Polynucleotide Analogues. 4.[1] Synthesis and Physicochemical Properties of Poly(deoxyuridylic acid) Analogues

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ABSTRACT: Poly[(2-uracil-1-yl-2,3-dihydrofuran)-*alt*-(maleic anhydride)] (3) was synthesized by a radical copolymerization of the relevant monomer pair. Its alternating sequence was confirmed by titration of functional groups and the NMR spectrum of the polymer. The number-average molecular weight was found to be 1840, which corresponded to 6.6 repeating units per chain. Hydrolysis of the copolymer gave poly-[(2-uracil-1-yltetrahydrofuran-4,5-diyl)-1,2-dicarboxyethylene] (4), an analogue of poly(2′-deoxyuridylic acid) in which the methylene phosphate groups were substituted by dicarboxyethylene groups. Polymer 4 showed a hyperchromicity of 30.1% in H_2O whereas a hypochromicity of 28.4% in DMF in comparison with the UV absorption of 2′-deoxyuridine. These changes in chromicity were due to base stacking depending on the relative geometry of the stacked chromophores. Consequently, two different conformations of polymer 4 were formed in H_2O and in DMF which stayed in equilibrium in solvent mixtures of H_2O–DMF. CD and ORD curves of polymer 4 showed several extrema with wavelengths and magnitudes different from those of the monomer. These differences were due to atactic addition polymerization at the dihydrofuran ring of the monomer. Additionally, polymer 4 exhibited a typical polyelectrolyte behavior in the concentration dependence of the reduced viscosity in aqueous solution. The sodium salt of polymer 4 migrated to the anode by electrophoresis and showed three separate bands, indicating that the polymer 4 was a mixture of hepta-, hexa-, and pentamers.

Introduction

Over the last decade there has been considerable interest in the synthesis of polynucleotide analogues (PNA) in an effort to elucidate natural polynucleotide structures and utilize their biological activities in the design of polymeric drugs for chemotherapy. There are several PNA synthetic methods, which include a method of attaching the nucleic acid bases (NAB) to vinyl monomers and polymerization of these monomers resulting in PNAs,[2-8] a coupling method for reacting NABs with the functional groups of the selected polymers,[9-20] and polycondensation of ω-hydroxy carboxylic acid or α-amino acids containing the NABs.[21-23] However, most of the reported PNAs exhibit neither good solubilities in water, due to the lack of hydrophilic groups, nor optical activities, due to the absence of sugar moieties on the polymer chain. The alternating sequences between nucleosides and phosphate, observed in natural polynucleotides, have rarely been realized in synthetic PNAs.

For therapeutic application of PNAs, either as an interferon inducer[24] or an antisense compound,[25] it is required for them to have resistance to hydrolysis caused by nucleases as well as good water solubility. In order to meet these requirements, many attempts have been made to replace phosphate groups in polynucleotides by alkylphosphonate[26,27] or phosphorothioate[28,29] groups, but it still remains unsolved.

Recently, we have reported the synthesis of several polynucleotide analogues[1] in which either the methylene phosphate groups were substituted by dicarboxyethylene groups or the furanose sugar moieties were replaced by pyranose rings. These PNAs were soluble in water, resistant to hydrolysis, and optically active. They contained alternating sequences along the polymer chain.

In line with the effort to obtain new PNAs closely resembling natural polymers and study their physicochemical properties, we have synthesized the monomer (*R*)-(−)-2-uracil-1-yl-2,3-dihydrofuran (1)[30] and copolymerized it with maleic anhydride to obtain an alternating copolymer, poly[{(*R*)-(−)-2-uracil-1-yl-2,3-dihydrofuran}-*alt*-{maleic anhydride}] (3). This product was hydrolyzed to give poly[(2-uracil-1-yltetrahydrofuran-4,5-diyl)(1,2-dicarboxyethylene)] (4). This polymer was an analogue to poly(deoxyuridylic acid), in which the methylene phosphate groups were substituted by dicarboxyethylene groups (Scheme I). In this paper we report on the synthesis, characterization, and physicochemical properties of the polymers.

Experimental Section

Materials. 2′-Deoxyuridine (Aldrich Co.) was recrystallized from ethyl acetate (mp 167 °C). Maleic anhydride was sublimed under vacuum. DMF was refluxed over anhydrous $CaCl_2$ and distilled under reduced pressure. AIBN was recrystallized from methanol. All other commercially available reagent chemicals were used without further purification.

Synthesis of Monomer. 2′-Deoxyuridine-5′-carboxylic acid was synthesized by the method of Zemlicka[31] and Moss.[32] The compound afforded a melting point (223 °C) and an NMR spectrum consistent with the literature.[31] (*R*)-(−)-2-Uracil-1-yl-2,3-dihydrofuran was synthesized according to the method of Zemlicka et al. The melting point and an NMR spectrum of the compound coincided with those reported in the literature.[31]

Copolymerization Procedure. The calculated amounts of monomers, solvent (DMF), and initiator (AIBN) were charged into the polymerization tubes (Table I), which were then immersed in a Dewar flask containing dry ice and acetone. After conventional freeze–thaw treatment under N_2, the tubes were sealed and placed in an oil bath at 100 °C for 48 h, as listed in Table I. The polymer was precipitated in benzene twice, collected, and dried in vacuum over P_2O_5 at 50 °C.

