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Properties of Synthetic Polydeoxyribonucleotide Complexes Containing Adenine and Bromouracil*

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ABSTRACT: The homopolymer polydeoxyribobromouridylic acid (dBrU) has been prepared using *Escherichia coli* DNA polymerase I. Two- and three-stranded base-paired homopolymer complexes with polydeoxyriboadenylic acid (dA) have been made, and interactions occurring between the complexes and their component strands have been studied at 0.1 M sodium ion. The thermal stabilities of the homopolymer pair dA·dBrU and the alternating copolymer d(A-BrU)·d(A-BrU) have been determined at pH 7 as a function of ionic strength, and at 0.1 M sodium ion as a function of pH. In addition, the thermal stabilities of dA·dT and d(A-T)·d(A-T) have been determined at 0.1 M sodium ion as a function of pH. For both d(A-BrU) polymers and d(A-T) polymers, stability is affected by base sequence. The homopolymer pair is more stable than the copolymer pair at all pH values and ionic strengths studied. Other polymer systems show no base sequence effect. Possible sources of the base sequence dependence are discussed.

Synthetic polynucleotides serve as model compounds for naturally occuring nucleic acids and afford the opportunity to study the reactivity of molecules having a relatively simple, repeating composition. Polymers can be studied which differ from one another in chemically simple ways. In this study, we have prepared the homopolymer dBrU[1] and the base-paired two- and three-stranded complexes dA·dBrU and dA·dBrU$_2$, and we have compared certain properties of these polymer complexes to those of closely related polymer complexes which differ only in the 5-carbon pyrimidine substituent or the 2′-hydroxy sugar substituent or in base sequence alone.

The properties of deoxy polymers containing BrU are of interest since BrU has been used extensively in biological experiments in several different connections. When BrU is incorporated into DNA, it acts as a mutagen, it increases the density of the DNA, and it sensitizes the DNA to uv inactivation *in vivo*. The effects on the properties and reactivities of DNA containing BrU should be understood in order to allow most effective use of BrU as a tool in biological experiments. BrU increases the thermal stability of DNA and synthetic polynucleotides. Certain aspects of this stabilization have been studied in the work reported here, with emphasis on the effect of base sequence on the increase in thermal stability.

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[1] Abbreviations used are: A, BrU, U, T: adenine, bromouracil, uracil, and thymine, respectively. Deoxynucleoside triphosphates, dXTP. Abbreviations for the synthetic polynucleotides and polynucleotide complexes are those of the IUPAC–IUB Commission (*Biochemistry 9*, 4025 (1970)), for example, dA·dBrU$_2$ denotes the three-stranded homopolymer complex composed of one polydeoxyadenylate strand and two polydeoxybromouridylate strands, while d(A-BrU) denotes the deoxy copolymer containing adenine and bromouracil in strictly alternating sequence. Classes of polymer complexes are also referred to. The "deoxy A-BrU polymers" refers to both copolymer and homopolymer complexes. T_m = the temperature at the midpoint of an absorbancy transition, pH_m = the pH at the midpoint of an absorbancy transition. ϵ_P is the molar extinction coefficient relative to phosphorus. The symbol 2 → 3 represents a transition from a two- to a three-stranded complex, with similar connotation for other types of transitions.

Materials

The nucleoside triphosphate dBrUTP was prepared by bromination of dUTP using a method similar to that described by Chamberlin (1962). The lithium salt of dUTP (10 μmoles) was dissolved in 0.5 ml of formamide. Five per cent bromine in CCl_4 was added dropwise to the solution until the bromine color persisted. After 3 min at room temperature, one drop of water-saturated phenol was added, the solution was chilled, and 4 ml of ice-cold ethanol was added to precipitate dBrUTP. After standing overnight at $-5°$, the mixture was centrifuged for 21 min at 17,300g and the precipitate was dissolved in water. Chromatographic purification was carried out at 5°. A column of Dowex 1 (Cl) (26 cm $\times$ 0.8 cm^2, 10% cross-linking) was made and washed thoroughly with 0.01 N HCl. The dBrUTP preparation was applied to the column. Elution was stepwise, using 30 ml of 0.01 N HCl, 100 ml of 0.1 N HCl–0.2 N NaCl, and 100 ml of 0.5 N HCl–0.5 N NaCl. The dBrUTP appeared in the final eluate. Optically pure fractions were pooled and neutralized. The nucleotide was concentrated and desalted by Ba precipitation and converted to the K^+ salt by treatment with Dowex 50 (K^+). The dBrUTP prepared had the following optical properties in 0.01 N HCl: λ_{max} 280; OD_{280}/OD_{260} = 1.91, OD_{290}/OD_{260} = 1.53, OD_{250}/OD_{260} = 0.50. The $\epsilon_{P,280}$ at pH 2 was determined to be 7.6×10^3, indicating a possible contamination by a nonultraviolet-absorbing phosphorylated compound (Duval and Ebel, 1964). Incorporation of the contaminant into dBrU polymer probably does not occur since the $\epsilon_{P,279}$ of the dBrU polymer was found to be 8.04×10^3 (see Results), suggesting that nonultraviolet-absorbing material in the dBrUTP preparation was not utilized by DNA polymerase. The apparent pK_a of dBrUTP as determined by spectrophotometric titration was 8.25.

