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# Conversions of Poly(2-aminodeoxyadenylate-5-halodeoxyuridylate) from B to A Forms in High Salt. An NMR and Circular Dichroism Study

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ABSTRACT: Proton one- and two-dimensional nuclear Overhauser enhancement (1D and 2D NOE) spectroscopy has been used to demonstrate that poly(d2NH$_2$A-d5IU) and poly(d2NH$_2$A-d5BrU) are converted from the B to the A conformation in high salt, as found previously for poly(d2NH$_2$A-dT) [Borah, B., Cohen, J. S., Howard, F. B., & Miles, H. T. (1985) *Biochemistry 24*, 7456-7462]. The 2D NOE and 1D NOE spectra exhibit strong base proton (H8,H6)-H3′ cross relaxation, suggesting short interproton distances. These results are indicative of a C3′-endo sugar pucker for both purine and pyrimidine residues in an A or closely related structure. The circular dichroism and UV spectra are consistent with the interpretation of an A conformation in high salt.

The conformational equilibrium between right-handed B-DNA and left-handed Z-DNA depends on several extrinsic and intrinsic factors. The level of hydration, ionic strength, temperature, specific ligands, and supercoiling are extrinsic factors that may influence the B-Z equilibrium. Intrinsic factors such as base sequence and covalent chemical modification of the base or of the backbone can also promote a right-handed B to left-handed Z transition. It has been reported that halogen substitution on the pyrimidine C5 position facilitates the B-Z transition (Malfoy et al., 1982). Methyl substitution of the pyrimidine C5 was shown to stabilize the Z form of alternating poly(dG-d5MeC) at physiological salt concentrations (Behe & Felsenfeld, 1981; Fujii et al., 1982). The efficiency of C5 substitution for stabilizing the left-handed form has been reported to increase in the order of iodo > bromo > methyl ≫ H (Jovin et al., 1983a). Bromination in the guanine C8 position also stabilizes Z-DNA (Moller et al., 1984), presumably by stabilizing the syn conformation present in the Z form.

The commonly used spectroscopic criteria, such as circular dichroism (CD), IR, Raman, and $^{31}$P NMR spectra, however, cannot unambiguously define structure in solution. Another method of identifying the handedness of a DNA duplex is based on the immunogenicity of Z-DNA. Z-DNA elicits the production of antibodies specific for the left-handed form in a variety of organisms (Lafer et al., 1981; Malfoy & Leng, 1981; Moller et al., 1982; Pohl et al., 1982). Such antibodies have been used to show the existence of the Z form in native DNA by the use of immunofluorescence microscopy (Nordheim et al., 1981; Jovin et al., 1983a,b; Lemeunier et al., 1982; Lipps et al., 1983; Morgenegg et al., 1983; Arndt-Jovin et al., 1985a,b) and competitive radioimmunoassay (Lafer et al., 1981; Rich, 1983; Jovin et al., 1983c). It was reported, for example, that poly(d2NH$_2$A-dT) in preliminary experiments interacts at high ionic strength with antibodies specific for Z-DNA, and it was indicated that this polymer has the structural characteristics of left-handed Z-DNA under those conditions (Jovin et al., 1983a). Preliminary results on poly(d2NH$_2$A-dT) with CD and $^{31}$P NMR also suggested a Z-like structure (Howard et al., 1984; Gaffney et al., 1982).

One-dimensional and two-dimensional nuclear Overhauser enhancement (1D and 2D NOE) spectroscopy provides a unique method for detailed conformational analysis of polydeoxynucleotides in solution (Assa-Munt & Kearns, 1984; Borah et al., 1985a,b). Recent work in this laboratory using 2D NOE spectroscopy has demonstrated that poly(d2NH$_2$A-dT) in high salt concentration adopts an A form or closely related structure (Borah et al., 1985b) rather than the Z form suggested in the reports cited above. The observed base proton-H3′ cross-peaks in the 2D NOE spectra indicated a 3′-endo sugar pucker for both the 2NH$_2$A and T nucleotides in an A conformation. This significant finding has prompted us to investigate two other alternating copolymers, poly(d2NH$_2$A-d5IU) and poly(d2NH$_2$A-d5BrU), using 1D and 2D NOE methods as well as CD and UV spectroscopy. We have analyzed the 1D and 2D NOE spectra of these polymers in low- and high-salt conditions and compared them with spectra characteristic of B, Z, and A forms (Borah et al., 1985b). Our conclusion is that both the poly(d2NH$_2$A-d5XU) copolymers also give an A form rather than a Z form in high salt. These results have consequences for the interpretation of anti-Z antibody binding to native DNA.

## Materials and Methods

*Materials.* 5-Bromodeoxyuridine 5′-triphosphate (lot no. 769031), 5-iododeoxyuridine 5′-triphosphate (lot no. 867041), poly(dA-dT) (lot no. 658192), and DNA polymerase I from *Eschericia coli* (lot no. 926-10) were from Pharmacia. Synthesis of 2-aminodeoxyadenosine 5′-triphosphate has been

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described (Howard & Miles, 1984).

