Poly(8-bromodeoxyadenylic acid): Properties of the Polymer and Contrast with the Ribopolynucleotide Analogue†

Eiko Nakagawa Kanaya, Frank B. Howard, Joe Frazier, and H. Todd Miles*

ABSTRACT: Introduction of the bulky 8-bromo substituent into adenine residues of polynucleotides has strikingly different consequences in the deoxy- and ribopolynucleotide series. Poly(r8BrA) was found in earlier studies to form a very stable double-helical self-structure but not to undergo interaction with potentially complementary polynucleotides. We find that poly(d8BrA), in contrast, does not form an ordered self-structure in 0.1 M Na^+ but appears to exist as an electrostatically expanded rigid rod with unusual circular dichroism (CD) properties at very low ionic strength. The deoxy polymer, moreover, readily forms double helices with either deoxy or ribo pyrimidine polynucleotides, studied by UV, CD, and IR spectroscopy. These complexes are destabilized, relative to those formed by poly(dA), possibly because energy is needed to convert the purine residues from a more stable syn to an anti conformation, required for heteroduplex formation. The CD spectrum of $(d8BrA)_n{\cdot}(dT)_n$ is similar to that of B DNA. The deoxy–ribo hybrids $(d8BrA)_n{\cdot}(rU)_n$ and $(d8BrA)_n{\cdot}(rBrU)_n$ have CD spectra resembling those of A DNA or RNA. Unlike other deoxy–deoxy pairs $(d8BrA)_n{\cdot}(dBrU)_n$, however, has a CD spectrum resembling RNA and other helices having the A form.

Bulky substituents in the 8-position of purine nucleotides tend to shift the rotational equilibrium about the glycosidic bond from the normally favored anti to the syn range of conformations [cf. Ikehara et al. (1969), Michelson et al. (1970), Tavale & Sobell (1970), and Howard et al. (1974)]. Studies of poly(8-bromoriboadenylic acid) showed it to have an all-syn conformation and revealed surprising properties of the ribo homopolymer (Howard et al., 1974, 1975; Govil et al., 1977, 1981). Poly(r8BrA) forms a highly stable self-structure (T_m = 57 °C in 0.1 M Na^+). The ordered form is helical and mutually hydrogen bonded at N1 and N6. The polymer is highly stacked in both the double-stranded and single-stranded forms. The homopolymer, however, exhibits no interaction with either poly(rU) or poly(rBrU).

Studies of natural and synthetic nucleic acids have shown that, whereas RNA duplex helices have only the A or A′ conformation, DNA helices can have a variety of structures: A, B, C, D, Z, and variants of these [cf. Sundaralingam (1973), Sasisekharan (1973), Chandrasekharan et al. (1980), Wang et al. (1979), Drew et al. (1980, 1981), and Zimmerman (1980) and references cited therein]. Deoxyribose evidently permits in DNA polymers a wider range of torsional angles or combinations of torsional angles, which allow a greater degree of bending or flexibility in deoxy- than in ribopolynucleotides. We have therefore prepared and investigated the deoxypolymer poly(d8BrA) to see whether the unusual properties of the ribo analogue are a necessary consequence of 8-bromo substitution or whether quite different macromolecular conformations may result from introduction of the deoxyribose moiety.

Materials and Methods

dATP (20 mg) was brominated by the procedure of Ikehara & Uesugi (1969) with saturated bromine water (0.31 mL) in 1 M acetate buffer (1.3 mL, pH 4.0). After 16 h, $NaHSO_3$ (5 mg) was added, and the solution was diluted to 20 mL and applied to a column of desalting charcoal (Wako Jungaku Kogiyo Co. Ltd., Osaka, Japan; 5 mL), which was washed with water (1 L). The nucleotide was eluted with ethanol–water–concentrated ammonia (50:48:2; 100 mL) and the solvent evaporated. Recovery was 300 AU at 263 nm or about 60% yield from dATP.

Initial experiments at 25 °C indicated that the acid chromatographic solvent below resulted in extensive depurination of the deoxynucleotide. Chromatography in a cold room (6 °C) largely avoided this problem.

The nucleotide recovered from charcoal was applied to a column (0.9 × 5 cm) of anion-exchange resin (Bio-Rad AG 1-X2) (chloride form, 100–200 mesh). After being washed with water, the column was eluted (cold room) with a linear gradient of 1 L of 3 mM HCl solution and 1 L of 5 mM NaCl plus 3 mM HCl. Fractions (22 mL) corresponding to 1474–1760 mL of (190 AU) eluate were pooled and desalted with charcoal. Recovery was 156 AU.

The product was completely dephosphorylated with crude venom of *Crotalus adamanteus* to give 8-bromodeoxyadenosine [R_f 0.47 on silica gel thin-layer chromatography (TLC), $CHCl_3$–MeOH, 10:1]. No deoxyadenosine (R_f 0.19) was detected. The following chromatographic information was also used to monitor the reaction and purification. R_f values on silica gel system noted above were as follows: 8-bromoadenine, 0.41; deoxyadenosine, 0.26; 8-BrdATP, 0. R_m values on cellulose thin-layer electrophoresis (50 mM triethylammonium bicarbonate, pH 7.5, 500 V, 8 mA, 1 h) were as follows: 8-bromoadenine, 0.16; deoxyadenosine, –0.11; dATP, 1.0; d8BrATP, 0.95.

Polymerization. 8-BrdATP was incubated with terminal deoxynucleotidyl transferase (TdT; Bollum, 1966; Willis et al., 1980) at 34 °C for 60 h. Reaction mixtures (total volume 0.5, 1, or 2 mL) contained the following materials at the indicated concentrations: TdT, 600 units/mL (Willis et al., 1980); 8-BrdATP, 2.5 mM; $p(dA)_7$, 0.6 AU/mL; 2-mercaptoethanol, 2 mM; potassium phosphate, pH 7, 5 mM; potassium chloride, 80 mM; potassium cacodylate, pH 7.5, 200 mM; magnesium chloride, 8 mM; zinc sulfate, 0.34 mM; ethylenediaminetetraacetic acid (EDTA), 0.17 mM; bovine serum albumin (BSA), 0.84 mg/mL; glycerol, 8.3% by volume.