Titration. Analysis of anhydride groups in polymer 3 was performed by dissolving polymer 3 in DMF and titrating the solution with a solution of sodium methoxide (0.1 N) in DMF–methanol with the aid of a potentiometer.[33] Carboxyl groups in polymer 4 were titrated as their aqueous solution with NaOH (0.1 N).

Hydrolysis of Polymer 3. A 0.1-g portion of polymer 3 was stirred in 20 mL of 0.1 M NaOH for 1 h at room temperature. The solution was acidified to pH = 3.5 with 0.1 M HCl and the solvent removed by evaporation under reduced pressure. After the residue was dissolved in DMF, the sodium chloride was removed by filtration. Polymer 4 was obtained by precipitation in benzene and dried in vacuum (yield 80%).

Measurements. ^1H NMR (300 or 60 MHz) and ^{13}C NMR (25 MHz) spectra were measured on Bruker AM-300 and Varian-

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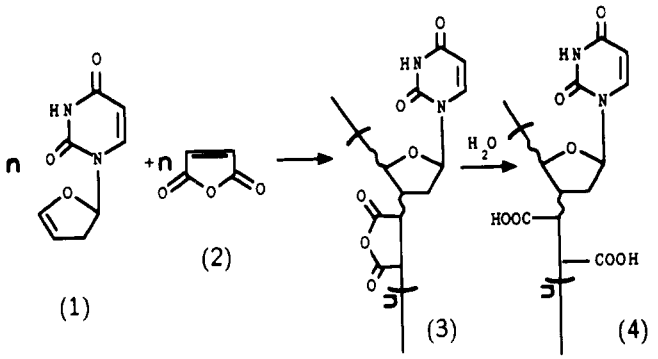
Scheme I



T60 spectrometers, respectively. ORD and CD spectra were recorded on a Jobin Yvon spectropolarimeter Model CD6 with 10-mm cells at concentrations of 10^{-4} M for monomer and 10^{-5} M base residues for polymer at room temperature. Viscosities of polymer solutions were measured by a Ubbelohde viscometer at 30 °C in H_2O or in DMF.

UV absorption was recorded using a Hitachi spectrophotometer equipped with a water circulation type cell unit at 25 ± 0.1 °C. The concentrations of solutions were approximately 10^{-4} M for monomer and 10^{-5} M for polymer base residues. The number-average molecular weight of polymer **3** was measured using a vapor pressure osmometer (Knauer Co.) at 90 °C in DMF.

Hyper- or Hypochromicity. The percent hyper- or hypochromicity (h, %) was calculated from eq 1 where ϵ_p and ϵ_m denote the molar extinction coefficients of the base residue of the polymer

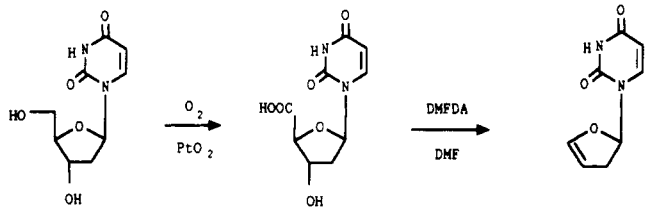
$$h\,(\%) = 100[(\epsilon_p - \epsilon_m)/\epsilon_m] \quad (1)$$

and 2′-deoxyuridine in relevant solvents (H_2O or DMF). The positive value of h is hyperchromicity whereas a negative value means hypochromicity.

Electrophoresis. Thin-layer electrophoresis was carried out on a cellulose sheet (25 cm × 2 cm, Toyo Roshi Kaisha, Sheet No. 51A), which was immersed in a 0.1 M phosphate buffer solution of pH = 7.4 at a constant 250 V for 3 h. The separated bands were identified in an I_2 chamber.

Results and Discussion

Synthesis and Optical Properties of Monomers. The monomer (*R*)-(−)-2-uracil-1-yl-2,3-dihydrofuran was synthesized according to Zemlicka et al.[31] This method involved oxidation of 2′-deoxyuridine followed by decarboxy dehydration of the resulting 2′-deoxyuridine-5′-carboxylic acid.



DMFDA : Dimethyl formamide dineopentyl acetal

Since the chiral atom C_1' in 2′-deoxyuridine remained intact during the reaction, its chirality was retained to render the monomer optically active. The CD and ORD spectra of the monomer **1** as well as their data are given in Figures 2 and 3 and Table II.

