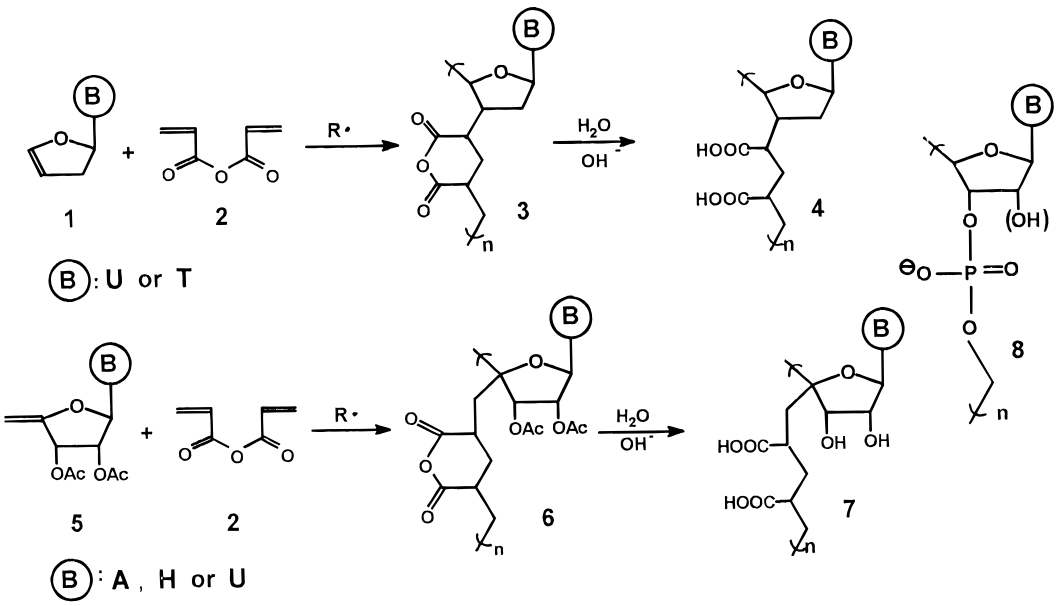

Table 1. Copolymerization Data at 90 °C for 24 h

polymer	monomer	feeding mole ratio	solvent (concn, mol/L)[a]	AIBN (mol %)	yield (%)	M_n[b]
3U	**1U**:AA[c]	2.5:1	benzene (0.5)	1	24.5	5200
3T	**1T**:AA	3:1	benzene (1)	2	28	7500
6U	**5U**:AA	2:1	DMF (2.4)	1	80	10200
6A	**5A**:AA	2:1	DMF (1.5)	2	37	7500
6H	**5H**:AA	2:1	DMF (1.5)	2	58	6800

[a] The total monomer concentration in the solvent. [b] Number-average molecular weight of the hydrolyzed product (**4** or **7**) measured by GPC. [c] Acrylic anhydride monomer.

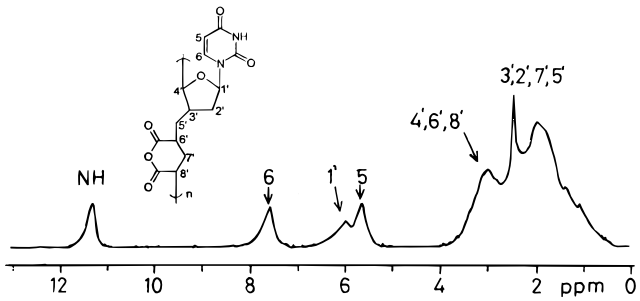

Figure 1. ^{1}H NMR spectrum of **3U** in DMSO-d_6.

in which the methylene phosphate groups of the nucleic acids were substituted by dicarboxyalkylene groups. In most polynucleotide analogues synthesized previously, the furanose rings were connected by 1,2-dicarboxyethylene groups,[1] which were shorter than the methylene phosphate groups of the natural polymers. When the methylene phosphate groups were substituted by 1,3-dicarboxybutylene for polymer **4** and 2,4-dicarboxypentylene for **7**, the polymers showed properties quite similar to those of the natural polymers.