The reaction was stopped by adding 5 μL/mL concentrated ammonia. After extraction with 0.87 mL of chloroform–isoamyl alcohol (62:25), the water solution was applied to 1

† From the Laboratory of Molecular Biology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Building 2, Room 201, Bethesda, Maryland 20205.
Received December 13, 1983.

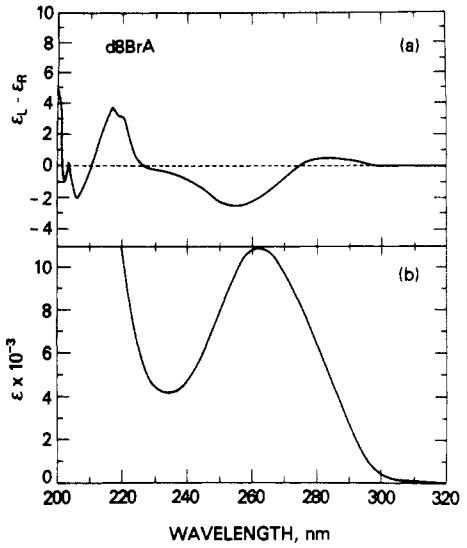

FIGURE 1: CD and UV spectra of poly(d8BrA) in 0.1 M Na^+–0.002 M sodium cacodylate, pH 7.0, T = 25 °C.

mL of DEAE-cellulose. The column was washed with 40 mL of 0.5 M NaCl to remove BSA [subsequently confirmed by IR analysis of D_2O solution; BSA has bands at 1652, 1572, and ~1550 (sh) cm^{-1}, which were absent in solutions of the polynucleotide]. The polymer was then eluted with 10 mL of 1 M NaCl, and salt was removed on a Sephadex G-50 column (2.8 × 55 cm). Yield was 18%, on the basis of triphosphate. Gel electrophoresis of polymer labeled at the 5′-end with ^{32}P showed the bulk of the material to have a chain length between 70 and 80 residues.

5-BrdUTP was polymerized with TdT at 34 °C for 60 h in solutions (1 mL) containing the following materials: TdT, 600 units/mL; 5-BrdUTP, 2 mM; $p(dT)_7$, 0.6 OD/mL; 2-mercaptoethanol, 0.2 mM; $CoCl_2$, 1.2 mM; potassium phosphate, 5 mM; potassium chloride, 40 mM; potassium cacodylate, 20 mM, pH 7.5; zinc sulfate, 0.34 mM; EDTA, 0.17 mM; BSA, 0.84 mg/mL; glycerol, 8.3% by volume. The reaction was stopped by adding 5 μL of concentrated ammonia. After extraction with 0.9 mL of chloroform–isoamyl alcohol (62:25), the aqueous solution was applied to a 10-mL column of DEAE-cellulose, chloride form. The column was washed with 40 mL of 0.4 M NaCl to remove BSA and 5-BrdUTP. The polymer was eluted with 50 mL of 1 M NaCl. Sephadex G-50 chromatography was used to remove NaCl. Yield was 37%. Absence of BSA was confirmed by infrared spectroscopy.

IR spectra were measured with a Perkin-Elmer 580B spectrophotometer interfaced to an LDACS distributed computer system (Powell et al., 1980). UV spectra and melting curves were measured with a Cary 118 spectrophotometer interfaced to and controlled by an LDACS computer. Circular dichroism (CD) spectra were measured with a JASCO J-500A spectropolarimeter, also interfaced to an LDACS computer.

Results

Poly(d8BrA). The UV spectrum of poly(8BrA) (Figure 1; Table I) in 0.1 M Na^+ has λ_{max} 262.2 nm and ϵ_{max} 10940, compared to monomer values of 263 nm and 15000. Poly(rBrA), in contrast, has λ_{max} 259 nm and ϵ_{max} 7200, both markedly changed from monomer values by stacking and hypochromism of an ordered self-structure (Howard et al., 1974). Temperature profiles of poly(dBrA) in 0.3, 0.1, and 0.03 M Na^+ are broad and noncooperative with an absorbance increase over the range 5–92 °C of ~16% (supplementary material Figure 1; see paragraph at end of paper regarding supplementary material). The poly(rBrA) profiles are cooperative and show absorbance increases in excess of 60% over the same temperature range (Howard et al., 1974).

Table I: Spectroscopic Data

material	Ultraviolet λ_{max} (nm)	ϵ_{max}	λ_{min} (nm)	ϵ_{min}
8-bromo-2′-deoxyadenosine	264		235	
$(d8BrA)_n$ (pH 7)	262.2	10940	234	4130
$(d8BrA)_n \cdot (rU)_n$	260.4	9000	233	3300
$(d8BrA)_n \cdot (dT)_n$	262.2	7940	235.6	2740

material	Circular Dichroism λ_{max} (nm)	$\epsilon_L - \epsilon_R$	λ_{min} (nm)	$\epsilon_L - \epsilon_R$
$(d8BrA)_n$	216	+3.6		
	284.4	+0.6	254	−2.8
$(d8BrA)_n \cdot (r5BrU)_n$			206	−13
	218.4	+2.0		
	262.4	11.9		
	~285 (sh)	+1		
	297	+0.5		
$(d8BrA)_n \cdot (d5BrU)_n$	218.8	+3.0	206	−9
	262.6	+7.2	246.8	−2.3
	~285 (sh)	~2		
	~298 (sh)	~1		
$(d8BrA)_n \cdot (rU)_n$	219.8	+2.7	206.6	−5.5
	263	+5.4	241.8	−0.6
$(d8BrA)_n \cdot (dT)_n$	220.6	+4.1	207	−4.0
	263	+0.4	250.6	−6.0
	281	+2.5	268.4	−0.6
	288.6	+3.1		

material	Infrared ν_{max} (cm^{-1})	ϵ_{max}
$(d8BrA)_n \cdot (dT)_n$ (1:1, pD 7.0, 5 °C)	1694	357
	1662.5	404
	1632	499
	1571	110
$(d8BrA)_n \cdot (rU)_n$ (1:1, pD 7.0, 6 °C)	1688.5	349
	1670	442
	1632	412
	1568	70