Copolymerization and Hydrolysis of the Copolymer. The radical copolymerization of monomers **1** and **2** is expected to give an alternating copolymer since (1) the radical copolymerization of 2,3-dihydrofuran with maleic anhydride has been known to produce alternating copolymers by forming charge-transfer complexes of the monomer pairs during the copolymerization process[34,35] and the electron-donating character of the vinyl ether group of **1** is negligibly influenced by uracil-1-yl groups on the C_2 position of the 2,3-dihydrofuran, (2) copolymerization of maleic anhydride with (*R*)-(−)-thymin-1-yl-2,3-dihydrofuran, in which the thyminyl group replaced the uracilyl group in monomer **1**, has also been known to produce alternating sequences,[1] and (3) monomer **1** as well as **2** is not homopolymerizable under the conditions used.

Table I
Copolymerization Data in DMF at 100 °C for 48 h and Characterization of the Copolymer

concn (mol/L)						
1[a]	2[b]	AIBN	yield (%)	$[\eta]$[c] (dL/g)	M_n[d]	MA[e] (%)
1.52	1.52	0.06	74			
1.79	1.79	0.07	77	0.17		
1.85	1.85	0.07	80	0.19	1840	50.4

[a] (*R*)-(−)-2-Uracil-1-yl-2,3-dihydrofuran. [b] Maleic anhydride. [c] Intrinsic viscosity measured in DMF at 30 °C. [d] Number-average molecular weight measured by VPO in DMF at 90 °C. [e] Mole percent of maleic anhydride incorporated into the copolymer.

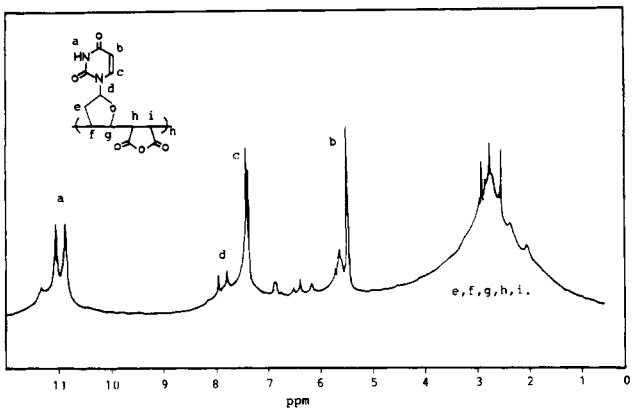


Figure 1. ^{1}H NMR spectrum of polymer **3** in DMSO-d_6.

The copolymerization of **1** with **2** was carried out with different monomer concentrations in the presence of the radical initiator (AIBN) in DMF at 100 °C. The data are given in Table I. Higher yields were obtained with higher initial monomer concentrations. This copolymer (**3**) was a yellowish powder, soluble only in highly polar solvents such as DMF and DMSO, and was insoluble in THF, acetone, acetonitrile, chloroform, and ethyl acetate as well as other less-polar solvents.

Simple hydrolysis of polymer **3** in distilled water at room temperature gave poly[(2-uracil-1-yltetrahydrofuran-4,5-diyl)(1,2-dicarboxyethylene)] (**4**) without difficulty. The completion of the hydrolysis was monitored by following the disappearance of the characteristic IR peaks at 1810 cm^{-1} (C=O) and at 1220 cm^{-1} (C—O—C) for anhydride groups of polymer **3**. Polymer **4** was soluble in water, DMF, and DMSO and insoluble in other nonpolar organic solvents.

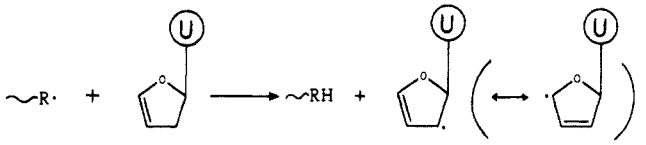
Characterization of the Copolymers. The anhydride groups incorporated into polymer **3** and succinic acid moieties in polymer **4** were found to be 50.4 and 49.2 mol %, respectively, by titrating them with sodium methoxide[33] and aqueous NaOH (0.1 N), respectively. These results indicated that polymer **3** had alternating sequences.

The ^{1}H NMR spectrum of polymer **3** is shown and the chemical shifts of the protons are assigned in Figure 1. The peaks of double bond protons (δ = 6.52 and 5.09) in the dihydrofuran ring of monomer **1** disappeared. The integration values for proton peaks corroborated the alternating structure of copolymer **3**. However, the integral value of the peak at δ = 11.0 was a little higher than the calculated value of imide protons, which was attributed to protons of carboxylic acid formed by hydrolysis of the

anhydride groups during purification of the polymers. The imide protons were split into three peaks around $\delta = 11.0$ at room temperature. When it was recorded at an elevated temperature (80 °C), the peaks merged into one broad peak. The splitting of the peaks was attributable to the hydrogen bonding of the imide protons either by self-association or with the anhydride groups, since formation of hydrogen bond shifts proton signals downfield.[36]

It was known that, during radical homopolymerization of 1-vinyluracil, cyclopolymerization occurred by reaction of the radical chain end with the double bond of uracil, so that the double bonds disappeared and consequently the ultraviolet absorption at 265 nm of the uracil groups decreased.[37] However, the double bond of uracil was found to be intact in this polymerization system, since equivalent integration values of olefin protons and UV absorption of uracil groups were obtained in characterization of polymer 3.