*Synthesis of Poly(d2NH$_2$A-d5BrU).* A 20-mL reaction mixture contained the following components: $1 \times 10^{-3}$ M 2-aminodeoxyadenosine 5′-triphosphate; $1 \times 10^{-3}$ M 5-bromodeoxyuridine 5′-triphosphate; $5 \times 10^{-3}$ M magnesium chloride; $1 \times 10^{-3}$ M 2-mercaptoethanol; and 845 units of DNA polymerase I from *E. coli*. Progress of the reaction in a 0.3-mL aliquot was followed by the decrease in absorbance at 287 nm (1-mm path length; 37 °C) monitored with the Cary Model 118 spectrophotometer–LDACS system [cf. Howard and Miles (1984)]. The remainder of the reaction mixture was incubated separately in a 37 °C bath. After 20 h, absorbance decreased by 16% and the reaction mixture was extracted with 10 mL of a 1:1 mixture of chloroform and phenol equilibrated with 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8.0, and containing 0.1% 8-hydroxyquinoline (Maniatis et al., 1982). The aqueous solution was dialyzed against 2-L volumes of 1 M NaCl–0.001 M ethylenediaminetetraacetate (EDTA) (24 h), 1 M NaCl (two changes, 48 h), and water (five changes, 96 h). Poly(d2NH$_2$A-d5BrU) was recovered in 26.9 mL of water and stored at −20 °C; yield was $8.48 \times 10^{-3}$ mmol (21.2%). The molar extinction coefficient was determined as described previously (Muraoka et al., 1980) with a 5-fold reduction in scale. $\epsilon_{277} = 4580 \pm 100$ (three determinations) for poly(d2NH$_2$A-d5BrU) in 0.002 M sodium cacodylate, pH 7.0–0.1 M NaCl. Selective degradation of the primer template by the 5′-exonuclease activity of DNA polymerase I resulted in complete removal of poly(dA-dT) from the isolated polymer (Klett et al., 1968; Howard et al., 1984). No transition assignable to poly(dA-dT) was observed in melting curves of poly(d2NH$_2$A-d5BrU), indicating the absence of detectable primer template.

*Synthesis of Poly(d2NH$_2$A-d5IU).* Poly(d2NH$_2$A-d5IU) was synthesized in 37.8% yield in a manner similar to the synthesis of the 5-Br analogue. For this copolymer, $\epsilon_{283} = 4480 \pm 120$ (six determinations) in 0.002 M sodium cacodylate, pH 7.0–0.1 M NaCl. Poly(dA-dT) was also shown to be absent from poly(d2NH$_2$A-d5IU).

*Sonication of Polymers.* A solution of $1.06 \times 10^{-2}$ mmol of poly(d2NH$_2$A-d5IU) (polymer P) in 2.5 mL of $4 \times 10^{-3}$ M sodium phosphate buffer, pH 7.2, was sonicated at 10% power output for 3.4 h while the temperature was held at 5 °C. The solution was centrifuged at 10000 rpm for 25 min. After 0.04 mL of 1.0 M NaCl was added, the supernatant was freeze-dried and the residue dissolved in 0.6 mL of $D_2O$ (99.96 atom %). The sonicated polymer was freeze-dried twice more from 0.4 mL of $D_2O$ and finally dissolved in 0.4 mL of $D_2O$ (99.96 atom %, Merck) for 2D NOE measurements. For observations at high salt, the sample was freeze-dried and dissolved in 0.21 mL of $D_2O$ to which 0.19 mL of 8.42 M $NaClO_4$ in $D_2O$ was added. Poly(d2NH$_2$A-d5BrU) ($8.48 \times 10^{-3}$ mmol of polymer P) was sonicated in a similar manner.

*Methods.* UV spectra were measured with a Cary Model 118 spectrophotometer interfaced to an LDACS computer system (Powell et al., 1980). CD spectra were recorded with a JASCO J-500A spectropolarimeter connected to the LDACS system.

Proton 2D NOE experiments were performed at 270 MHz by using a GE NMR spectrometer with a Bruker superconducting magnet, interfaced with a Nicolet 1280 computer and a 293C pulse programmer. 2D NOE spectra were collected with a combination of three nonselective 90° pulses (Kumar et al., 1980) and a mixing time of 25 ms. The free induction decays (FIDs) consisted of 512 data points in the $t_2$ dimension with a sweep width (SW) of 2500 Hz and 64 $t_1$ values incremented by the delay ($^1/_2$SW). Pure absorption mode spectra were collected by using a phase-cycling routine that allowed spectra to be recorded in quadrature mode. The FIDs were apodized by a Gaussian function with 10-Hz line broadening in both dimensions. Transient proton NOE spectra were obtained at 400 MHz by using a Varian XL-400 spectrometer with an Oxford superconducting magnet. Spectra were recorded with irradiation of a selected resonance for 15–25 ms. A control spectrum with off-resonance irradiation was obtained simultaneously under identical conditions and automatically subtracted from the on-resonance spectrum to give an NOE difference spectrum.

## Results

*2D NOE Spectra of Poly(d2NH$_2$A-d5IU) and Poly(d2NH$_2$A-d5BrU). (1) Spectra in Low Salt.* The contour plots of the 2D NOE spectra of these two alternating copolymer duplexes in 0.1 M NaCl solution are shown in Figure 1. The projections of the diagonal peaks, which represent spin magnetization that does not cross-relax during the mixing time, are shown on each axis. The assignments of resonances are given on the basis of previous assignments in analogous sequences (Borah et al., 1985a,b). Major cross-peaks are observed in both cases, showing cross relaxations between H8–(H2′,H2″), H6–(H2′,H2″), H1′–(H2′,H2″), and H3′–(H2′,H2″).

*(2) Spectra in High Salt.* 2D NOE spectra of poly(d2NH$_2$A-d5IU) in 4 M $NaClO_4$ using a 25-ms mixing time are shown in Figure 2. $NaClO_4$ was used in this case since the polymer is insoluble at such high NaCl concentrations. In high-salt solution the H8 resonance undergoes a downfield shift, and the chemical shift separation between the H8 and H6 resonances increases from ca. 0.2 ppm in low salt to 0.46 ppm in high salt. The HDO signal has also shifted upfield leaving the two H3′ resonances well resolved. The 2D NOE contour plots show cross-peaks for interactions of the sugar (H2′, H2″) protons with the base (H8, H6) protons and with the H1′ and H3′ resonances, which also appear in the low-salt contour plots. The most noticeable new features in the high-salt 2D NOE spectra are two cross-peaks arising from H8–H3′ and H6–H3′ interactions (Figure 2). Figure 3 shows cross sections of the 2D NOE contour plot of Figure 2, also illustrating the cross-peaks for base proton (H8,H6)–H3′ and base proton (H8,H6)–(H2′,H2″) interactions.