DNA polymerase was purified from *Escherichia coli* B according to the procedure of Jovin *et al.* (1969). Fraction 7 was used in all procedures described below. The preparation had a specific activity of 9600 units/mg of protein and was stored in liquid nitrogen.

The homopolymer complex dA·dT was synthesized as described previously (Riley *et al.*, 1966), using rA·rU as template for DNA polymerase.

The homopolymer complex dA·dBrU was synthesized using dA·dT as template for DNA polymerase, with dATP and dBrUTP supplied as substrates. The dA·dT preparation used was highly viscous and proved to be a poor template for dA·dBrU synthesis unless "activated" by treatment with pancreatic DNase. The reaction mixture contained 0.82 μmole of dA·dT, 0.03 μg of pancreatic DNase (Worthington one-time crystallized), 700 μmoles of Tris buffer (pH 7.0), 70 μmoles of $MgCl_2$, and 17 μmoles of mercaptoethanol in a final volume of 3.8 ml. The mixture was incubated at 45° for 30 min, then heated at 65° for 15 min. The DNase was completely inactivated by the heat treatment. Although its viscosity was reduced, the dA·dT retained high molecular weight after this treatment ($\geq$25 S) but is assumed to have sustained single-strand breaks. For the synthesis of dA·dBrU, 5 μmoles of dATP, 5 μmoles of dBrUTP, and 1100 units of DNA polymerase were added and the volume was brought to 14 ml. The mixture was incubated further at 45°. The course of the reaction was followed by monitoring the absorbance at 260 and 280 mμ. When the absorbance reached a minimum, usually after 150 min, the reaction was terminated by the addition of 280 μmoles of EDTA (pH 7). Longer incubation often led to contamination of the preparation with the alternating copolymer dABrU. The reaction mixture was dialyzed against two changes each of 5.0 M NaCl–0.1 M EDTA, 0.1 M NaCl–10^{-5} M EDTA, and finally 0.01 M Tris (pH 7.0)–10^{-5} EDTA. The preparation was examined by Cs_2SO_4 density gradient equilibrium centrifugation in the analytical ultracentrifuge at both neutral and alkaline pH. At pH 7.0, the dA·dBrU product was widely separated from the dA·dT template (ρ = 1.60 and 1.42, respectively). At pH 10, the dA·dBrU complex was dissociated. Using an input density of ρ = 1.50, the dissociated homopolymer dA was visualized at 1.39. Only those preparations were used which had no detectable material banding at the density of alkali-denatured copolymer d(A-BrU) (ρ = 1.56). (Under the experimental conditions used, the limit of detection of contaminating material was about 3%). The dissociated homopolymer dBrU was visualized at a density of approximately 1.72 in a separate experiment using higher Cs_2SO_4 concentrations. The homopolymer strands were separated on a preparative scale by centrifuging the dialyzed reaction mixture in Cs_2SO_4 at ρ = 1.50 and pH 10.0 in a SW-41 Ti Spinco rotor at 148,000g at 20° for 3 days. Tubes were punctured and the dBrU and dA fractions recovered were dialyzed as described above, then stored in a liquid nitrogen refrigerator. Yields of dA and dBrU after strand separation were 1.5 and 1.8 μmoles, respectively.

The alternating copolymers d(A-T) and d(A-BrU) were prepared in a primed synthesis with DNA polymerase using the procedures of Schachman *et al.* (1960) and Wake and Baldwin (1962). The alternating copolymers r(A-U) and r(A-BrU) were the kind gift of Dr. Michael Chamberlin.

Methods

Thermal melting experiments were carried out in a thermostatically controlled cell housing in a Zeiss PMQ II spectrophotometer, essentially as described earlier (Inman and Baldwin, 1962; Riley *et al.*, 1966). To ensure attainment of temperature equilibrium in the sample solution, at least 15 min was allowed between measurements. Polymer solutions were dialyzed against the chosen solvent overnight before use. Sodium phosphate buffer was used at pH values below 8.0, sodium borate buffer was used at pH values of 8.0 and above. The pH of the solution was measured both before and after thermal melting. Sodium phosphate and sodium borate concentrations are expressed in terms of sodium ion concentration. Sodium phosphate buffers contained 0.01 M sodium phosphate and additional NaCl to give the stated sodium ion concentration. Sodium borate buffers contained only borate as the anion. All buffers contained 10^{-5} M NaEDTA.

pH melting experiments were carried out in 3-ml cuvets in the spectrophotometer using microelectrodes, titrating the polymer solutions with NaOH as described previously (Riley and Paul, 1970).

"Mixing curves" were determined by a modification of the method of continuous variations (Job, 1928) as previously described (Riley *et al.*, 1966). At least 10 min was allowed between additions.