The number-average molecular weight ($\bar{M}_n$) of polymer 3 was measured by vapor pressure osmometry (VPO) at 90 °C in DMF. As the polymer was free from intermolecular hydrogen bonding at this temperature, any abnormality due to the association of polymer molecules was not observed during VPO measurement. $\bar{M}_n$ was found to be 1840, which corresponded to 6.6 repeating units per chain. The low molecular weight of the polymer was attributable to transfer reactions on the monomer, as shown below. The allyl and/or allyloxy radicals, formed by hydrogen transfer from the monomer to the active center, are very stable due to the formation of resonance hybrids. These stable free radicals can start the copolymerization anew.[34] This point was confirmed by the presence of the $\delta = 6.52$ absorption peak of dihydrofuran in the ^{1}H NMR spectrum of polymer 3 corresponding to the presence of a trace amount of vinyl protons (Figure 1).



where ~R· is a radical chain end.

CD and ORD Spectra. ORD and CD measurements are sensitive probes for molecular conformation and in some instances are related to the secondary structure of macromolecules. CD spectra of 2′-deoxyuridine, monomer 1, and polymer 4 are shown in Figure 2, and their data are summarized in Table II. The CD spectrum of monomer 1 showed two negative Cotton effects at 211 and 253 nm and a positive one at 228 nm, which were not coincident with the UV maximum (263 nm). These Cotton effects were quite different from those of 2′-deoxyuridine; their signs were opposite to each other at close wavelengths. The disappearance of chirality on the C_3' and C_4' carbon atoms of the furanosyl ring of 2′-deoxyuridine by formation of a double bond caused a significant change in the CD spectrum.

The CD curve of poly(uridylic acid)[43] has shown a magnitude larger in circular dichroism with the same sign of Cotton effect than that of uridylic acid.[44] This difference is due to the ordered structural formation of the polymer (Table II).[45] As two chiral atoms at C_3' and C_4' arise during the polymerization of monomer 1, a larger magnitude of CD bands is expected if the polymerization proceeds stereoregularly. The CD curve of polymer 4 showed three extrema (213, 228, and 252 nm) with different wavelengths from those of the monomer 1 or of 2′-deoxyuridine and reduced magnitude.

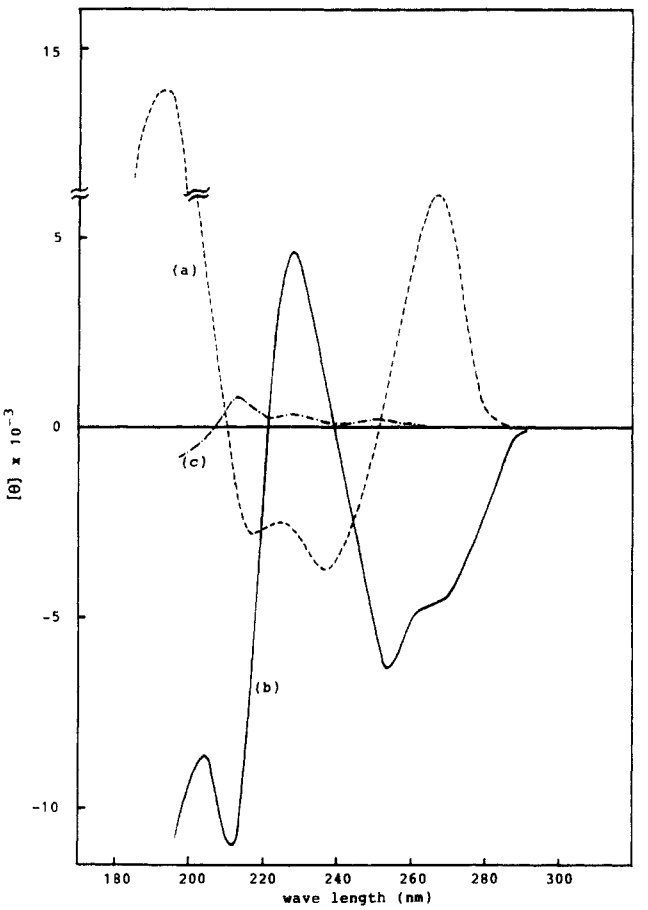
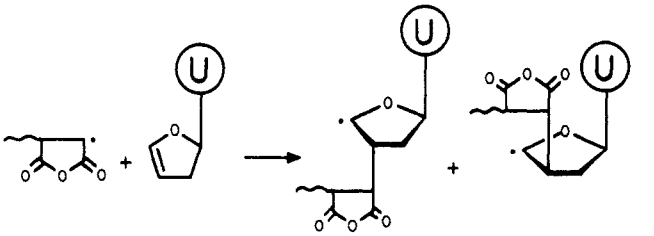


Figure 2. CD spectra in H_2O: (a) 2′-deoxyuridine; (b) monomer 1; (c) polymer 4.