*1D NOE Spectra of Poly(d2NH$_2$A-d5BrU) in High Salt.* Figure 4 shows the 400-MHz one-dimensional (1D) NOE difference spectra of poly(d2NH$_2$A-d5BrU) in 4 M $NaClO_4$. Two spectra were recorded by successive irradiation, one off-resonance and another on-resonance, for 20 ms. The difference spectra are shown in Figure 4, when the purine (Pu) H8 and the pyrimidine (Py) H6 were irradiated consecutively. Significant NOEs to the H3′ resonances are clearly observed in both cases.

*CD and UV Spectra.* The CD spectrum of poly(d2NH$_2$A-d5IU) in 0.1 M $NaClO_4$ has a broad, composite first negative band with unresolved contributions at 279 and ~292 nm (Figure 5a). We attribute these to the $B_2u$ transitions observed in UV spectra of d2NH$_2$A and d5IU and 278 and 288 nm, respectively, and note that exciton splitting of these bands appears to be weak or absent. Very weak maxima and minima appear at 265 and 257 nm, respectively, possibly reflecting splitting of the (usually weak) $B_1u$ transition of d2NH$_2$A [cf. Howard et al. (1976)]. The positive band at 222 nm presumably results from exciton splitting of the $E_1u$ transition of d2NH$_2$A at 214 nm.

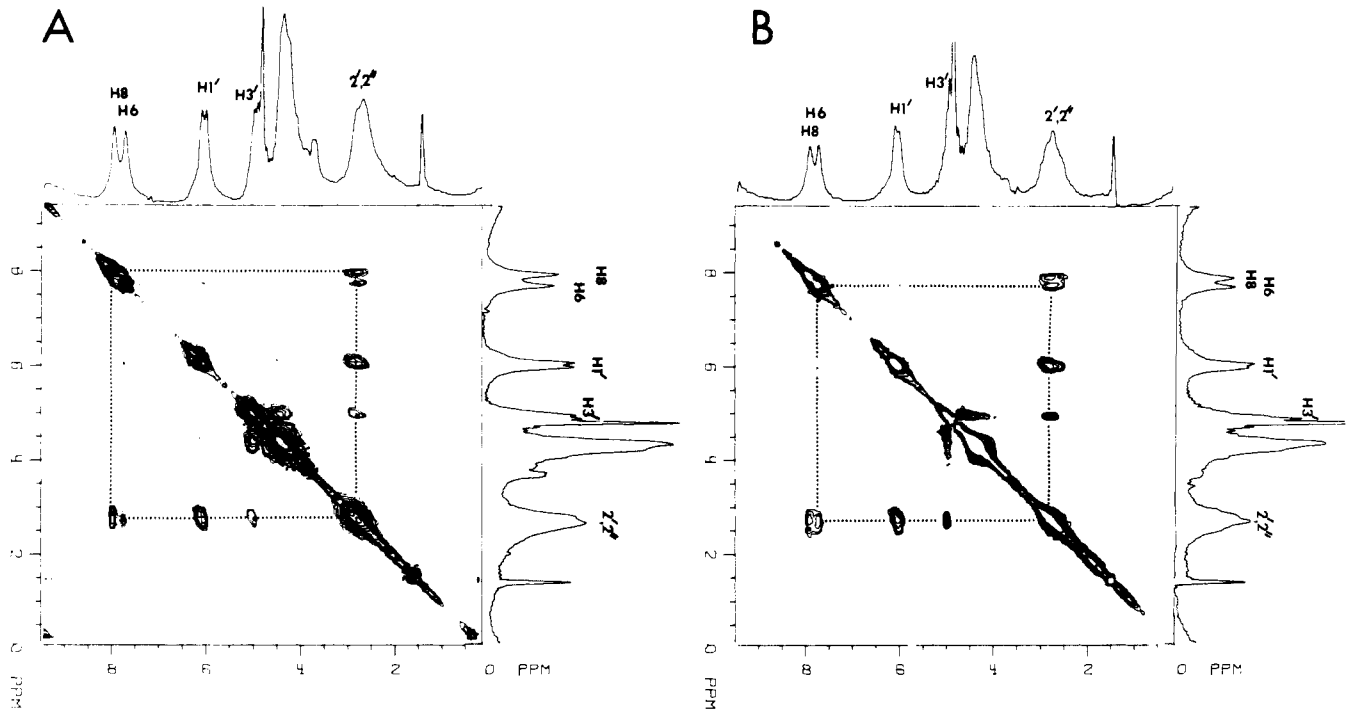


FIGURE 1: Contour plots of absorption-mode 2D NOE spectra at 270 MHz of double-stranded (A) poly(d2NH$_2$A-d5IU) and (B) poly(d2NH$_2$A-d5BrU) in 0.1 M NaCl. The mixing time in both cases was 25 ms. The dotted lines join cross-peaks for pairs of interacting protons shown on both axes.

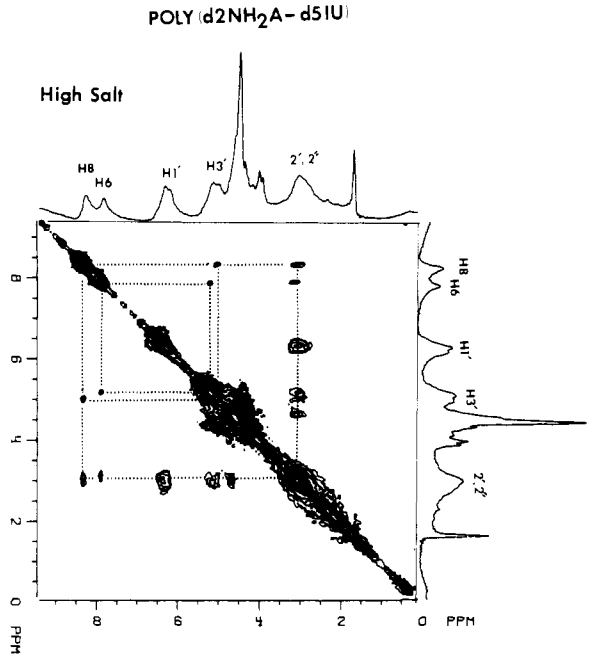


FIGURE 2: Contour plot of absorption-mode 2D NOE spectra at 270 MHz of poly(d2NH$_2$A-d5IU) in 4 M $NaClO_4$. Mixing time was 25 ms.