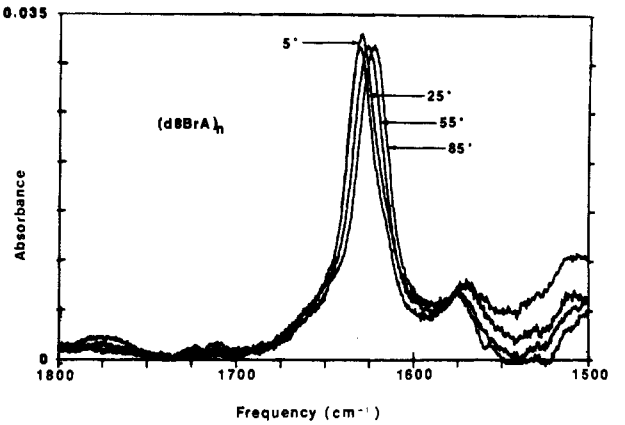

FIGURE 2: Infrared spectrum in D_2O of 0.0055 M poly(d8BrA), 0.1 M NaCl, 0.01 M sodium cacodylate, pD 7. The strong BrA ring vibration at 1631.5 cm^{-1} (5 °C) shifts to slightly lower frequency at higher temperature, but the intensity is independent of temperature. The spectrum of 8BrADP is very similar, but the spectrum of poly(r8BrA) exhibits splitting of the ring vibration and a large reduction in intensity (Howard et al., 1975). Molar absorbance of the strong band is 1090 L mol^{-1} cm^{-1} at 25 °C.

The infrared spectrum of poly(dBrA) in 0.1 M Na^+ shows a single strong band at 1631 cm^{-1} at 4 and 25 °C, which shifts slightly to lower frequency with increasing temperature (Figure 2). This spectrum (and its temperature dependence) is similar to that of the monomer 8-BrADP and totally different from that of poly(r8BrA), which exhibits a splitting of the ring

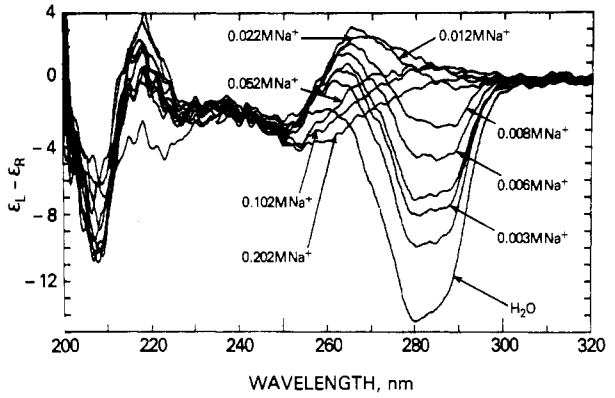

FIGURE 3: CD spectra of poly(d8BrA). The lowest curve is measured in distilled water. All other solutions are buffered with 0.002 M sodium cacodylate, pH 7.0, and contain varying amounts of NaCl. Figures refer to total Na^+ contributed by both salts. T = 25 °C.

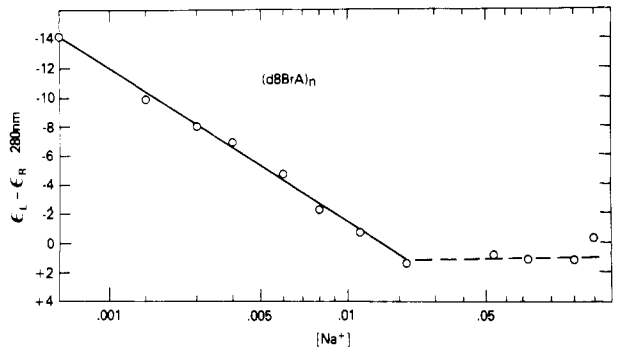

FIGURE 4: Dependence of intensity of 280-nm extrumum on log [Na^+]. Dependence is linear over the range $\sim 10^{-4}$–10^{-2} and becomes independent of [Na^+] above $\sim$0.02 M Na^+.

vibration (ν_{max} = 1632 and 1617 cm^{-1}) below the melting range of the helix (Howard et al., 1974, 1975).

The CD spectrum of poly(d8BrA) exhibits a striking dependence on [Na^+] at very low ionic strength (<10^{-3}–10^{-2} M Na^+) (Figure 3), and spectra in this range are entirely different from that observed in 0.1 M Na^+ (Figure 1). The magnitude of the intense negative band at 280 nm exhibits a linear dependence on log [Na^+] over the range 10^{-3}–10^{-2} M Na^+ and above 0.02 M Na^+ becomes relatively constant (Figure 4).

Interaction of Polynucleotides. (A) Electronic Spectra. In view of the failure of poly(r8BrA) to interact with other polynucleotides, one of our first objectives with poly(d8BrA) was to determine whether it formed double helices with appropriate pyrimidine polymers. Ultraviolet spectra of 1:1 mixtures of poly(d8BrA) with each of the polymers poly(dT), poly(rU), poly(rBrU), and poly(dBrU) were measured and compared with computer summations of the two components measured separately under the same conditions. Similarly, CD spectra of 1:1 mixtures were measured and compared with computer summations of the components (Figures 5 and 6). In all cases, marked departure of the experimental curves from the summations clearly indicated that interaction had occurred. Further conclusions from these spectra are presented under Discussion.

(B) Infrared Spectra. Infrared spectra of polynucleotides are quite distinct from one another and have resolved bands that can be assigned to vibrations of particular parts of the bases such as the carbonyl groups. The spectra are highly sensitive to base-pairing interactions and are used here to demonstrate specific complex formation and to provide evidence on stoichiometry [cf. Miles (1971, 1978) and references cited therein].

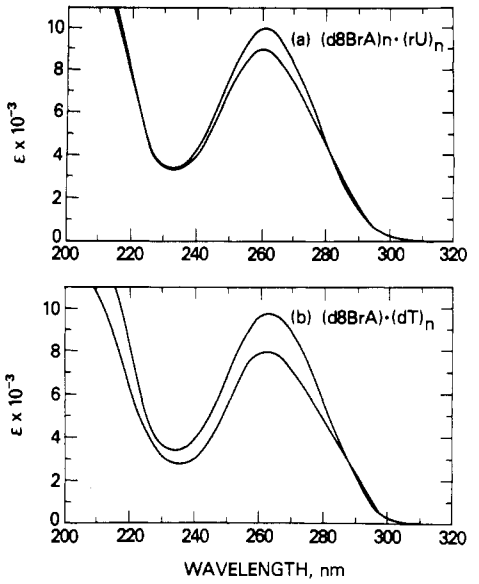

FIGURE 5: UV spectra of 1:1 mixtures of $(d8BrA)_n$ with (a) $(rU)_n$ and (b) $(dT)_n$. The lower curve of each pair is observed; the upper is the summation of spectra of the components. [Na^+], 0.1 M; Cacodylate buffer, 0.002 M, pH 7; T = 25 °C.