The formation of complementary base-paired complexes between both poly(ribo- and poly(deoxyribonucleotide)s is well-known, which is generally determined by continuous variation mixing curves.[13] In Figure 3 are shown the curves for **4U**−natural poly(deoxyriboadenylic acid) of 40 bases[14] and **7A**−natural poly(uridylic acid)[14] at room temperature and the neutral pH in the presence of 0.01 M Mg^{2+} and 0.1 M Na^+. The former showed the highest hypochromicity at the mole ratio of **4U**:poly(dA) = 1:1, whereas that for the latter appeared

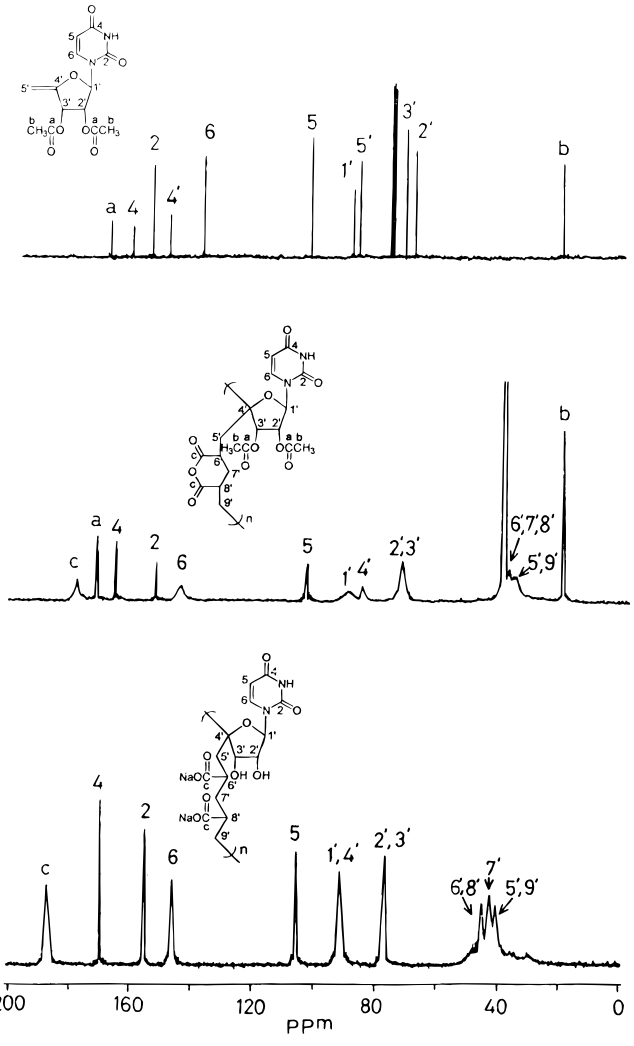

Figure 2. 125 MHz ^{13}C NMR spectra of **5U** in $CDCl_3$, **6U** in DMSO-d_6, and **7U** in D_2O.

at **7A**:poly(rU) = 2:1, indicating that they formed **4U**−poly(dA) double-stranded and **7A**−poly(rU)−**7A** triple-stranded complexes. The corresponding natural polymer complexes are well documented.[15]

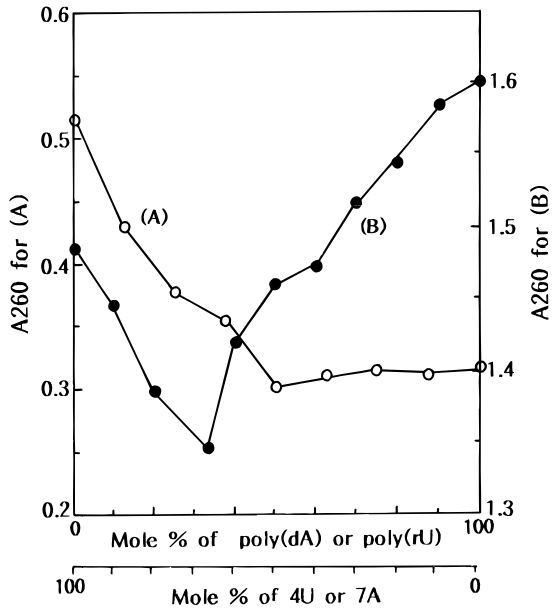

Figure 3. Continuous mixing variation curves: (A) for poly(dA) and **4U** after 3 days at room temperature; (B) for poly(rU) and **7A**, which was heated for 20 min at 90 °C and then kept for 3 days at room temperature in Tris buffer (pH 7.4) containing 0.01 M Mg^{2+} and 0.1 M Na^{+}.