These results suggested that an atactic addition polymerization occurred at the double bonds of monomer 1. Since 5′-deoxyuridine showed a CD curve similar to that of the 4′-epimer of 5′-deoxyuridine (Table II),[46] the chirality of the C_4' atom had little effect on the CD curve of the 5′-deoxyuridine. The sign of the Cotton effect of the furanosyl ring in polymer 4 seems to be therefore determined by the C_3' configuration. As shown, the maleic anhydride chain end will attack C_3' of monomer 1 to form epimers, as the resulting oxyalkyl radicals are more stable than alkyl radicals. Several extrema with a reduced magnitude of the CD spectrum of polymer 4 were therefore due to overlapping of the circular dichroism with different signs arising from the epimers.



ORD curves of 2′-deoxyuridine, monomer 1, and polymer 4 are shown in Figure 3, and their data are given in Table II. Monomer 1 showed neither a peak nor crossover point but two troughs at 222 and 273 nm that were quite different from those of 2′-deoxyuridine with two peaks (204 and 285 nm) and one trough at 254 nm. The replacement of C_4' hydroxymethyl and C_3' hydroxy groups by a double bond on the furanosyl ring had a significant effect on the ORD curve. Polymer 4 showed two troughs at 210 and 259 nm and a peak at 240 nm with negligible amplitudes. The difference in wavelengths of extrema from those of

Table II
Optical Data of Monomer and Polymers at 25 °C

compd	solvent	UV λ_{max} (nm) (10^{-3} ϵ)	h^a (%)	CD extrema $\lambda(10^{-3}[\theta])$	ORD extrema $\lambda(10^{-3}[m])$
2′-deoxyuridine	H_2O	263 (9.50)		194 (13.9), 218 (−2.88) 237 (−3.7), 268 (6.14)	204 (5.0), 254 (−3.2) 285 (1.4)
	DMF	267 (9.35)			
monomer 1	H_2O	263 (10.78)		204 (−8.6), 211 (−11.8) 228 (4.59), 253 (−6.36)	222 (−20.8), 247 (−0.8) 273 (−9.6)
	DMF	267 (9.4)			
polymer 3	DMF	268 (9.92)			
polymer 4	H_2O	261 (12.36)	+30.1	213 (0.8), 228 (0.4) 252 (0.2)	210 (−1.5), 240 (0.15) 259 (−0.3)
	DMF	268 (6.69)	−28.4		
uridine[44]	H_2O	261 (10.1) 205 (9.8)		266 (8.6), 238 (−4.0) 215 (−5.0)	
poly(uridiylic acid)[43]	H_2O	261 (9.2)		269 (11.13), 244 (−5.46)	258 (−18.25), 282 (8.05)
5′-deoxyuridine[46]	H_2O			268 (8.1), 240 (−4.0) 215 (−4.0), 194 (8.5)	
4′-epimer of 5′-deoxyuridine[46]	H_2O			270 (4.0), 243 (−5.5) 215 (−7.0), 195 (8.0)	

[a] Positive value is hyperchromicity whereas negative value means hypochromicity.

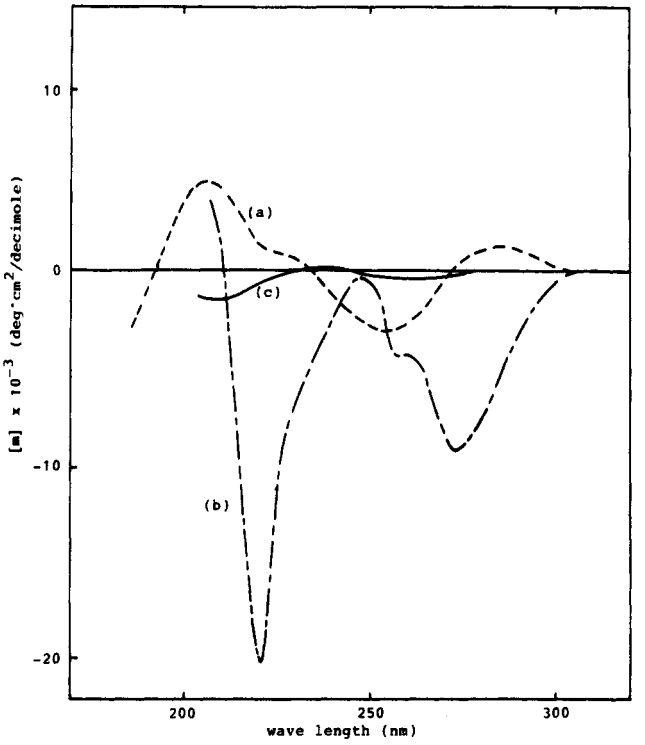


Figure 3. ORD spectra in H_2O: (a) 2′-deoxyuridine; (b) monomer 1; (c) polymer 4.