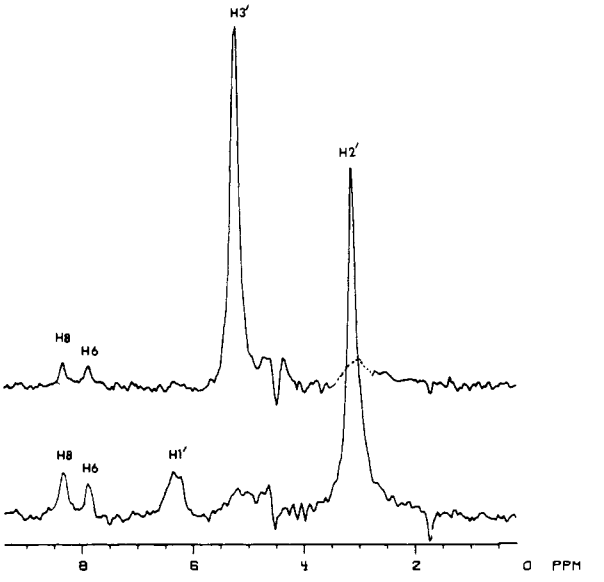


FIGURE 3: Cross sections of absorption-mode 2D NOE contour plots of poly(d2NH$_2$A-d5IU) (shown in Figure 2). The top trace shows the diagonal peak at H3' and two cross-peaks for *intra*nucleotide H8–H3' and H6–H3' interactions. The bottom trace shows the diagonal peak at H2' and cross-peaks for *inter*nucleotide H8–H2' and H6–H2' interactions as described in the text.

With increasing concentration of $NaClO_4$, a relatively intense negative band develops at 291 nm (Figure 5a). Isoelliptic points at 257 and 274 nm indicate that two components are present during the salt-induced conformational transition. The midpoint of the transition monitored at 293 nm occurs at 0.80 M $NaClO_4$ (Figure 6).

The CD spectrum of poly(d2NH$_2$A-d5BrU) in 0.05 M $NaClO_4$ (figure 5b) is similar to that poly(d2NH$_2$A-d5IU) in having a broad first negative band of low intensity ($\lambda_{min}$ = 272 nm, $\epsilon_L - \epsilon_R = -1.23$). The $B_2U$ transition of d5BrU occurs at the same wavelength (279 nm) as the $B_2u$ transition of d2NH$_2$A, and there is no apparent resolution of the contributions made by these two. Again, exciton splitting appears to be absent. The strong positive band at 222 nm is assigned to splitting of the $E_1u$ transition of d2NH$_2$A at 214 nm.

As the $NaClO_4$ concentration is increased, a new negative band at 287 nm and a positive band at 263 nm appear (Figure 5b). This new pair may result from exciton splitting of either of the two transitions at 279 nm assigned to d2NH$_2$A or d5BrU, or possibly from both. During the salt-induced conformational change, two isoelliptic points appear at 248 and 274 nm, indicating the presence of two components. The midpoint of transition monitored at 286 nm occurs at 0.78 M $NaClO_4$ (Figure 6).

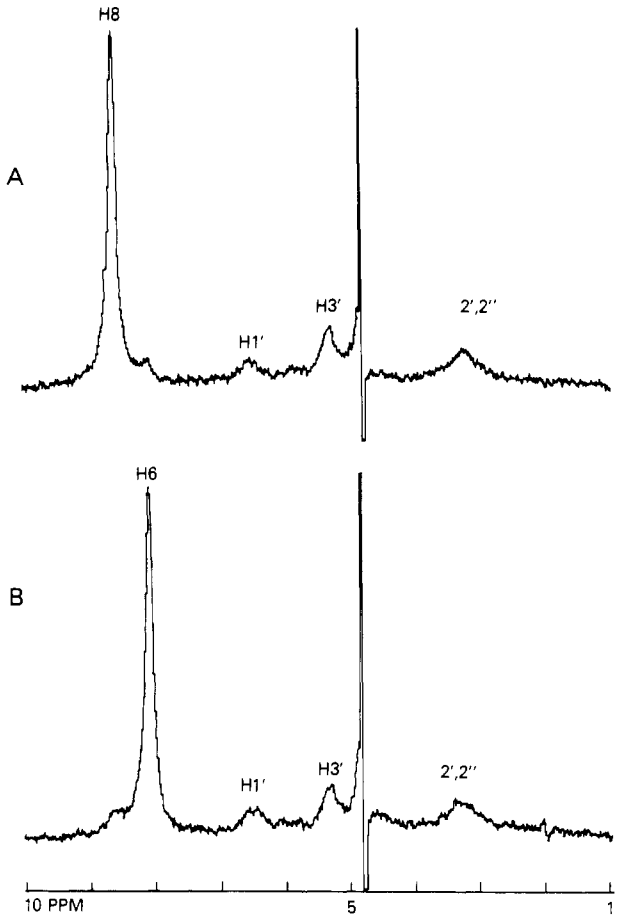


FIGURE 4: One-dimensional NOE difference spectra at 400 MHz of poly(d2NH$_2$A-d5BrU) in 4 M $NaClO_4$. Two spectra were obtained by successive irradiation for 20 ms, one off-resonance and another on-resonance. The difference spectra are shown in (A), when the Pu H8 was irradiated, and in (B), when the Py H6 was irradiated. Significant NOEs are observed for H8–H3′ and H6–H3′ interactions.