The following points are relevant to the present study. Infrared spectra of all AU homopolymer double helices that we have observed (about 10 examples) have the following characteristics in common: the strong A ring vibration at 1625 cm^{-1} decreases in intensity ($\sim$40–60%) from the random coil value; there are two bands above 1650 cm^{-1}, assigned to the C2=O stretch ($\sim$1690 cm^{-1}) and to a coupled mode having significant contribution from the C4=O stretch ($\sim$1670 cm^{-1}); the first carbonyl band usually decreases in frequency from the random coil value, and the latter increases in all examples observed; both carbonyl bands undergo intensity changes (Miles & Frazier, 1964; Howard et al., 1971; Ikeda et al., 1970; Ishikawa et al., 1972; Miles, 1971, 1975). Three-stranded AU helices, in contrast, have much weaker bands arising from A ring vibrations than do the double helices, and they have three bands above 1650 cm^{-1} rather than two (references cited above). Infrared spectra of mixtures of poly(d8BrA) with poly(dT), poly(rU), and poly(rBrU) (Figures 7 and 8 and supplementary material Figure 2) exhibit the properties described above for double helices.

The infrared spectrum of a 1:1 mixture of poly(d8BrA) and poly(dT) has strong bands at 1694, 1663, 1632 cm^{-1} and a weak A ring vibration at 1572 cm^{-1} (Figure 7). The 1694-cm^{-1} band is assigned to a C2=O stretch of T and the 1663 cm^{-1} also to a T band with considerable C4=O stretching character. The spectrum is quite similar to that of poly(dA)·poly(dT) (ν_{max} = 1695, 1661, 1642, 1621, and 1574 cm^{-1}; H. T. Miles and J. Frazier, unpublished results) except that the latter shows resolution of the T and A ring vibrations at 1642 and 1621 cm^{-1}, respectively. The unresolved 1632-cm^{-1} band in Figure 7 is apparently a composite of T and A ring vibrations since the frequency is the mean of 1642 and 1621 cm^{-1} observed in poly(dA)·poly(dT). The molar absorbance at 1632 cm^{-1}, moreover, is 499, a value consistent with the sums of absorbances of the resolved A and T bands in poly(dA)·poly(dT) (467) and poly(rA)·poly(rT) (453).

The infrared spectrum of a 1:1 mixture of poly(dBrA) and poly(rU) has bands at 1689 (U C2=O), 1669 (mixed mode with some U C4=O character), 1632 (A ring vibration), and 1570 cm^{-1} (A ring vibration) (Figure 8). The spectrum resembles that of poly(rA)·poly(rU) (Miles & Frazier, 1964)

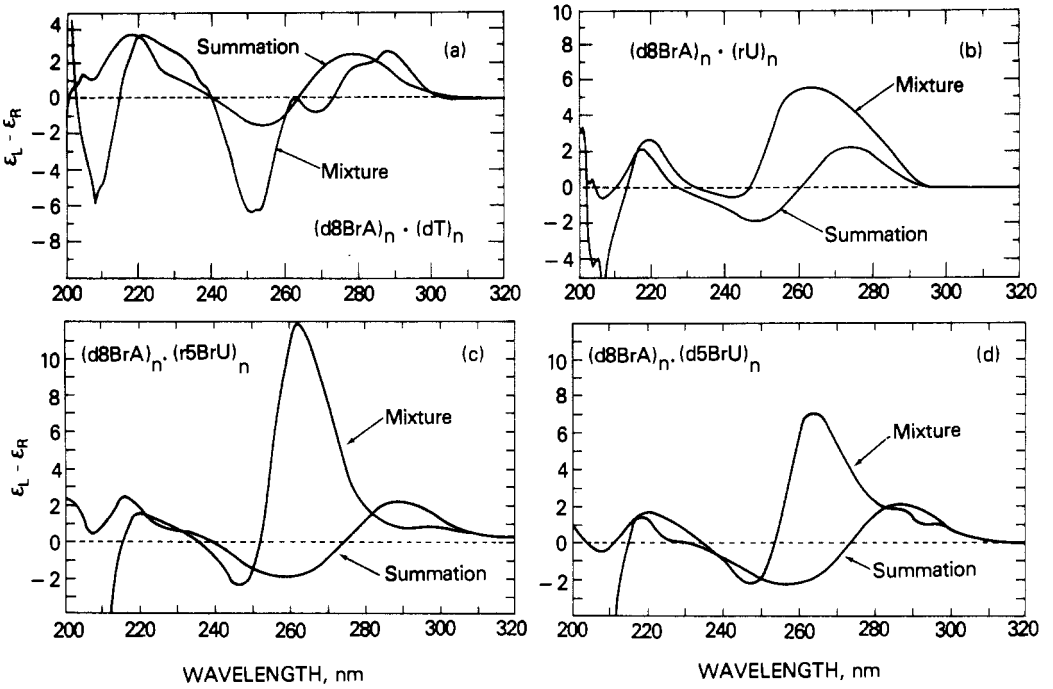

FIGURE 6: CD spectra of 1:1 mixtures of the indicated polymers. The wide deviations from summation spectra show that complex formation has occurred. Conditions are the same as those of Figure 5.

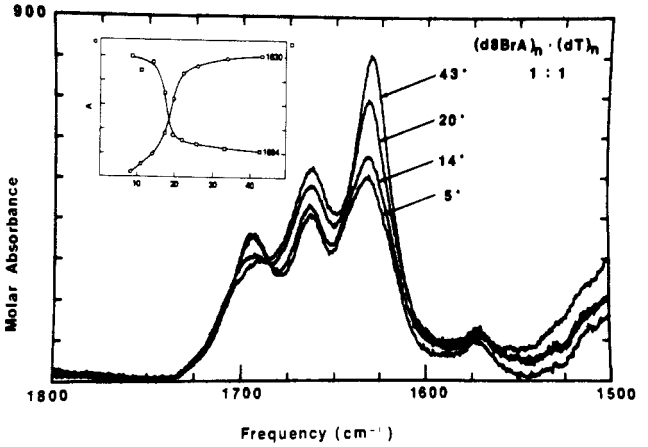

FIGURE 7: IR spectra in D_2O of $(d8BrA)_n \cdot (dT)_n$ as a function temperature. Concentration of each polymer was 0.005 M; [NaCl] was 0.1 M; sodium cacodylate buffer concentration was 0.01 M, pD 7. (Inset) IR melting curves at frequencies assigned to dT (1694 cm^{-1}) and d8BrA (1630 cm^{-1}).