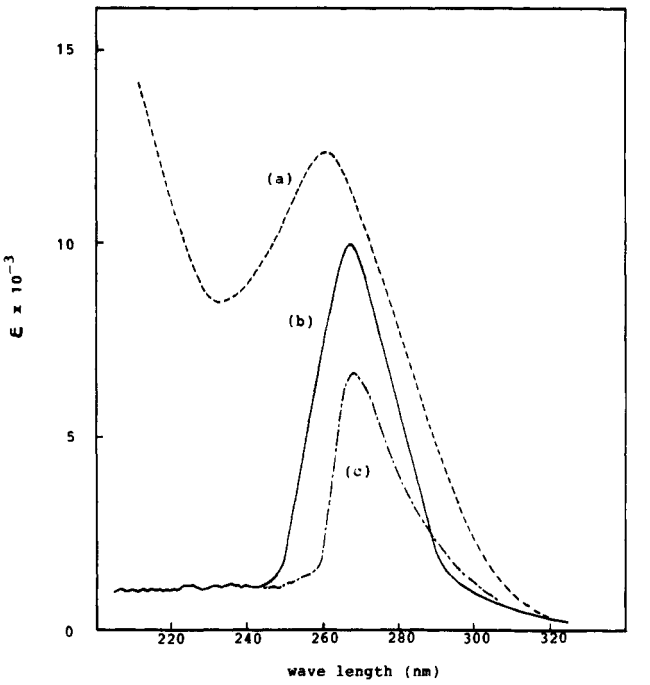


Figure 4. UV spectra: (a) polymer 4 in H_2O; (b) polymer 3 in DMF; (c) polymer 4 in DMF.

monomer 1 and reduction of their amplitudes can be explained as resulting from the reasons described above in CD spectra.

Ultraviolet Absorption. UV data for 2′-deoxyuridine and monomer 1 are summarized in Table II. Due to the solvent effect 2′-deoxyuridine had different absorption maxima as well as extinction coefficients in different solvents, i.e., 263 nm (ϵ = 9500) in H_2O and 267 nm (ϵ = 9350) in DMF at 25 °C. Monomer 1 also showed different values: 263 nm (ϵ = 10 780) in H_2O and 267 (ϵ = 9400) in DMF at 25 °C. This enormous change in extinction coefficient for monomer 1 in different solvents was not caused by base pairing or stacking of the molecules, because the measuring conditions—increases in temperature (60 °C) and decreases in concentration—made very little difference to the absorption (less than 2%). The double bond in the sugar ring seems to play a significant role in the UV absorption of the uracil group in organic and aqueous solution. A similar phenomenon was also found in the compound 2-(uracil-1-ylmethyl)-3,4-dihydro-2*H*-pyran, which showed UV absorption; 269 nm (ϵ = 12 930) in H_2O and 269 nm (ϵ = 10 770) in DMF.[38]

UV spectra of polymers 3 and 4 in different solvents are shown in Figure 4, and their data are summarized in Table II. Polymer 3 gave an extinction coefficient (ϵ = 9920) close to that of 2′-deoxyuridine in DMF, whereas polymer 4 showed a hyperchromicity of 30.1% in H_2O and a hypochromicity of 28.4% in DMF, compared with those of 2′-deoxyuridine in relevant solvents, respectively. It is notable that hyperchromicity has rarely been found in aqueous solutions of polynucleotides or nucleic acids though it was predicted theoretically.

According to Tinoco[39] and Rhodes[40] theory, induced dipole–dipole interactions in the chromophores of nucleic acid bases can result in either hypochroism or hyperchroism, depending on the relative geometry of the stacked chromophores. Hypochroism is common to systems with chromophores stacked one on top of another like a deck of cards, while systems with chromophores in a head-to-tail aggregate are generally prediced to be hyperchromic.[41,42] Polymer 4 was composed of uracil-1-ylfuranoids as hydrophobic groups and dicarboxyethylenes as hydro-