Nucleic acid concentrations are given in terms of total nucleotide phosphate. In some cases, results are presented as optical density per mole of the total nucleotide phosphate present in a mixture, or mean molar absorptivity [ϵ_P].

Results

The homopolymer dBrU had the following spectral proper-

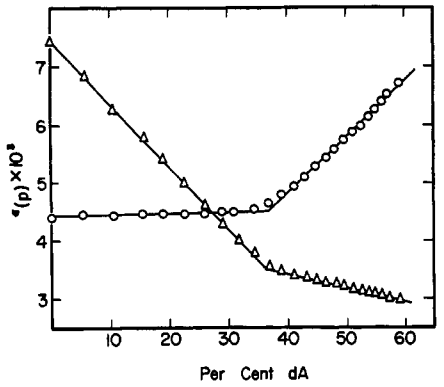

FIGURE 1: Mixing curve for dA and dBrU at pH 7.0 in 0.1 M sodium ion. Increasing amounts of dA were added to a solution of dBrU in 0.1 M sodium phosphate buffer (pH 7.0) at 25°. 10 min was allowed between each addition. (○) 260 mμ and (△) 285 mμ.

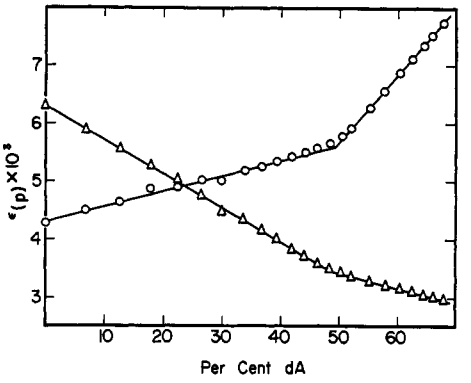

FIGURE 2: Mixing curve for dA and dBrU at pH 9.1 in 0.1 M sodium ion. Increasing amounts of dA were added to a solution of dBrU in 0.1 M sodium borate buffer (pH 9.1) at 25°. 10 min was allowed between each addition. (○) 260 mμ and (△) 285 mμ.

ties: ϵ_P at λ_{max} 279, 8.04×10^3 at pH 7; $OD_{280}/OD_{260} = 1.84$, $OD_{290}/OD_{260} = 1.46$, $OD_{250}/OD_{260} = 0.54$. The pK_a of the polymer, determined by spectrophotometric titration, was 8.6; $s_{20,w}$ at pH 7 was 17.5 S. Upon heating at neutral pH, in 0.1 M sodium ion, the polymer showed less than 5% change in absorbance at λ_{max}. This fact suggests that dBrU possesses little or no order under these conditions.

Determination of the extinction coefficient of the preparations of dA used in these studies showed that the previously reported value of $\epsilon_{P,260}$ for dA of 8.4 (Riley *et al.*, 1966) was low. We have used the value of $\epsilon_{P,260} = 10.0$ as reported by Ts'o *et al.* (1966) in these experiments.

Mixtures of dA and dBrU in 0.1 M sodium ion exhibited hypochromicity, indicating the formation of one or more ordered complexes. The degree of hypochromicity observed upon mixing dA and dBrU in 1:1 and 1:2 proportions at pH 7 in 0.1 M sodium ion was 20 and 27% respectively at 260 mμ, and 30% in both cases at 280 mμ.

The stoichiometry of complex formation was investigated by determining "mixing curves" in 0.1 M sodium ion at two pH values. When dA was added to dBrU at pH 7.0, a single break was observed at either 260 or 280 mμ at about 36% dA (Figure 1), indicating formation of the three-stranded complex dA·$dBrU_2$. The absence of a discontinuity at 50% dA implies either that the two-stranded complex dA·dBrU did not form under the conditions of the experiment or that the spectral relationships are such that the mean molar absorptivity of dA·dBrU is equal to that of 0.5dA·$dBrU_2$ + 0.5dA at the wavelengths observed. A 1:1 mixture of dA and dBrU was prepared and stored for 2 weeks at 15°. Over this interval of time, little if any change ($\lesssim$3%) in the optical absorbance of the sample was detected. Presence or absence of a 1:1 complex cannot be concluded from this observation. In the case of rA + rU, the mean molar absorptivity of 0.5rA·rU_2 + 0.5rA is equal to that of rA·rU at 260 mμ (Stevens and Felsenfeld, 1964; Blake *et al.*, 1967); thus no discontinuity is visible at 33% rA in a mixing curve monitored at 260 mμ. In the case of rA + rBrU, the mean molar absorptivity of 0.5rA·$rBrU_2$ + 0.5rA is nearly the same as that of rA·rBU at all wavelengths over the range 220–300 mμ (Riley and Paul, 1970). Thus absence of a discontinuity at 50% dA in the mixing curve for dA and dBrU does not give information on the formation of a two-stranded complex under these conditions.