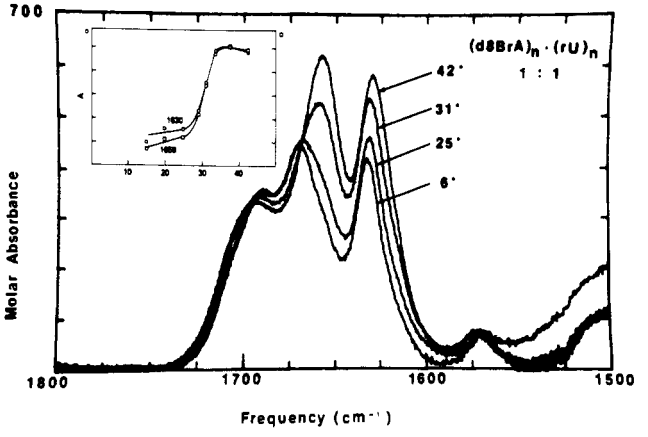

FIGURE 8: IR spectra in D_2O of $(d8BrA)_n \cdot (rU)_n$ as a function of temperature. Concentration of each polymer was 0.005 M; [NaCl] was 0.1 M; sodium cacodylate buffer concentation was 0.01 M, pD 7. (Inset) IR melting curves at frequencies assigned to rU (1658 cm^{-1}) and d8BrA (1630 cm^{-1}).

except that the first two bands listed are at slightly lower frequencies in the present case.

The spectrum of a mixture of poly(d8BrA) and poly(rBrU) (~37% BrA) (supplementary material Figure 2) resembles that of poly(rA)·poly(rBrU). The former complex has BrU C=O bands at 1690 and 1668 cm^{-1} and an A ring vibration at 1626 cm^{-1}. The latter complex has corresponding bands at 1690, 1670, and 1629 cm^{-1} (Ishikawa et al., 1972). The triple helix poly(rA)·2poly(BrU) has (like other A·2U helices) three bands above 1650 cm^{-1} at 1694, 1677, and 1652 cm^{-1} and a very weak (ϵ <50), unresolved A ring vibration near 1620 cm^{-1} (Ishikawa et al., 1972). The absence of the cited spectral features (supplementary material Figure 2) and resemblance to the spectrum of poly(rA)·poly(rBrU) indicate the helix is two stranded rather than three stranded.

Poly(d8BrA) is, in fact, incapable of forming a triple helix for the stereochemical reason outlined below. The large bromine van der Waals radius of 1.96 Å prevents the formation of a hydrogen bond between uracil N3 and bromoadenine N7, as several reports have pointed out (Kyogoku et al., 1967; Kim & Rich, 1968; Tavale et al., 1969). An estimate of the N3···N7 distance when the uracil 2-oxygen is in van der Waals contact with the 8-bromo of adenine is ~3.5 Å, a value outside the hydrogen-bonding range (Kim & Rich, 1968). Alternatively, if we place a Br in the 8-position of adenine in hydrogen-bonding schemes from the crystallographic report of Sakore et al. (1969), the Br8···O2 distance is ~2.3 Å for Hoogsteen pairing and ~2.5 Å for reverse Hoogesteen pairing. Since the sum of the van der Waals radii of Br and O is 3.35 Å (Pauling, 1960), these contacts are clearly not allowed.

Thermal Transitions. Ultraviolet melting curves of the complexes formed by poly(d8BrA) with pyrimidine polynucleotides show sharp cooperative transitions (Figure 9). Salt-dependence curves were determined for two of the complexes, poly(d8BrA)·poly(dT) and poly(d8BrA)·poly(rU), and the slopes of $dT_m/d \log [Na^+]$ were both found to be 19 °C, a typical value for double helices in the ribo or deoxyribo series (Figure 10). The transition temperatures of the new complexes and those of appropriate reference polymers are summarized in Table II.

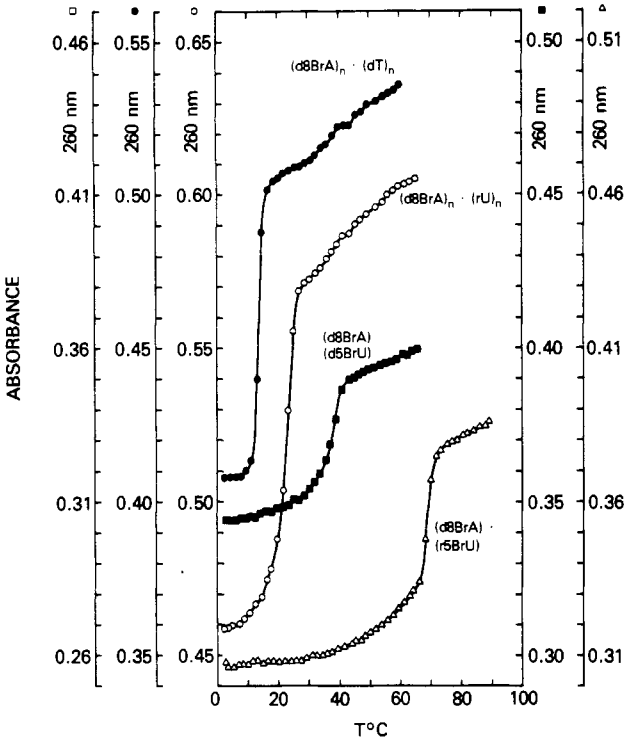

FIGURE 9: UV melting curves of indicated complexes in 0.1 M NaCl–0.002 M sodium cacodylate, pH 7.