Formation of the double helical structure of **4U** with poly(dA) was confirmed by surface-enhanced Raman spectroscopy (SERS). As well documented in the previous studies,[16] we observed the variation of the band intensities associated with the polymer backbone (sugar−dicarboxyalkylene and sugar−phosphate) and nucleic acid base moieties during the conformational change from two separated single strands to a double helical structure. As shown in Figure 4, strong bands for the polymer backbone at 2950 cm^{-1} and for adenine moieties at 730 and 1330 cm^{-1} were initially observed in the SERS spectrum of the mixed solution of **4U** and poly(dA). A significant decrease of the band intensities associated with the adenine moieties at 730 and 1330 cm^{-1} occurred 3 days after mixing, while constant intensity of the band at 2950 cm^{-1} from the polymer backbone was maintained. This is indicative of the close proximity of the backbone on the Ag film substrate and further out in the case of adenine moieties as a result of the formation of a double helix by base-pairing. Spectrum b in Figure 4 resembles that of the double helix of the natural poly(dU) and poly(dA).[16b]

When the complex solution was heated slowly, the base pairs broke and finally the strands were separated, which was accompanied by an increase of UV absorption. The melting profiles of the complexes are shown in Figure 5, which are also quite similar to those of the relevant natural complexes. T_m of **4U**−poly(dA) was found to be 62 °C, a little lower than that (65 °C) of the natural polymer complex, poly(dA)−poly(dU),[15a] in 0.434 M Na^{+}. While the natural triplex [poly(rA)−poly(rU)−poly(rA)] is transformed *via* a duplex [poly(rA)−poly(rU) + poly(rA)] (T_m: 32 °C) to single strands (T_m: 44 °C),[15c] the triplex **7A**−poly(rU)−**7A** was melted directly to the individual strands [**7A** + **7A** + poly(rU)] at T_m of 80 °C. Compared with poly(rA), **7A** contained additional $C_{3'}$−OH groups, which elevated the T_m of the triplex.[15a]

In conclusion, we found that the radical copolymerization of acrylic anhydride with vinyl ethers proceeded by an alternating cyclocopolymerization mechanism. This method enabled us to synthesize novel DNA and

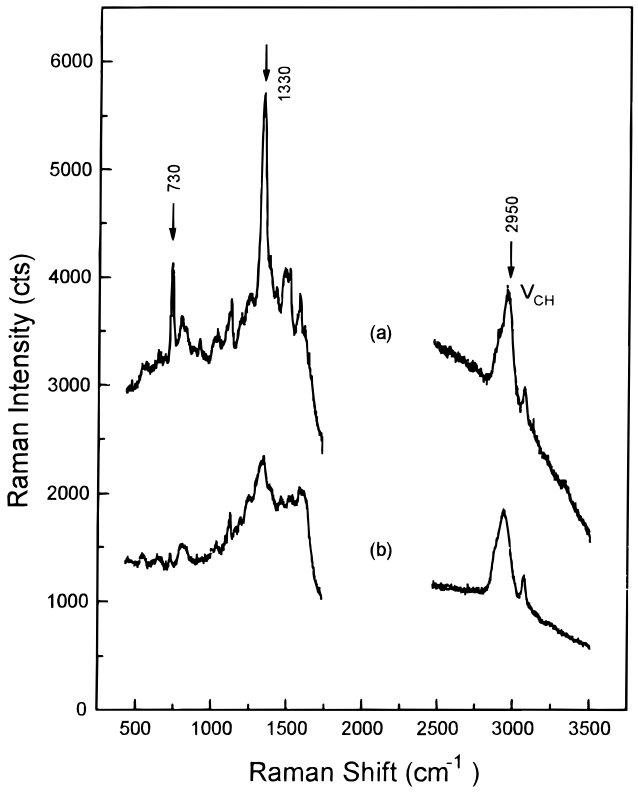

Figure 4. SERS spectra of the mixed solution of **4U** and poly(dA). Spectra were taken at 10 min (a) and 3 days (b) after mixing of the two components. Experimental conditions: laser excitation wavelength, 514.5 nm; power, 10 mW at the sample; integration time, 10 scans at 1 s/scan.