Table I: Spectroscopic Data

| | Ultraviolet[a] [$NaClO_4$] (M) | $\lambda_{max}$ (nm) | $\epsilon_{max}$ | $\lambda_{min}$ (nm) | $\epsilon_{min}$ |
|---|---|---|---|---|---|
| poly(d2NH$_2$A-d5BrU) | 0.05 | 278 | 4580 | 267 | 4320 |
| | | 263 | 4360 | 243 | 2690 |
| poly(d2NH$_2$A-d5BrU) | 6.12 | 280 | 4640 | 245 | 2630 |
| poly(d2NH$_2$A-d5IU) | 0.1 | 283 | 4480 | 266 | 3830 |
| | | 262 | 3890 | 248 | 3260 |
| poly(d2NH$_2$A-d5IU) | 6.12 | 283 | 4490 | 265 | 3460 |
| | | 261 | 3480 | 251 | 3180 |

| | Circular Dichroism[a] [$NaClO_4$] M | $\lambda_{max}$ (nm) | $\epsilon_L - \epsilon_R$ | $\lambda_{min}$ (nm) | $\epsilon_L - \epsilon_R$ |
|---|---|---|---|---|---|
| poly(d2NH$_2$A-d5BrU) | 0.05 | 218 | 5.51 | 272 | −1.23 |
| poly(d2NH$_2$A-d5BrU) | 6.12 | 221 | 3.51 | 234 | −1.01 |
| | | 263 | 1.32 | 287 | −3.55 |
| poly(d2NH$_2$A-d5IU) | 0.10 | 222 | 6.44 | 257 | −0.84 |
| | | 265 | −0.70 | 279 | −1.46 |
| poly(d2NH$_2$A-d5IU) | 6.12 | 222 | 4.60 | 253 | −0.97 |
| | | 266 | −0.16 | 290 | −3.66 |

[a] Conditions: 0.002 M sodium cacodylate, pH 7.0, 20 °C.

The UV spectra of the alternating poly(d2NH$_2$A-dPy) polymers in high and low salt are shown in Figure 7 (cf. Table I). The spectrum of poly(d2NH$_2$A-dT) in low salt has a maximum at 260 nm and a shoulder at ~280 nm. The former is assigned to the $B_2u$ transition of dT and the $B_1u$ transition of d2NH$_2$A and the latter to the $B_2u$ transition of d2NH$_2$A

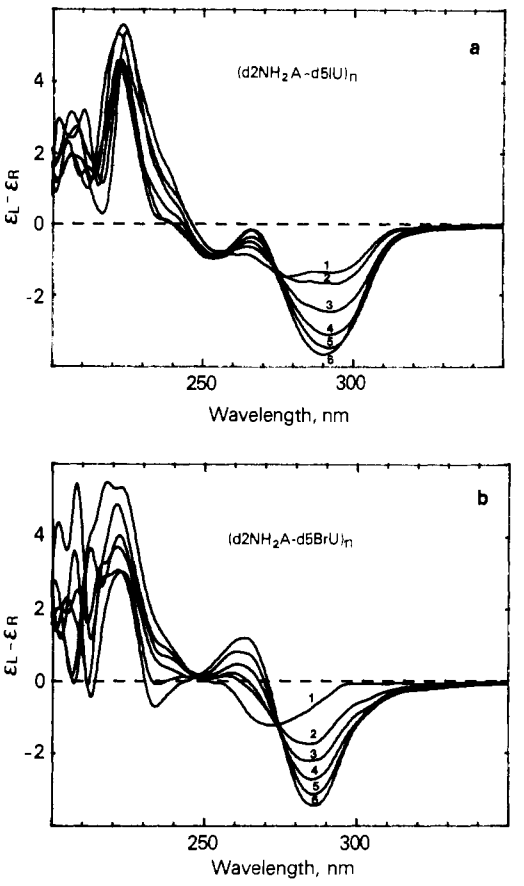


FIGURE 5: (a) CD spectra of poly(d2NH$_2$A-d5IU) at the following concentrations of $NaClO_4$: 0.01 (1), 0.32 (2), 0.87 (3), 1.99 (4), 3.97 (5), and 6.12 M (6); the buffer was sodium cacodylate, 0.002 M, pH 7.0, 20 °C. (b) CD spectra of poly(d2NH$_2$A-d5BrU) at the following concentrations of $NaClO_4$: 0.05 (1), 0.56 (2), 0.77 (3), 1.41 (4), 2.57 (5), and 5.03 M (6); the buffer was sodium cacodylate, 0.002 M, pH 7.0, 20 °C. In both cases, each spectrum is the average of nine runs and has been smoothed, base line corrected, and plotted by a computer.

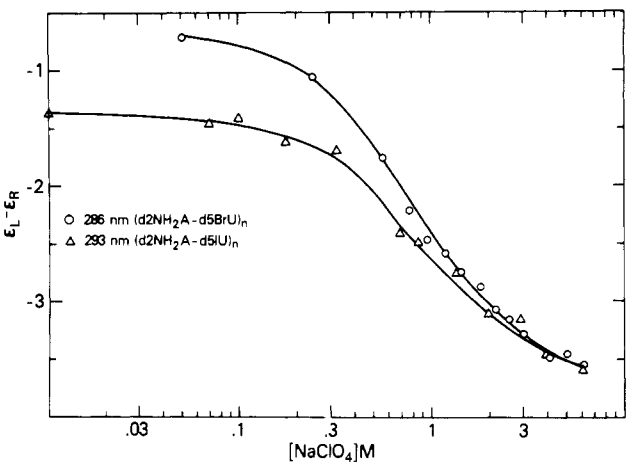


FIGURE 6: Dependence of $\epsilon_L - \epsilon_R$ at 286 (○) and 293 nm (△) on log [$Na^+$] for poly(d2NH$_2$A-d5BrU) (○) and poly(d2NH$_2$A-d5IU) (△). Midpoints of transition occur at 0.78 M $NaClO_4$ for poly(d2NH$_2$A-d5BrU) and at 0.80 M $NaClO_4$ for poly(d2NH$_2$A-d5IU). Data are taken from CD spectra of Figure 5 and other CD spectra not shown.