When a mixing curve was carried out at pH 9.1, a single discontinuity was observed at 50% dA (Figure 2), indicating the formation of the two-stranded complex dA·dBU but no formation of the three-stranded dA·dBU_2 under these conditions.

The simplest interpretation of these observations is that (1) both two- and three-stranded complexes form at pH 7 at appropriate ratios of dA and dBrU, (2) one strand of dBrU is dissociated from dA·$dBrU_2$ with a pH_m between 7.0 and 9.1, and (3) only the two-stranded complex is stable at pH 9.1.

Spectral measurements are consistent with this view. Spectra of 1:1 and 1:2 mixtures are given in Figure 3. The mean molar absorptivities of dA·dBrU were compared to those calculated for 0.5dA·BrU_2 + 0.5dA and were found to be nearly the same over the wavelength range 220–300 mμ.

To test the hypothesis that both dA·dBrU and dA·$dBrU_2$ are stable in 0.1 M sodium ion at pH 7, dA and dBrU were mixed in both 1:1 and 1:2 ratios at 5° and these solutions were subjected to temperature melting. The profile of the melting curve for the 1:1 mixture showed the single spectral transition expected for the dissociation of two-stranded dA·dBrU (2 → 1) (Figure 4a). The profile of the melting curve for the 1:2 mixture showed two transitions referable to a two step dissociation of three-stranded dA·dBU_2 (3 → 2, 2 → 1) (Figure 4b). The T_m of the second step of the melting

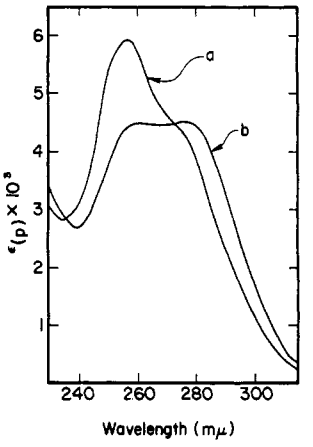

FIGURE 3: Spectra of dA:dBrU and dA:$dBrU_2$ in 0.1 M sodium phosphate buffer (pH 7) at 25°. (a) dA:dBrU and (b) dA:$dBrU_2$.

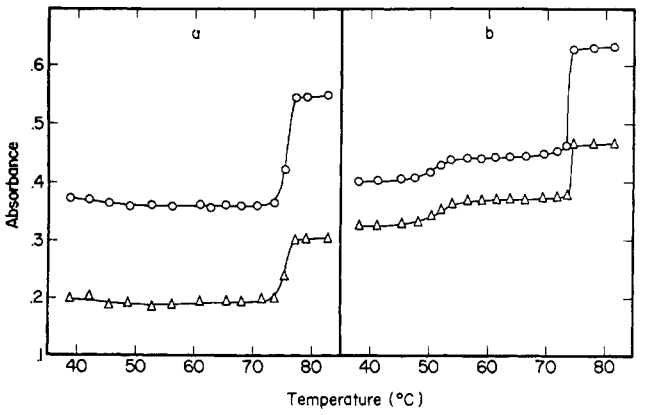

FIGURE 4: Temperature meltings of 1:1 and 1:2 mixtures of dA and dBrU in 0.1 M sodium phosphate buffer (pH 7.1). (a) 1:1 mixture and (b) 1:2 mixture. (○) 260 mμ and (△) 285 mμ.

TABLE I: Thermal Stabilities of Homopolymer and Copolymer Polynucleotide Complexes.[a]

Polymer System	T_m (copolymer) (°C)	T_m (homopolymer) (°C)	ΔT_m[b] (°C)
r(A-U)	66	56	+10
d(A-U)	58[c]	52[d]	+6
d(A-T)	61	68.5	−7.5
d(A-BrU)	69.5	76.5	−7

[a] All determinations were made in 0.10 M sodium ion at pH 7. [b] T_m(copolymer) − T_m(homopolymer). [c] Data from Chamberlin *et al.* (1963). [d] Data from Zmudzka *et al.* (1969).

of the three-stranded complex was the same as the T_m of the single step of the melting of the two-stranded complex. That this transition is indeed a 2 → 1 transition rather than a 3 → 1 transition is shown by the fact that the magnitude of the salt dependence of the transition is characteristic of 2 → 1 transitions rather than 3 → 1 transitions (see below). Thus, as in other synthetic homopolymer systems, the nature of the complex formed is dictated by the stoichiometry of the mixture, and 1:1 mixtures of dA and dBrU in 0.1 M sodium ion at pH 7 contain the two-stranded complex, dA·dBrU.

There is no evidence in the melting profile of the two-stranded complex for a 2 → 3 type of disproportionation reaction as was observed for rA·rBrU in 0.1 M sodium ion at neutral pH (Riley and Paul, 1970). The simple 2 → 1 transition observed upon heating dA·dBrU is unlike the 2 → 3 → 1 sequence seen for rA·rBrU. Entirely consistent are the differences seen upon heating the two three-stranded complexes. Whereas dA·dBrU$_2$ undergoes a two-step 3 → 2 → 1 dissociation, rA·rBrU$_2$ melts with a single 3 → 1 dissociation.