Table II: Thermal Transitions

material	T_m (°C) (0.1 M Na^+)
(a) $(dA)_n \cdot (dT)_n$	68.8
(b) $(d8BrA)_n \cdot (dT)_n$	13.9
(c) $(d8BrA)_n \cdot (d5BrU)_n$	37.8
(d) $(dA)_n \cdot 2[(rU)_n]$	45.2
(e) $(d8BrA)_n \cdot (rU)_n$	21.4
(f) $(d8BrA)_n \cdot (r5BrU)_n$	68.1
(g) $(dA)_n \cdot (d5BrU)_n$	76.5

Infrared temperature profiles (Figures 7 and 8, inserts) provide unambiguous evidence of heteroduplex formation. Frequencies at which only one of the components absorb are selected for plotting: 1694 cm^{-1} for dT and 1630 cm^{-1} for dBrA (Figure 7); 1650 cm^{-1} for rU and 1630 cm^{-1} for dBrA (Figure 8); 1655 cm^{-1} for rBrU and 1630 cm^{-1} for dBrA (supplementary material Figure 2). Parallel melting of the two bands then demonstrates that the observed transition results from specific interaction of the two polymers, rather than, for example, formation of helical self-structures [for further discussion, cf. Miles (1971)].

Discussion

The following properties indicate that poly(d8BrA) at moderate ionic strength has a single-stranded, nonregular structure: (a) the UV spectrum is relatively little changed from that of the monomer: (b) the temperature profiles are not cooperative; (c) the infrared spectrum in the double-bond region and its temperature dependence closely resemble those of the monomer and are quite different from those of any base-paired polynucleotide. This result is in interesting contrast to results observed with two other 8-substituted poly(A)'s in the ribo series: poly(r8BrA) forms a regular, double-stranded, base-paired helix (Howard et al., 1974, 1975; Govil et al., 1977, 1981) and poly(r8MeA) forms a regular, single-stranded helix without base pairing (Limn et al., 1983). In both of the latter cases, complementary heteroduplex formation appears to be

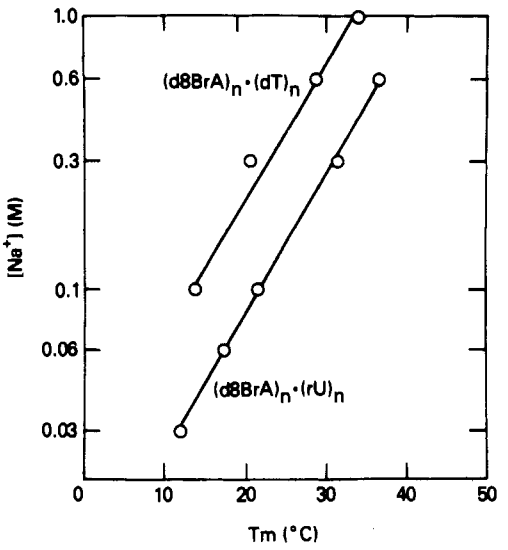

FIGURE 10: Salt-dependence curves of $(d8BrA)_n \cdot (dT)_n$ and $(d8BrA)_n \cdot (rU)_n$. For both complexes, $dT_m/d \log [Na^+] = 19$ °C.

completely blocked. Poly(d8BrA), in contrast, readily forms heteroduplex helices, though these are of lower stability than those of unsubstituted polymers (see below).

The striking dependence of the CD spectrum on $[Na^+]$ at very low ionic strength (and the parallel but much smaller changes in the UV) is probably not due to a transition between double helix and random coil, as with poly(r8BrA). The changes occur below the range of $[Na^+]$ necessary to screen interstrand repulsion of double helices, and the magnitude of the CD changes is in the opposite direction from that expected for helix formation. Similarly, formation of an acid helix analogous to that formed by poly(rA) appears quite unlikely. The pK of monomeric 8-bromoadenosine is ~2.9, whereas the solution is buffered at pH 7.0. Salt-dependence curves of such acid structures, moreover, though they have negative slopes, exhibit no discontinuities above 0.01 M Na^+, such as that seen in Figure 4. The residual $p(dA)_7$ terminating the chains (<5%) is too small in amount and the chain length of 7 is too short to account for the observed behavior. We suggest that the changes observed in Figure 3 arise from electrostatic expansion [cf. Rosenberg & Studier (1969) and Bloomfield et al. (1974)] of the polymer chain at very low ionic strength and from the effect on this expansion of screening counterions. Polyelectrolyte chain expansion occurs in the same range of cation concentration (10^{-2}–10^{-3} or lower) as the large CD changes observed here. Rosenberg & Studier (1969), for example, show a large expansion of single-stranded DNA, as reflected by the increase in specific viscosity, and a smaller change for native DNA. Their curve of η vs. log $[Na^+]$ for single-stranded DNA, while not linear overall, can be approximated reasonably well by a straight line over the range 10^{-2}–10^{-3} M Na^+. We find that the magnitude of the intense negative CD band is also linear in log $[Na^+]$ over the range ~10^{-4}–10^{-2} M (Figure 4). An electrostatic expansion of the chain is thus expected in this range of ionic strength, but the question remains why this change should produce the large changes seen in the CD spectra. We suggest the bases are disposed regularly along the rigid rod at a constant distance and rotation angle about the rod from one base to the next. If, as appears likely, rotation about the glycosidic bond is restricted by the bulky Br atom, the bases could be aligned in a regular array, approximately parallel to each other and favorably disposed for interaction of the highest wavelength π–π^* transition. As $[Na^+]$ increases above 10^{-3} M, the electrostatic expansion of the chain is

progressively relaxed by counterion screening, and the proportion of random coil increases. The proportion of bases favorably disposed for electronic interaction decreases, and cancellation of CD contributions of opposite sign may further decrease the observed magnitude of the 280-nm extremum.