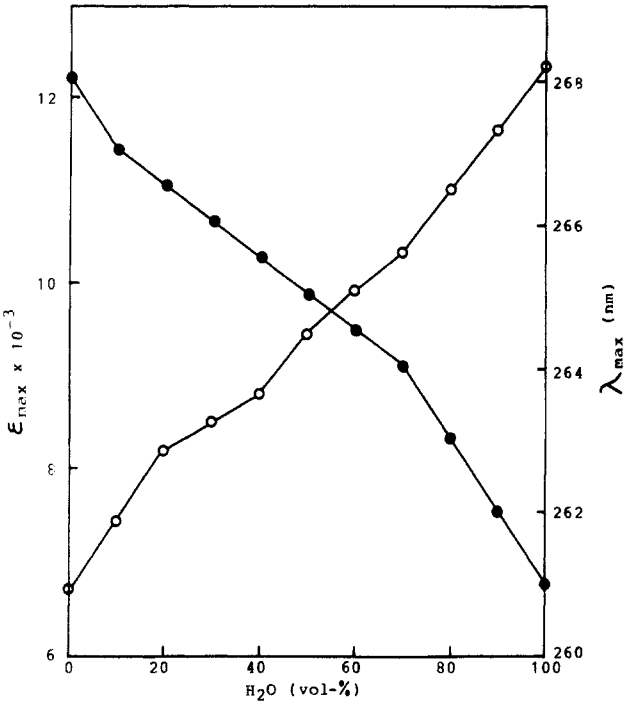


Figure 5. ϵ_{max} and λ_{max} of polymer 4 as a function of H_2O–DMF solvent compositions at the concentration of 4.1989×10^{-5} residue mol/L at 25 °C: ϵ_{max} (open circle) and λ_{max} (solid circle).

philic groups. The carboxyl groups of polymer 4 in aqueous solution protruded outward, interacting with the aqueous environment. Consequently, the polymer formed a conformation such that the chromophores aggregated into head-to-tail order in H_2O to cause the hyperchromicity. To the contrary, the uracil groups in polymer 4 were stacked one upon another in DMF to cause hypochromicity. Since the UV extinction of the polymer 4 in DMF is about half (ϵ = 6690, λ_{max} = 268 nm) of that (ϵ = 12 360, λ_{max} = 261 nm) in H_2O, two different stacking forms, hyperchromic in H_2O and hypochromic in DMF, seem to be formed.

The UV extinction and maximal wavelength of polymer 4 were measured in solvent mixtures of DMF–H_2O after equilibration for 24 h at 25 °C and plotted in Figure 5. When the water content was increased in the solvent mixture, the extinctions were increased while wavelengths of absorption maxima were blue-shifted. At a solvent composition of water 50 vol %, the extinction was equal to that of free uracil as well as the mean value of the two forms.

In order to confirm whether an equilibrium state can be attained at the same solvent composition, starting from either one of the two forms, polymer 4 was dissolved in H_2O or in DMF and the time-dependent extinction changes were measured after diluting with DMF or H_2O, respectively (Figure 6). The equilibrium value of extinction was attained quickly from the hypochromic form whereas very slowly from hyperchromic form after 270 min. Polymer 4 contained carboxyl groups and their interactions with water, which compel the polymer to change its conformation, seem to be stronger than those of hydrophobic groups of the polymer with the organic solvent.

Polyelectrolyte Behavior. The sodium salt of polymer 4 is a polyelectrolyte, and the polyanions migrate to the anode in an electrical field. The mobility (V) of charged polymers is proportional to the net charge (Z) and is inversely proportional to the two-thirds power of molecular weight (M)[47]

$$V = KZM^{-2/3} \quad (2)$$

where K is a constant. A thin-layer electrophoresis

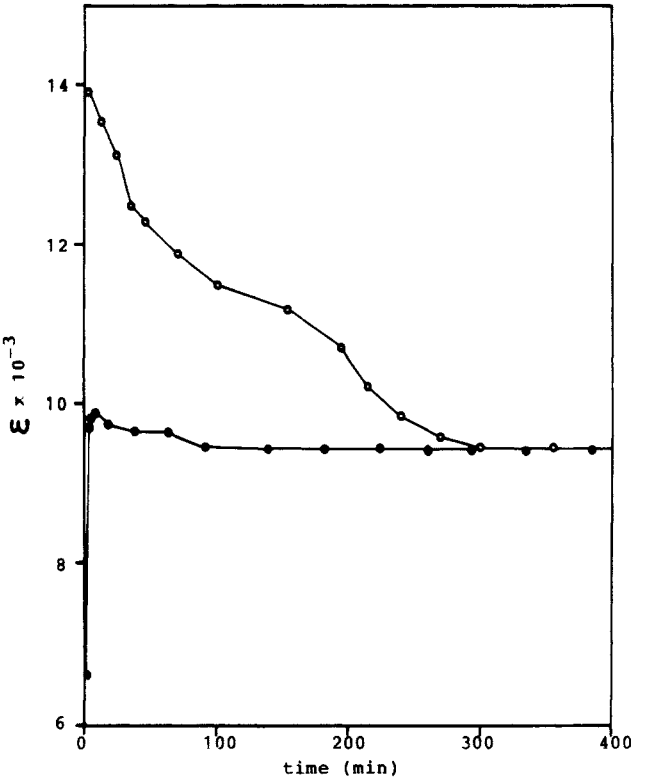


Figure 6. Time-dependent approach to the equilibrium values of extinction coefficient (ϵ) at 265 nm in H_2O–DMF (1:1 v/v) at 25 °C starting with form A (open circle) and form B (solid circle).