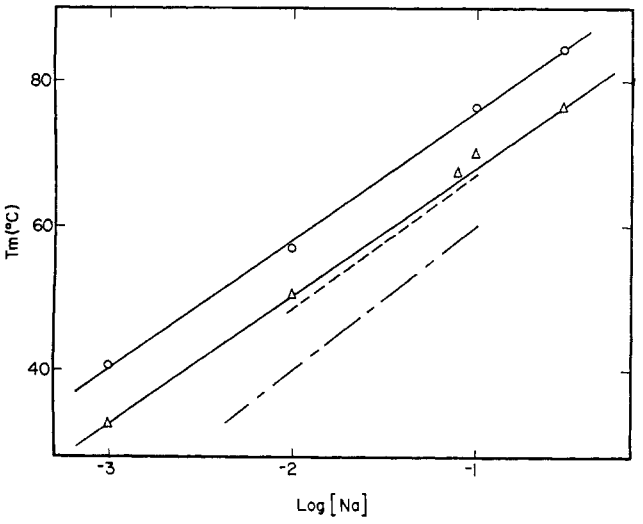

FIGURE 5: Variation of T_m with sodium ion concentration for d(A-BrU) and d(A-T) polymer complexes. Sodium phosphate buffer at pH 7 was used for all determinations. (○) dA·dBrU, (△) d(A-BrU)·d(A-BrU); (- - -) dA·dT, from data Riley *et al.* (1966), and (– – –) d(A-T)·d(A-T), data from Inman and Baldwin (1962).

Thus, at 0.1 M sodium ion and pH 7, dA·dBrU undergoes a simple 2 → 1 dissociation, while the rA·rBrU does not.

The availability of the homopolymer pair dA·dBrU and the alternating copolymer d(A-BrU)·d(A-BrU), both capable of a simple 2 → 1 helix–coil transition, made possible a study of the effect of base sequence on the dissociation of two-stranded deoxy polymer complexes containing only A and BrU. The analogous two-stranded polymer complexes containing A and T have also been examined from this point of view.

The thermal stabilities of dA·dBrU and d(A-BrU)·d(A-BrU) were determined at pH 7 at sodium concentrations ranging from 10^{-3} to 0.7 M Na (Figure 5). The slope, dT_m/d log [Na], had a value of 18° for both polymer complexes. The departure from linearity observed earlier for d(A-BrU) (Inman and Baldwin, 1962) may have resulted from utilization of a preparation of lower molecular weight than the preparation used in these experiments. The value of dT_m/d log [Na] for the two-stranded deoxy A-BrU homo- and copolymers is about the same as that determined previously at pH 7 for other two-stranded synthetic polymer systems and for double-stranded DNA (Record, 1967a). It would appear, then, that the electrostatic forces affecting the helix–coil transitions of the deoxy A-BrU polymer complexes are not remarkable and are similar in magnitude to those observed for other two-stranded polynucleotides.

Although salt dependence is normal, the relative magnitude of the thermal stabilities of the homo- and the copolymer complexes in both deoxy A-BrU and deoxy A-T systems is different from that observed in other polymer systems. In the case of ribo and deoxy polymer complexes containing A and U, the thermal stability of the copolymer is greater than that of the homopolymer (Richards and Simpkins, 1968; Zmudzka *et al.*, 1969; Chamberlin *et al.*, 1963). However, in the case of deoxy A-BrU and A-T polymers, the T_m value for each copolymer complex is *less* than the T_m of the corresponding homopolymer complex at all salt concentrations examined. Representative T_m values determined at 0.1 M sodium ion and neutral pH are presented in Table I.

The thermal stabilities of the deoxy A-BrU and A-T polymers were studied further as a function of pH at constant ionic strength. T_m values were determined as a function of pH in 0.1 M sodium ion, and pH_m values were determined by alkaline titration at room temperature (23 ± 2°). These data are presented in Figure 6. At pH 7, the T_m values for the polymer complexes containing BrU are higher than those for

TABLE II: Alkaline Titration Characteristics of Homopolymer and Copolymer Complexes.

	pK_a[a]	pH_m	$pH_m - pK_a$	$\Delta(pH_m - pK_a)$[b]
d(A-T)·d(A-T)	10.0	11.06	1.06	−0.07
dA·dT		11.13	1.13	
d(A-BrU)·d(A-BrU)	8.25	9.57	1.32	−0.13
dA·dBrU		9.70	1.45	
r(A-U)·r(A-U)[c]	9.8	10.9	1.1	
rA·rU[c]	9.7	10.4	0.7	0.4
r(A-BrU)·r(A-BrU)[d]	8.5	10.1	1.6	0.4
rA·rBrU[d]		9.7	1.2	

[a] pK_a values for the deoxy polymers are those of the pyrimidine nucleoside phosphates. The value for dTMP is taken from Hurst *et al.* (1953). The value for dBrUTP was determined in the course of this study. pK_a values for the ribo polymers are those of the pyridmidine homopolymer. [b] $(pH_m - pK_a)$(copolymer) − $(pH_m - pK_a)$(homopolymer). [c] Data taken from Richards and Simpkins (1968). [d] Data taken from Riley and Paul (1970).