From the thermal transitions of the double helices reported here (Figures 7–9; Table II), an important result stands out. Whereas the stabilizing effect of a pyrimidine 5-bromo substituent is well-known [cf. Michelson & Monny (1967) and Howard et al. (1969)] and is also exemplified in Table II, the adenine 8-bromo substituent is markedly destabilizing in all cases. Thus, the difference in T_m between a complex containing dA and one containing d8BrA is 55, 24, and 38 °C for the pairs a–b, d–e, and g–c, respectively. The high transition temperature of poly(r8BrA) (T_m = 57 °C in 0.1 M Na^+) and that of the monomer–polymer helix 5′-8BrGMP·$(rBrC)_n$ (T_m = 45 °C in 2 M Na^+; Howard et al., 1972) indicate that purine 8-bromo substitution is not necessarily destabilizing. We suggest that nonbonded steric constraints are primarily responsible for the destabilizing effect of the 8-bromo substituent observed in the present study. Bulky substituents in the purine 8-position are known to favor the syn conformation [cf. Ikehara et al. (1969), Michelson et al. (1970), Tavale & Sobell (1970), and Howard et al. (1974)]. In the case of poly(r8BrA), both the random coil and the helix have only the syn conformation (Howard et al., 1974, 1975; Govil et al., 1977, 1981), but with poly(r8MeA) it appears that both syn and anti forms exist in the same chain (Limn et al., 1983). We suggest that the reason for the difference between facile heteroduplex formation with poly(d8BrA) and lack of interaction with poly(r8BrA) may lie in accessibility of the anti conformation in the former case but not in the latter. Upon the basis of similarities of their CD spectra to those of helices having known configurations, the poly(d8BrA) complexes reported here appear to have either A or B conformations, depending upon the complementary polymer (see below). We suggest that the glycosidic conformation in all of these is anti. A previous paper (Govil et al., 1981) suggested that poly(r8BrA) does not react with poly(rBrU) because the helix axis (between N1 and N6 of BrA) in its preferred conformation cannot be brought into coincidence with the axis of an A-form poly(rBrU) strand (near an extension of a line drawn through N3 and C4 and more than 1 Å away from the C4 carbonyl). In the present case, we suggest that poly(d8BrA) is less rigid than its ribo analogue and that a syn–anti equilibrium in the random coil can be displaced toward the anti form by reaction with a complementary polynucleotide that requires the latter conformation. Presumably, other torsional angles would change to accommodate the duplex helix. The free energy required to drive the conformational equilibrium of poly(d8BrA) to values favorable for helix formation would then be reflected in the observed lower stability of the resulting heteroduplex.

The CD spectra of Figure 6 provide suggestive evidence on the conformations of the four helices represented. Uncertainties in the interpretation of CD data, however, are too large to permit a structure to be established on this basis alone [cf. discussion in Howard et al. (1976)]. The spectrum of poly(d8BrA)·poly(dT) shows a general resemblance to B-form DNA helices with a positive band centered at about 285 nm and a negative band of roughly equal area at 250 nm [for relevant background on DNA and RNA spectra, cf. Yang & Samejima (1969), Johnson & Tinoco (1969), Bush & Brahms (1973), and Bloomfield et al. (1974)]. The B conformation is to be expected from the pairing of two deoxypolymers at moderate ionic strength. On the basis of several examples (Milman et al., 1967; O'Brien & MacEwan, 1970; Arnott, 1970), hybrid pairs would be expected to have the A conformation, though a B-like form has also been observed for poly(rA)·poly(dT) under highly hydrated conditions (Zimmerman & Pheiffer, 1981). The hybrid pairs poly(d8BrA)·poly(rU) and poly(d8BrA)·poly(rBrU) have strong positive bands at 263 nm and much weaker negative bands at 247 or 241 nm, patterns similar to those of typical RNA spectra. A more surprising result from these four helices is the CD spectrum of poly(d8BrA)·poly(dBrU) (Figure 6d). Here the spectrum resembles spectra of RNA or A DNA, with an intense positive band at 263 nm and a much weaker negative band at 247 nm. All deoxy–deoxy duplexes previously reported are believed to have the B conformation at moderate ionic strength. The CD spectrum in Figure 6, however, suggests that this helix may have the A conformation.

Supplementary Material Available

Two figures showing temperature profiles of poly(8BrA) in 0.3, 0.1, and 0.03 M Na^+ and infrared spectra of a mixture of $(d8BrA)_n$ and $(r5BrU)_n$ as a function of temperature (2 pages). Ordering information is given on any current masthead page.

Registry No. d8BrA, 14985-44-5; $(d8BrA)_n$, 90968-89-1; $(d8BrA)_n$·$(rU)_n$, 90968-90-4; $(d8BrA)_n$·$(dT)_n$, 90968-91-5; $(d8BrA)_n$·$(r5BrU)_n$, 90968-93-7; $(d8BrA)_n$·$(d5BrU)_n$, 90968-95-9.

References

Arnott, S. (1970) *Prog. Biophys. Mol. Biol. 21*, 267–319.

Bloomfield, V. A., Crothers, D. M., & Tinoco, I. (1974) in *Physical Chemistry of Nucleic Acids*, pp 389–406, Harper and Row, New York.

Bollum, F. J. (1966) in *Procedures in Nucleic Acid Research* (Cantoni, G., & Davies, D., Eds.) Vol. 1, pp 284–295, Harper and Row, New York.

Bush, C. A., & Brahms, J. (1973) in *Physico-Chemical Properties of Nucleic Acids* (Duchesne, J., Ed.) pp 147–186, Academic Press, New York.

Chandrasekaran, R., Arnott, S., Banerjee, A., Campbell-Smith, S., Leslie, G., & Puigjaner, L. (1980) *ACS Symp. Ser. No. 141*, 483–502.

Drew, H. R., Takano, T., Tanaka, S., Itakura, K., & Dickerson, R. E. (1980) *Nature (London) 286*, 567–573.

Drew, H. R., Wing, R. M., Takano, T., Broka, C., Tanaka, S., Itakura, K., & Dickerson, R. E. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2179–2183.

Govil, G., Fisk, C., Howard, F. B., & Miles, H. T. (1977) *Nucleic Acids Res. 4*, 2573–2592.

Govil, G., Fisk, C., Howard, F. B., & Miles, H. T. (1981) *Biopolymers 20*, 573–603.

Howard, F. B., Frazier, J., & Miles, H. T. (1969) *J. Biol. Chem. 244*, 1291–1302.

Howard, F. B., Frazier, J., & Miles, H. T. (1972) *J. Biol. Chem. 247*, 6733–6735.

Howard, F. B. Frazier, J., & Miles, H. T. (1974) *J. Biol. Chem. 249*, 2987–2990.

Howard, F. B., Frazier, J., & Miles, H. T. (1975) *J. Biol. Chem. 250*, 3951–3959.