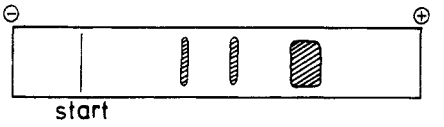


Figure 7. Electrophoresis diagram of sodium salt of polymer 4 at a constant 250 V for 2.5 h in a pH = 7.4 buffer solution.

diagram for a sodium salt of 4, developed on an electrophoresis cellulose sheet immersed in a buffer solution (pH = 7.4), is shown in Figure 7.

The increase of one repeating unit in polymer 4 is accompanied by an increase of two net charges. According to the relationship of eq 2, the effect of net charge on mobility is higher by a one-third power than that of molecular weight. The polymer band at the farthest distance from the starting line, therefore, has a higher degree of polymerization than the next band. However, a polymer initiated and terminated by maleic anhydride will have more carboxylate groups than a polymer which is initiated and terminated by dihydrofuran derivatives. The former will migrate farther than the latter when the polymers have very similar molecular weights.

Polymer 4 showed three bands. The band at the farthest distance from the starting line was broad and intense whereas the other two bands were thin. As the repeating units per polymer chain were found to be 6.6 by VPO, the polymer was a mixture of hepta-, hexa-, and pentamers, in which the latter two oligomers were contained in small portions. The sharp molecular weight distribution was attributable to the transfer reactions mentioned above, which cut off high molecular weight, and to the purification process after copolymerization and hydrolysis of the polymers, which resulted in dissolution of the low molecular weight portion.

Reduced viscosities of the sodium salt of copolymer 4 as a function of concentration in H_2O are shown in Figure 8. It exhibited typical polyelectrolyte behavior for reduced viscosity decreasing at the beginning and thereafter

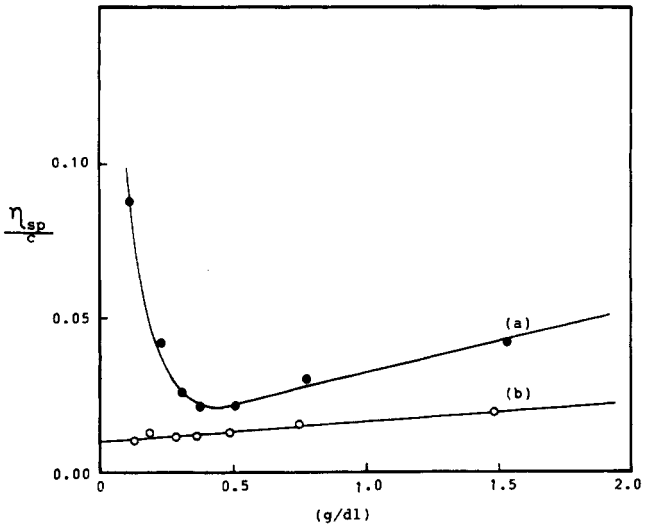


Figure 8. Reduced viscosity as a function of concentration: (a) polymer 4 in H_2O; (b) sodium salt of polymer 4 in 5% NaCl aqueous solution at 30 °C.

increasing steeply with continuous dilution. By addition of neutral salts they retained normal behavior.[48] The intrinsic viscosity of copolymer 4 in aqueous NaCl solution (5%) was rather low compared with that of polymer 3, as given in Table I. This point was attributable to the chain flexibility of polymer 4 caused by cleavage of anhydride groups in polymers 3 as well as to the association of polymer 3 resulting from the intermolecular hydrogen bonding in DMF, which was confirmed by the NMR spectrum of polymer 3, as described above.

Conclusions

A new alternating copolymer was synthesized by a radical copolymerization of 2-uracil-1-yl-2,3-dihydrofuran with maleic anhydride. After characterization of its structure, the copolymer was hydrolyzed to give poly[(2-uracil-1-yl-tetrahydrofuran-4,5-diyl)(1,2-dicarboxyethylene)], an analogue to poly(2-deoxyuridylic acid) in which the methylene phosphate groups were substituted by dicarboxyethylene groups. As the polymer contained hydroxyl groups and furanosyl sugar moieties on its chain, the polymer had good solubility in water and optical activity, both of which had been hardly found in the synthetic polynucleotide analogues.

The polymer solutions in DMF and H_2O showed high hypo- and hyperchromicity, respectively, due to the base stacking depending on the relative geometry of the stacked uracil rings in different solvents. CD and ORD curves of the polymer showed several extrema of reduced magnitudes and wavelengths different from those of the monomer and 2-deoxyuridine. These differences were caused by atactic addition polymerization at the dihydrofuran ring of the monomer. The sodium salt of the polymer showed a typical polyelectrolyte behavior, i.e., the migration of the polyanion to the anode in an electrical field and the steep increase in the reduced viscosity of the aqueous polymer solution by continuous dilution. All of the physicochemical properties of the polymer were similar to those of natural polynucleotides.

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