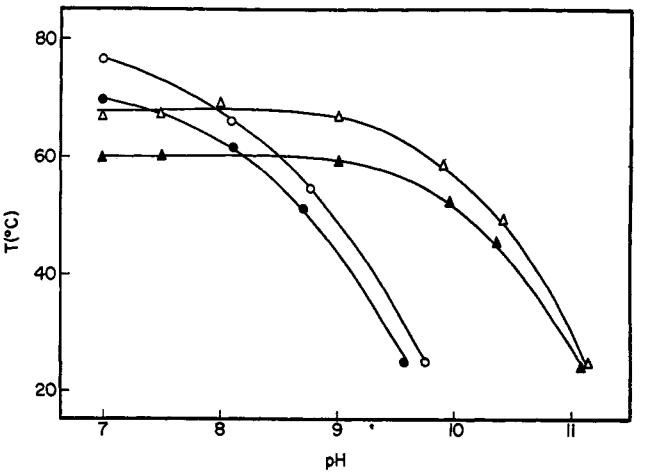

FIGURE 6: Variation of T_m with pH for d(A-BrU) and d(A-T) polymer complexes. All solutions contained 0.1 M sodium ion. Values at 24–25° were determined by titration of unbuffered solutions with NaOH. All other values were obtained by heating buffered solutions. (○) dA·dBrU, (●) d(A-BrU), (△) dA:dT, and (▲) d(A-T).

polymer complexes containing T. At higher pH values, complexes containing T become more stable than complexes containing BrU, a consequence of the difference in the pK_a values of dBrUMP and dTMP. The complexes containing T have higher pH_m values than do complexes containing BrU.

The values of $pH_m - pK_a$ are greater for the deoxy A-BrU polymers than for the deoxy A-T polymers (Table II), reflecting the greater stability of the d(A-BrU) base pair as compared to the d(A-T) base pair. This observation is in line with expectations arising from theoretical treatment of polynucleotides as ionizable polyelectrolytes (Record, 1967b).

As at pH 7, for both pairs of polymer complexes, the homopolymer complex is more stable than the copolymer complex at all pH values up to and including the pH_m. The T_m's tend to converge as the pH approaches the pH_m, and this effect is more pronounced in the case of the T polymers, but the T_m of the homopolymer complex remains higher than that of the copolymer complex throughout this pH range, and the value $(pH_m - pK_a)$(copolymer) − $(pH_m - pK_a)$(homopolymer) is negative (Table II). These results are to be contrasted to the findings for the ribo A-U and ribo A-BrU polymers. In both these systems, the value $(pH_m - pK_a)$(copolymer − $(pH_m - pK_a)$(homopolymer) is positive (Richards and Simpkins, 1968; Riley and Paul, 1970; Table II).

In summary, the deoxy A-BrU and deoxy A-T polymers differ from other synthetic polymers containing A and a pyrimidine in that both the values of T_m(copolymer–homopolymer) and $(pH_m - pK_a)$(copolymer–homopolymer) are negative rather than positive.

Discussion

Both two- and three-stranded deoxy homopolymer complexes containing A and BrU have been prepared and studied. Some of the characteristics of this system can be compared to those of other base-paired polynucleotide complexes.

The deoxy A-BrU homopolymer complexes differ from the ribo A-BrU complexes in that the stability of the three-stranded complex relative to the two-stranded complex is not as great in the deoxy system as in the ribo system. In the ribo system, a 2 → 3 disproportionation reaction occurs at 0.1 M sodium ion and at concentrations as low as 10^{-3} M sodium ion (Riley and Paul, 1970; M. Riley, unpublished observations). In the deoxy system, no 2 → 3 disproportionation reaction occurs at 0.1 M sodium ion, and the relatively large difference between the T_m's of the 3 → 2 and 2 → 1 transitions leads one to predict that no 2 → 3 transition will occur in solutions at sodium concentrations of 1.0 M or less.

A similar situation exists in the case of the ribo and deoxy A-U homopolymers. For the ribo A-U polymers, a 2 → 3 reaction takes place at sodium ion concentrations greater than 0.15 M (Stevens and Felsenfeld, 1964). For the deoxy A-U polymers, the 2 → 3 reaction only occurs at sodium ion concentrations of 0.8 M or higher (Zmudzka *et al.*, 1969). A greater degree of counterion charge neutralization seems necessary to maintain a three-stranded deoxy homopolymer complex as opposed to a three-stranded ribo homopolymer complex.