Howard, F. B., Frazier, J., & Miles, H. T. (1976) *Biochemistry 15*, 3783–3795.

Ikehara, M., & Uesugi, S. (1969) *Chem. Pharm. Bull. 17*, 348–352.

Ikehara, M., Tazawa, I., & Fukui, T. (1969) *Biochemistry 8*, 736–743.

Johnson, W. C., & Tinoco, I. (1969) *Biopolymers 7*, 727–749.
Kim, S. H., & Rich, A. (1968) *Proc. Natl. Acad. Sci. U.S.A. 60*, 402–408.
Kyogoku, Y., Lord, R. C., & Rich, A. (1967) *Proc. Natl. Acad. Sci. U.S.A. 57*, 250–257.
Limn, W., Uesugi, S., Ikehara, M., & Miles, H. T. (1983) *Biochemistry 22*, 4217–4222.
Michelson, A. M., & Monny, C. (1967) *Biochim. Biophys. Acta 149*, 88–95.
Michelson, A. M., Monny, C., & Kapuler, A. U. (1970) *Biochim. Biophys. Acta 217*, 7–15.
Miles, H. T. (1971) in *Procedures in Nucleic Acid Research* (Cantoni, G., & Davies, D., Eds.) Vol. 2, pp 205–232, Harper and Row, New York.
Milman, G., Langridge, R., & Chamberlin, M. J. (1967) *Proc. Natl. Acad. Sci. U.S.A. 57*, 1804–1810.
O'Brien, E. J., & MacEwan, A. W. (1970) *J. Mol. Biol. 48*, 243–261.
Olson, W. K., & Sussman, J. (1982) *J. Am. Chem. Soc. 104*, 270–278.
Pauling, L. (1960) in *The Nature of the Chemical Bond*, p 260, Cornell University Press, Ithaca, NY.
Powell, J. I., Fico, R., Jennings, W. H., O'Bryan, E. R., & Schultz, A. R. (1980) *Proceedings of the IEEE Computer Society, 21st International Conference*, pp 185–190, IEEE Computer Society, Los Angeles.
Rosenberg, A. H., & Studier, F. W. (1969) *Biopolymers 1*, 765.
Sakore, T. D., Sobell, H. M., Mazza, F., & Kartha, G. (1969) *J. Mol. Biol. 43*, 385–406.
Sasisekharan, V. (1973) *Jerusalem Symp. Quantum Chem. Biochem. 5*, 247–260.
Sundaraligam, M. (1973) *Jerusalem Symp. Quantum Chem. Biochem. 5*, 417–455.
Tavale, S. S., & Sobell, H. M. (1970) *J. Mol. Biol. 48*, 109–123.
Tavale, S. S., Sakore, T. D., & Sobell, H. M. (1969) *J. Mol. Biol. 43*, 375–384.
Wang, A. H. J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., & Rich, A. (1979) *Nature (London) 282*, 680–686.
Willis, J. E., Krocker, W. D., & Hassell, A. M. (1980) *Prog. Clin. Enzymol.* 272–277.
Yang, J. T., & Samejima, T. (1969) *Biochem. Biophys. Res. Commun. 33*, 739–744.
Zimmerman, S. B. (1982) *Annu. Rev. Biochem. 51*, 395–427.
Zimmerman, S. B., & Pheiffer, B. H. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 78–82.

Altered Chromatin Conformation of the Higher Plant Gene Phaseolin†

Michael G. Murray* and Wayne C. Kennard

ABSTRACT: Micrococcal nuclease, DNase I, and S1 nuclease were used to probe alterations in the chromatin conformation of phaseolin genes which encode the major seed storage proteins of the French bean (*Phaseolus vulgaris* L. cv. Tendergreen). Comparisons were made between cotyledon tissue where the genes are expressed and leaf tissue where the genes are not expressed. The nucleosomal repeat length of leaf chromatin is 191 base pairs (bp). However, in the cotyledon, a tissue which undergoes extensive DNA synthesis leading to endopolyploidy, the repeat length is considerably shorter (177 bp). Phaseolin sequences are organized into the same repeat length as the total chromatin in each respective tissue. Phaseolin genes are preferentially sensitive to DNase I in cotyledons relative to leaves. DNase I hypersensitive sites could not be identified in the flanking DNA of phaseolin genes in chromatin. Although S1 nuclease hypersensitive sites could be identified in DNA sequences flanking the phaseolin genes when analysis was performed on pure, supercoiled DNA, they could not be demonstrated in chromatin.

Abundant evidence derived from animal systems suggests an important role for chromatin structure in the regulation of gene expression [reviewed in Mathis et al. (1980), Elgin (1981), and Weisbrod (1982)]. Transcriptionally active sequences are in an altered conformation which renders them preferentially sensitive to nucleases and particularly to DNase I. Distinct nuclease hypersensitive sites are frequently found in DNA flanking transcriptionally active sequences. High mobility group proteins are preferentially associated with active chromatin and appear in part to be able to confer preferential DNase I sensitivity. In contrast, little is known in higher plants beyond basic levels of chromatin organization [reviewed in Thompson & Murray (1981) and Spiker (1984)]. Only one published study has examined whether transcriptionally active sequences show a modified chromatin structure. In wheat germ, active sequences are preferentially sensitive to DNase I, and proteins apparently analogous to high mobility group proteins are released (Spiker et al., 1983). Few higher plant gene systems have been characterized in sufficient detail to permit more extensive analysis.

We present here the first studies in higher plants on the chromatin conformation of a specific gene. The gene family examined, phaseolin, encodes the major storage protein of the French bean (*Phaseolus vulgaris* L. cv. Tendergreen). Phaseolin expression is under strict developmental control; protein accumulation begins in midmaturation cotyledons and ultimately comprises about 50% of the seed protein (Sun et al., 1978). Phaseolin polypeptides are encoded by a highly conserved multigene family consisting of 7–10 genes, and 7 different genomic clones have been isolated (Hall et al., 1983a; D. R. Talbot et al., unpublished results). Although sexual crosses show phaseolin genes to be tightly linked (Brown et

† From the Agrigenetics Advanced Research Division, Agrigenetics Corporation, Madison, Wisconsin 53716. *Received January 23, 1984.* This is paper 23 from the Agrigenetics Advanced Research Division.

0006-2960/84/0423-4225$01.50/0