[cf. Howard et al. (1976)]. In high salt the maximum decreases in intensity, and hyperchromism of the second transition brings it to almost equal intensity with the peak at 262 nm. The spectrum is again hyperchromic with respect to that of the low-salt form above 290 nm.

The UV spectrum of poly(d2NH$_2$A-d5BrU) has resolved maxima at 263 and 278 nm, differing from the previous case

Table II: Interproton Distances (Å) Computed for Different DNA Models

| | purine *intra*nucleotide interactions | | | | purine *inter*nucleotide interactions | | | |
|---|---|---|---|---|---|---|---|---|
| model | H8–H1′ | H8–H2′ | H8–H2″ | H8–H3′ | H8–H1′ | H8–H2′ | H8–H2″ | H8–H3′ |
| B-DNA | 3.69 | *2.14* | 3.53 | 4.10 | *2.83* | 3.71 | *2.40* | 4.95 |
| A-DNA | 3.63 | 3.77 | 4.51 | *2.84* | 4.06 | *1.82* | 3.30 | 3.45 |
| Z-DNA | *2.32* | 3.98 | 4.28 | 5.26 | 7.31 | 7.80 | 8.48 | 6.94 |
| | pyrimidine *intra*nucleotide interactions | | | | pyrimidine *inter*nucleotide interactions | | | |
| model | H6–H1′ | H6–H2′ | H6–H2″ | H6–H3′ | H6–H1′ | H6–H2′ | H6–H2″ | H6–H3′ |
| B-DNA | 3.58 | *1.92* | 3.35 | 3.83 | *2.81* | 3.81 | *2.42* | 4.92 |
| A-DNA | 3.51 | 3.42 | 4.35 | *2.68* | 4.01 | *1.76* | 3.13 | 3.45 |
| Z-DNA | 3.54 | 3.27 | 4.35 | 4.64 | 5.61 | 2.98 | 4.21 | 3.45 |

because the strong $B_2u$ transition of d5BrU is at 278 nm (coinciding with $B_2u$ of $d2NH_2A$) rather than at 263 nm, as in dT. In high salt the spectral changes are similar: a decrease of intensity at 263 nm and a slight increase at 281 nm (Figure 7). There is also a 3-nm blue shift of the 210-nm peak. In the spectrum of poly($d2NH_2A$-d5IU), the long-wavelength peak is shifted still further to the red (283 nm) by the $B_2u$ transition of dIU at 288 nm. The second peak remains at 261 nm, assigned to the $B_1U$ transition of $d2NH_2A$. This peak also undergoes a reduction of intensity in high salt, but the long-wavelength peak shows no significant change (Figure 7). The latter result, contrasted with the small hyperchromism in poly($d2NH_2A$-d5BrU) and the larger change in poly($d2NH_2A$-dT), suggests that the salt-induced increase of intensity of the long-wavelength peak may arise from an effect of conformation on the $B_2u$ transition of $d2NH_2A$ and the decrease at 260 nm from an effect on the $B_1u$ transition of the same base.

## Discussion

*Conformation from NMR Spectra.* The use of one-dimensional and two-dimensional NOE measurements provides an important method to elucidate the conformation of DNA in solution. The power of the method lies in the fact that, with appropriate choice of parameters, a single 2D NOE experiment can provide a network of interproton connectivities that can qualitatively define the conformation of DNA in solution. Recent work in this laboratory and elsewhere has revealed that 2D NOE spectra can be used to assign B, Z, and A conformations of polydeoxynucleotides in solution (Assa-Munt & Kearns, 1984; Borah et al., 1985a,b). Several secondary structural features of DNA conformation, such as sugar pucker, syn vs. anti nucleotide conformation, and helical sense, can be probed by 1D and 2D NOE measurements. The determination of the secondary structure of poly($d2NH_2A$-d5IU) and poly($d2NH_2A$-d5BrU) will be illustrated by the application of these NMR methods.

Detailed analysis of the 2D NOE spectra reveals that, in low salt (0.1 M NaCl, 10 mM phosphate), the alternating copolymers adopt a right-handed B form. The most important connectivities that characterize the B form are those between the base protons and the H2′,H2″ resonances. Both intranucleotide (H8,H6)–H2′ and internucleotide (H8,H6)–H2″ distances are short (2–2.4 Å) in a right-handed B form (Table II) and give rise to very strong cross-peaks in the 2D NOE spectra. Such interactions are found to be present in the conformation in low salt concentrations of poly(dA-dT) (Assa-Munt & Kearns, 1984; Borah et al., 1985a), poly(dG-d5MeC) (Borah et al., 1985a), and poly($d2NH_2A$-dT) (Borah et al., 1985b). In the case of poly($d2NH_2A$-d5IU) in low salt concentration, the features of the 2D NOE spectra can also be interpreted on the basis of a B conformation (Figure 1). Both H8 and H6 protons give cross-peaks with the 2′,2″ sugar protons. However, as the H2′,H2″ resonances are not resolved, the intra- and internucleotide contributions to the cross-peaks cannot be distinguished. In Z-DNA, the purine nucleotide adopts a syn conformation in which the H8 proton is in close