Both bromine and methyl group substituents at the pyrimidine 5-carbon confer stability on polynucleotide complexes. Both thermal stability (Table I) and stability to alkali denaturation (Table II) are affected in the order U < T < BrU. This stabilization may be composed of both proximity-dependent and -independent components. The data presented here indicate that at least one component of the stabilization is base sequence dependent.

In the case of the ribo A-U polymers, it has been shown that thermal stabilities are not grossly affected by base sequence. The relationship between the T_m values for the ribo A-U homopolymer and copolymer complexes has been analyzed by Richards and Simpkins (1968). These authors showed that the free-energy changes of the homopolymer and copolymer helix–coil transition reactions are expected to differ to the extent that the dissociated homopolymer rA retains partial order, whereas the dissociated copolymer r(A-U) is not ordered. The expected difference in ΔG values was evaluated and was found to correspond well with the observed value of ΔT_m(copolymer–homopolymer) of +6°. The authors have

TABLE III: Spectral Characteristics of Copolymer Coils.

Copolymer	Extinction Coeff at λ_{max}		% Hypochromicity
	Coil	Nucleotides	
d(A-BrU)[a]	8.5	9.8	13
d(A-T)[a]	9.6	12.3	22
r(A-U)[b]	10.4	12.4	16

[a] Data from Inman and Baldwin (1962). [b] Data from Chamberlin *et al.* (1963).

concluded that the stabilities of the ribo A-U complexes are not greatly affected by the base sequence of the polynucleotides.

The homopolymer complex dA·dU has recently been prepared and thermal stability studied as a function of ionic strength (Zmudska *et al.*, 1969). The alternating copolymer d(A-U) was prepared and studied earlier (Chamberlin *et al.*, 1963). The ΔT_m(copolymer–homopolymer) for the deoxy A-U polymer complexes is about the same as for the ribo A-U polymers (Table I). Thus, according to the analysis of Richards and Simpkins, the deoxy A-U polymers show little or no effect of base sequence on complex stability. The partial order present in single-stranded dA must account for the lower T_m of the homopolymer complex.

The deoxy A-BrU and A-T polymer complexes differ from the deoxy A-U polymers in that the ΔT_m(copolymer–homopolymer) value is negative. When adjusted for the free-energy difference in the products of the dissociation reaction, the dA·dBrU and dA·dT homopolymer complexes are more stable than the corresponding copolymer complexes to the extent of an increment in T_m of about 13°. Thus it appears that base sequence has a small but finite effect on the thermal stability of deoxy A-BrU and A-T polymer complexes.

The stabilities of the deoxy A-BrU and A-T polymers to alkaline denaturation are also anomalous. In the ribo A-U system, Richards and Simpkins (1968) have shown that a difference in $pH_m - pK_a$ values for copolymer and homopolymer of +0.4 can be accounted for in terms of the order present in dissociated rA strands, and that the experimental values are consistent with the absence of a base sequence effect on stability to alkaline denaturation. For the r(A-BrU) polymers, an experimental value of $\Delta(pH_m - pK_a)$ of +0.4 was found (Riley and Paul, 1970), indicating an absence of base sequence effect at alkaline pH values. However, for both the deoxy A-BrU and A-T polymers studied here, the values of $\Delta(pH_m - pK_a)$ are negative and about 0.5 pH unit from that expected if no base sequence effect were present (Table II).

Therefore, the stabilization of deoxy polymers due to a bromine atom or methyl group substituent on the pyrimidine 5-carbon exhibits a base sequence dependent component. This component is not operable in ribo A-BrU polymers at alkaline pH values, implying that the base-sequence-dependent component is not independent of the nature of the sugar moiety.

The difference in stability of the copolymer and homopolymer complexes might be caused either by an anomalously low stability of the copolymer complex or by an anomalously high stability of the homopolymer pair. Lowered stability of copolymer complexes might be due to the presence of partial order in the coil forms. This does not seem to be the case, however, since the difference between the mean molar absorptivities of the dissociated copolymer d(A-BrU) and d(A-T) and their component nucleotides is not significantly different from the case of r(A-U) (Table III), and yet the copolymer complex r(A-U)·r(A-U) is more stable than the corresponding homopolymer complex rA·rU.

Increased stability of the homopolymer pair might derive from a difference in molecular geometry. No X-ray diffraction studies have been made of the dA·dBrU homopolymer. The copolymers d(A-T) and d(A-BrU) are known to have a conformation similar to the B form of DNA (Davies and Baldwin, 1963), and the homopolymer complex dA·dT is reported to have a different structure (Langridge, 1967). Perhaps both the dA·dT and dA·dBrU homopolymer complexes assume a more stable conformation than do the corresponding two-stranded copolymers.

Alternatively, an increased stability of the homopolymer pair might derive from stacking interactions between adjacent pyrimidine residues. The bromine or methyl substituents may alter the polarizability of the pyrimidine in such a way as to lead to enhanced electrostatic interactions. This interaction must have geometric requirements which are met in a helical complex but are not present in the disordered coil since neither dBrU nor dT polynucleotides appear to possess a significant degree of order as free single strands at the cation concentrations used in these experiments.