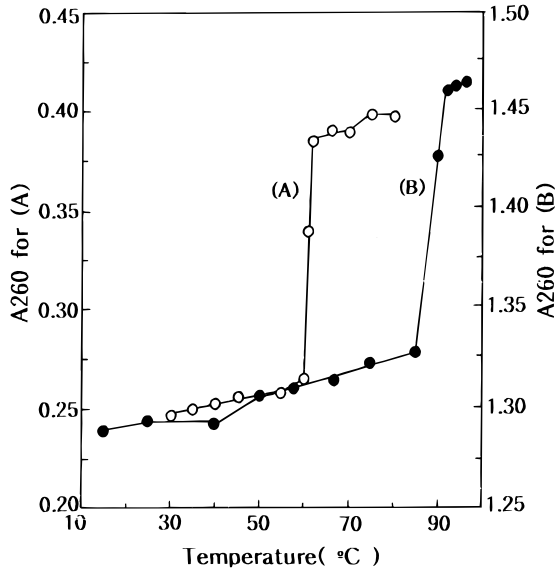

Figure 5. Absorbance−temperature profiles: (A) for poly(dA)−**4U** (1:1) complex and (B) for poly(rU)−**7A** (1:2) complex in Tris buffer (pH 7.4) containing 0.01 M Mg^{2+} and 0.1 M Na^{+}.

RNA analogues containing uracil, thymine, adenine, and hypoxanthine. These analogues have alkyl linkages between furanose rings, replacing the methylene phosphate groups of the natural polymers, and thus are resistant to the hydrolysis by nuclease in biological systems. They formed the base-paired complexes with the natural polymers of complementary bases. We are now investigating possibilities of their uses as antitemplates to inhibit DNA synthesis and/or RNA synthesis by binding to the enzymes (polymerases) and blocking their functions, as antigens to inhibit gene expression

by complexing with the double helical DNA and blocking its transcription, and as interferon inducers.

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(7) Characterization data of compound **5A**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.70 (d, 1H, $C_{1'}$), 6.2 (dd, 1H, $C_{2'}$), 6.4 (dd, 1H, $C_{3'}$), 4.6 (d, 1H, $C_{5'a}$), 4.4 (d, 1H, $C_{5'b}$), 8.4 (s, 1H, C_2), 8.4 (s, 1H, C_8), 2.1 (s, 3H, OAc), 4.2 (m, 2H, CH_2), 1.2−1.4 (m, 3H, CH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 88.06 ($C_{1'}$), 79.33 ($C_{2'}$), 71.47 ($C_{3'}$), 86.51 ($C_{4'}$), 69.17 ($C_{5'}$), 169.46 (NHCO), 61.21 (CH_2), 14.51 (CH_3), 20.52 (OCH_3 at $C_{2'}$), 20.31 (OCH_3 at $C_{3'}$), 169.35 (COO). Anal. Calcd for $C_{17}H_{19}N_5O_7$: C, 50.35; H, 4.89; N, 17.28. Found: C, 50.17; H, 5.01; N, 17.26.

(8) ^{13}C NMR data for the polymers (125 MHz, D_2O): (**4U**) δ 185.8, 184.1, 169.2, 154.2, 144.7, 104.5, 92.8, 88.5, 53.3, 50.5, 47.3, 39.9, 31.9; (**4T**) δ 185.7, 184.2, 169.0, 154.2, 144.8, 112.8, 89.1, 85.5, 52.3, 50.6, 49.3, 38.9, 31.1, 12.6; (**7A**) δ 186.4, 157.8, 157.1, 155.3, 142.7, 121.2, 91.0, 76.7, 65.6, 45.1, 41.9, 36.6; (**7H**) δ 180.9, 155.7, 147.9, 146.7, 139.2, 125.2, 86.5, 71.0, 65.8, 41.1, 36.7.

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(12) Poly(T), poly(thymidylic acid); poly(dU), poly(deoxyribouridylic acid); poly(rA), poly(riboadenylic acid); and so on.

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