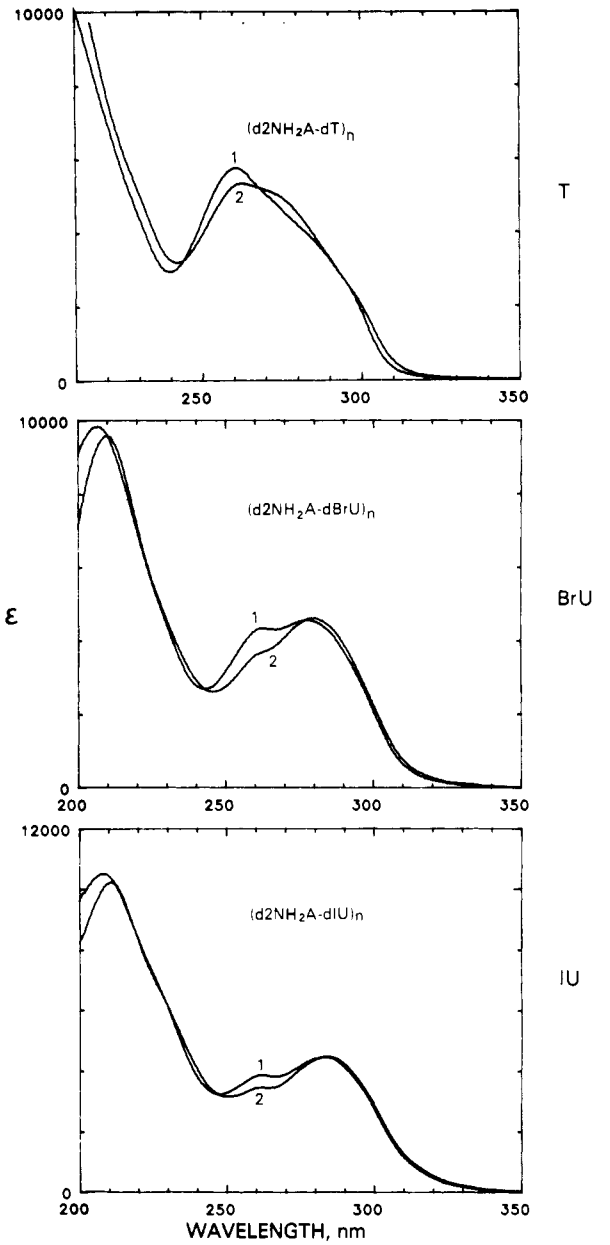


FIGURE 7: UV spectra of alternating poly($d2NH_2A$-dPy) polymers in high and low salt: poly($d2NH_2A$-dT) (top) in 0.1 M NaCl (1) and 4.03 M NaCl (2), in sodium phosphate buffer, 0.002 M, pH 7.5, 20 °C; poly($d2NH_2A$-d5BrU) (middle) in 0.05 M $NaClO_4$ (1) and 6.12 M $NaClO_4$ (2), in sodium cacodylate buffer, 0.002 M, pH 7.0, 20 °C; and poly($d2NH_2A$-d5IU) (bottom) in 0.1 M $NaClO_4$ (1) and 6.12 M $NaClO_4$ (2), in sodium cacodylate buffer, 0.002 M, pH 7.0, 20 °C.

proximity (2.32 Å) to the H1′ proton of the same nucleotide. A strong H8–H1′ cross-peak in the 2D NOE spectrum of poly(dG-d5MeC) in $Mg^{2+}$ solution shows that this copolymer is in the Z form (Borah et al., 1985a). The absence of such a cross-peak for poly(d2NH$_2$A-d5IU) in low salt therefore excludes a left-handed Z form as the structure of this polymer. The absence of base proton–H3′ interaction similarly excludes an A form for this polymer in low salt (Borah et al., 1985b).

In high salt, the 2D NOE spectrum of poly(d2NH$_2$A-d5IU) is strikingly different from that observed in low salt (Figure 2). In 4 M $NaClO_4$ the absence of cross-peaks for the H8–H1′ interaction again confirms that the high-salt form of poly(d2NH$_2$A-d5IU) is not a Z form. Two distinct cross-peaks appear in high salt for the H8–H3′ and H6–H3′ interactions. In our earlier work (Borah et al., 1985b), we have shown that these interactions are characteristic of an A conformation in solution. In an A form, the sugar pucker is 3′-endo and the nucleotide conformation is anti. This results in short H8–H3′ and H6–H3′ *intra*nucleotide distances (2.84 and 2.68 Å, respectively; Table II) that would give rise to strong NOE cross-relaxation peaks. In other common models of DNA conformations, such as B, alt-B, C, D, and Z, the *intra*nucleotide base proton–H3′ distances are longer than 3.6 Å (Table II). The *inter*nucleotide H8–H3′ and H6–H3′ distances for B- and Z-DNA are similarly long (>3.4 Å), so that no cross-peaks are expected at short mixing times from these interactions in these structures. In the A form the *inter*nucleotide base proton–H3′ distances are ca. 3.5 Å, also too long to give strong cross-peaks. Consequently, by elimination, the H8–H3′ and H6–H3′ cross-peaks that are observed in the high-salt spectra must arise from *intra*nucleotide cross relaxations for poly(d2NH$_2$A-d5IU) in an A form.

The evidence presented above clearly demonstrates that poly(d2NH$_2$A-d5IU) undergoes a salt-induced B to A transition in solution, similar to that for poly(d2NH$_2$A-dT) (Borah et al., 1985b). In the latter case, however, although the 2D NOE spectra clearly indicated an A form in high salt, the sugar pucker of the individual nucleotides could not be distinguished because the H8 and H6 resonances overlapped and the H3′ resonances also lacked resolution in high salt. In the case of poly(d2NH$_2$A-d5IU), the two base protons as well as the two H3′ resonances are fortunately resolved in the high-salt solution. The presence of two distinct H8–H3′ and H6–H3′ cross-peaks demonstrates that both the purine and pyrimidine sugar puckers are 3′-endo in the high-salt form.