Not all pyrimidine 5-carbon substituents stabilize the homopolymer complex. Both homopolymer and copolymer complexes containing 5-hydroxymethyluracil and adenine have been prepared by Patrick Cassidy. In this case, the ΔT_m(copolymer–homopolymer) in 0.1 M sodium ion at neutral pH is 26° (P. Cassidy, personal communication). The 5-hydroxymethyl substituent may offer steric hindrance to the assumption of a favorable conformation for the homopolymer complex.

A bromine substituent stabilizes polymer complexes containing cytosine. Both homopolymer and copolymer complexes containing the I–BrC base pair are more stable than the corresponding I–C complexes. In contrast with the finding here for A–BrU complexes, the stabilization conferred by bromine in the I–C system does not appear to exhibit base sequence dependence (Howard *et al.*, 1966).

In summary, we have found that the stabilization conferred either by the bromine atom or the methyl group on polymer complexes of the A-U type has a base-sequence-dependent component. This component might derive from differences in stacking interactions between adjacent pyrimidines in the ordered structure, or might derive from differences in molecular conformation. In either case, the nature of the sugar moiety must play a role, since ribo A-BrU polymers show no base sequence dependent effect at alkaline pH, while the deoxy A-BrU polymers do.

The observations made here may have pertinence to biological phenomena. There is an effect of neighboring base pairs on helix stability. It seems possible that sections of DNA containing runs of T (or BrU following experimental intervention) could exhibit properties that would distinguish these localized sections of the DNA from the bulk DNA. Perhaps the mechanisms of such phenomena as "position effects," "hot spots," or transcriptional punctuation can be attributed to such base-sequence-dependent differences.

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Studies on the Mechanism of Action of Aldosterone: Hormone-Induced Changes in Lipid Metabolism*

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ABSTRACT: Studies to elucidate the mode of action of aldosterone in the amphibian urinary bladder have been carried out. Within 20 min after its addition, aldosterone causes increased decarboxylation of [1-^{14}C]glucose and an alteration in the pattern of conversion of uniformly labeled [^{14}C]glucose into lipid, suggesting stimulation of the hexose monophosphate shunt and increased lipid synthesis. With [2-^{14}C]pyruvate as radioactive precursor aldosterone stimulates lipid labeling and increased incorporation of radioactive label into the 2 position of phospholipids during a 60-min incubation. No specific individual phospholipid class was preferentially labeled. Gas–liquid chromatography of the [^{14}C]fatty acids derived from phospholipids from toad bladders treated with aldosterone for 30 and 90 min revealed an increase in the specific activity of fatty acids of chain length up to 18:2. After 6 hr, phospholipid fatty acids from hormone treated tissue show an increase in the weight percentage and specific activity of several long-chain polyunsaturated fatty acids. In addition, pretreatment of bladders with a phospholipase A_2 containing solution significantly shortened the "latent period" observed before the aldosterone-induced increase in sodium transport. These results suggest that a fundamental action of aldosterone in the toad urinary bladder is to alter the fatty acid metabolism of membrane phospholipids.

The toad urinary bladder has been used as an *in vitro* system to elucidate the mode of action of the steroid hormone aldosterone (Sharp and Leaf, 1966; Edelman and Fimognari, 1968). Addition of aldosterone leads to an increase in the active transepithelial transport of sodium after a latent period of 45–120 min. Because the observed augmentation of sodium transport is prevented by pretreatment with actinomycin D or puromycin, the hormone has been thought to act *via* the synthesis of new RNA and protein molecules (Edelman *et al.*, 1963; Porter *et al.*, 1964). Two proposals have been advanced to explain the function of the hormonally induced protein(s): (1) the hormone increases sodium permeation across the mucosal cell surface; (2) the hormone increases the supply of energy for the active extrusion of sodium across the serosal cell surface (Sharp and Leaf, 1966; Edelman, 1968).

Our interest in the problem of aldosterone action arose from the results of an investigation of the effects of hyperbaric oxygen on toad bladder function (Allen and Rasmussen, 1971). In this study, it was observed that under appropriate metabolic conditions, high partial pressures of atmospheric oxygen led to a reversible inhibition of transcellular sodium transport. This inhibition was greatly potentiated by prior treatment of the tissue with aldosterone. Since the intracellular level of ATP rose, this indicated that the inhibition of transport resulted from either an inhibition of Na^+–K^+-activated ATPase or an inhibition of Na^+ entry. Work in other systems (O'Malley *et al.*, 1966; Schauenstein, 1967; Haugard, 1968) had led to the concept that lipid peroxide formation may be an important aspect of the biochemical events underlying oxygen toxicity. It therefore seemed possible that the potentiation of poisoning by aldosterone in the toad bladder might be due to an alteration in the lipid composition of the bladder cell membranes. In addition, aldosterone treatment causes an alteration of two other membrane-related processes. Goodman *et al.* (1969) found that pretreatment of the bladder with aldosterone led to the potentiation of the action of the peptide

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