As observed in Figures 2 and 3 for poly(d2NH$_2$A-d5IU) in high salt, there are two cross-peaks from the base protons to the H2′,H2″ region. The cross-peak intensities are stronger than those of the (H8,H6)–H3′ interactions. These cross-peaks are also consistent with an A-like structure. In A-DNA, the *inter*nucleotide H8–H2′ and H6–H2′ distances are short (Table II), compared to the *intra*nucleotide distances for base proton–(H2′,H2″) and *inter*nucleotide distances for base proton–H2″ interactions. The cross-peaks, therefore, can be assigned to the cross relaxation between a base proton (H8, H6) of one nucleotide and an H2′ proton of the neighboring nucleotide.

The one-dimensional NOE difference spectra of poly(d2NH$_2$A-d5BrU) also support the conclusion that this copolymer is in the A-like form in high salt (Figure 4). Although some contribution from spin diffusion cannot be ruled out, the presence of two strong NOE peaks for the H3′ resonances when the H8 and H6 protons were selectively irradiated strongly argues for the 3′-endo sugar pucker in an A-like conformation. NOE from H8 to H1′ is very weak, indicating that the Pu nucleotide is not syn as in Z conformation [cf. Figure 5B in Borah et al. (1985)].

*Conformation from Optical Spectra.* The unusual features of the CD spectra of poly(d2NH$_2$A-d5IU) and poly(d2NH$_2$A-d5BrU) in low salt are the negative first bands at high wavelength for the B conformation. With all previous examples of DNA, either natural (Kudyakov et al., 1978) or synthetic (Howard & Miles, 1983, 1984) containing d2NH$_2$A, the $B_2u$ transition has invariably led to a positive first band at ~290 nm in B-form helices and to a negative first extremum in A-form complexes. The A-form helices in this study are consistent with these observations but the B-form helices are not. The exceptional features cannot be ascribed to the use of $NaClO_4$; identical results are observed with low [NaCl]. We have used $NaClO_4$ because poly(d2NH$_2$A-d5IU) and poly(d2NH$_2$A-d5BrU) are insoluble at high [NaCl]. We note that, with prior examples, the $B_2u$ transition of d2NH$_2$A undergoes exciton splitting, in marked contrast to its apparent absence in the case of poly(d2NH$_2$A-d5IU) and poly(d2NH$_2$A-d5BrU). It is unclear what structural features prevent exciton splitting, but we suggest that its absence may account for the failure to observe positive bands in the spectra of these copolymers in the B form. These spectra clearly illustrate the unreliability of the sign of the long-wavelength CD bands as a criterion of the configuration of nucleic acids.

Though a number of DNA helices undergo changes in their electronic spectra in high ionic strength, the only other case in which the conformation of the high-salt form is known is the Z conformation, primarily of alternating dG-dC polymers. The UV spectral changes seen for poly(d2NH$_2$A-dPy) in high salt (Figure 7) are similar to those observed in the B–Z transition of dG-dC or dG-d5MeC (Pohl & Jovin, 1972; Behe & Felsenfeld, 1981) and might, like the CD, have suggested a Z conformation were it not for stronger evidence to the contrary. It is noteworthy that the similar UV spectral changes (especially the hypochromism at 260 nm) on going from B to A appear to be properties of alternating polymers. Homopolymers containing the same d2NH$_2$A-dT chromophores have been reported in both A and B forms (Howard & Miles, 1984), but in those cases the intensity at ~260 nm is the same or slightly higher for the A-form helices than for the B. Evidently the sequence is also important in determining the spectrum.

As noted above, optical spectroscopic methods may lead to erroneous conclusions regarding polymer conformations. In addition, the results of this and a previous report (Borah et al., 1985b) raise questions about deducing nucleic acid conformation from antibody binding. Since polymers in this series may respond positively to Z antibody in binding experiments, the possiblity exists that either the antibodies can bind to some A conformations or that the antibodies can themselves alter the DNA conformation so as to cause it to go to the Z rather than the A form.

*Conclusion.* The polynucleotides poly(d2NH$_2$A-d5XU), where X = Br and I, are both found to exist in the B form in low salt and in the A form in high salt concentrations.

**Registry No.** Poly(d2NH$_2$A-d5IU), 104576-78-5; poly(d2NH$_2$A-d5BrU), 104576-80-9.

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# Incorporation of a Synthetic Mitochondrial Signal Peptide into Charged and Uncharged Phospholipid Monolayers

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ABSTRACT: The interaction of the chemically synthesized 25-residue signal peptide of subunit IV of yeast cytochrome *c* oxidase with synthetic and natural phospholipids was studied by using a monolayer technique. Incorporation of the peptide into phospholipid monolayers was measured as surface area increase at constant surface pressure. The peptide was readily soluble in aqueous buffer, yet spontaneously inserted from an aqueous subphase into phospholipid monolayers up to limiting pressures of 30–40 mN/m. The incorporation of the positively charged peptide was strongly enhanced by the presence of negatively charged phospholipids. The molecular area of the signal peptide in monolayers was determined with a $^{14}C$-labeled signal peptide and was 560 ± 170 Å$^2$. This is consistent with a 25-residue $\alpha$-helical peptide incorporating with its long axis parallel to the plane of the monolayer. Incorporation isotherms into synthetic phosphatidylcholine and phosphatidylglycerol monolayers at different charge densities were analyzed in terms of a simple incorporation/binding model, involving (i) partitioning of the peptide into the monolayer and (ii) an in-plane binding reaction of the negatively charged phospholipids to the partitioned peptide.

In eucaroytes most proteins are encoded by the nucleus and translated on ribosomes in the cytoplasm. Many of them have to cross one or more membranes before they reach their final location inside or outside the cell. These include all secreted proteins and quite a large number of the polypeptides of chloroplasts and mitochondria. It has been recognized recently that N-terminal extensions ("signal", "leader", "transit" or "pre-" sequences) are responsible for intracellular sorting and the membrane translocation of these proteins. Most of them are synthesized as larger precursors, and the "signal" sequences are subsequently removed on the "trans" side of the membrane by specific proteases [for a review, see Wickner & Lodish (1985)]. One of the most intriguing questions relating to this process is how a polypeptide can select the correct target

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