Biochem., Biophys., Biol. 26B, 561-569.
Dall'Acqua, F., Terbogevich, M., Mariciani, S., Vedaldi, D., & Recher, M. (1978) *Chem.-Biol. Interact. 21*, 103-115.
Giessner-Rettre, C., & Pullman, B. (1976) *Biochem. Biophys. Res. Commun. 70*, 578-581.
Hearst, J. E. (1981) *J. Invest. Dermatol. 77*, 39-44.
Isaacs, S. T., Chun, C., Hyde, J. E., Rapoport, H., & Hearst, J. E. (1982) in *Trends in Photobiology* (Helene, C., Charlier, M., Montenay-Garestier, T., & Laustriat, G., Eds.) Plenum Press, New York and London.
Jain, S. C., Tsai, C. C., & Sobell, H. M. (1977) *J. Mol. Biol. 114*, 317-331.
Jain, S. C., Bhandary, K. K., & Sobell, H. M. (1979) *J. Mol. Biol. 135*, 813-840.
Kanne, D., Straub, K., Rapoport, H., & Hearst, J. E. (1982a) *Biochemistry 21*, 861-871.
Kanne, D., Straub, K., Hearst, J. E., & Rapoport, H. (1982b) *J. Am. Chem. Soc. 104*, 6754-6764.
Kroon, P. H., Kreishman, G. P., Nelson, J. H., & Chan, S. I. (1974) *Biopolymers 13*, 2571-2592.
Krugh, T. R., & Nuss, M. E. (1979) in *Biological Applications of Magnetic Resonance* (Shulman, R. G., Ed.) Academic Press, New York.
Land, E. J., Rushton, F. A. P., Beddoes, R. L., Bruce, J. M., Cernik, R. J., Dawson, S. C., & Mills, O. S. (1982) *J. Chem. Soc., Chem. Commun. 1*, 22-23.
Lee, C.-H., & Tinoco, I. (1978) *Nature (London) 274*, 609-610.
Lerman, L. S. (1961) *J. Mol. Biol. 3*, 18-30.
Li, H. J., & Crothers, D. M. (1969) *J. Mol. Biol. 39*, 461-477.
Miller, K. J., Macrea, J., & Pycior, J. F. (1980) *Biopolymers 19*, 2067-2089.
Neidle, S. (1979) *Prog. Med. Chem. 16*, 151-221.
Neidle, S., Achari, H., Taylor, G. L., Berman, H. M., Carrell, H. L., Glusker, J. P., & Stallings, W. C. (1977) *Nature (London) 269*, 304-307.
Patel, D. J. (1979) *Acc. Chem. Res. 12*, 118.
Patel, D. J., & Tonelli, A. E. (1974) *Biopolymers 13*, 1943-1964.
Parrish, J. A. (1981) *J. Invest. Dermatol. 77*, 167-171.
Parsons, B. J. (1980) *Photochem. Photobiol. 32*, 813-821.
Peckler, S., Graves, B., Kanne, D., Rapoport, H., Hearst, J. E., & Kim, S.-H. (1982) *J. Mol. Biol. 162*, 157-172.
Piette, L. H., & Anderson, W. A. (1959) *J. Chem. Phys. 30*, 899-908.
Reddy, B. S., Seshadri, T. P., Sakore, T. D., & Sobell, H. M. (1979) *J. Mol. Biol. 135*, 787-812.
Reuben, J., Baker, B. M., & Kallenbach, N. R. (1978) *Biochemistry 17*, 2915-2919.
Ribas Prado, F., & Giessner-Prettre, C. (1981) *Theor. Chem. (N.Y.) 1*, 81-98.
Rich, A., Quigley, G. J., & Wang, H. H.-J. (1980) in *Nucleic Acid Geometry and Dynamics* (Sarma, R. H., Ed.) Pergamon Press, New York.
Sakore, T. D., Reddy, B. S., & Sobell, H. M. (1979) *J. Mol. Biol. 135*, 763-785.
Sobell, H. M. (1980) in *Nucleic Acid Geometry and Dynamics* (Sarma, R. H., Ed.) Pergamon Press, New York.
Song, P. S., & Tapley, K. J., Jr. (1979) *Photochem. Photobiol. 29*, 1177-1197.
Stemple, N. R., & Watson, W. H. (1972) *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem. B28*, 2485-2489.
Straub, K., Kanne, D., Hearst, J. E., & Rapoport, H. (1981) *J. Am. Chem. Soc. 103*, 2347-2355.
Tsai, C. C., Jain, S. C., & Sobell, H. M. (1977) *J. Mol. Biol. 114*, 301-315.
Wakelin, L. P. G., & Waring, M. J. (1980) *J. Mol. Biol. 144*, 183-214.
Wang, H. M.-J., Nathans, J., van der Marel, G., van Boom, J. H., & Rich, A. (1978) *Nature (London) 276*, 471-474.
Wang, H. H.-J., Quigley, G. J., & Rich, A. (1979) *Nucleic Acids Res. 6*, 3879-3891.

Poly(d2NH$_2$A-dT): Two-Dimensional NMR Shows a B to A Conversion in High Salt

Babul Borah,[‡] Jack S. Cohen,*[,‡] Frank B. Howard,[§] and H. Todd Miles[§]

Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, and Laboratory of Molecular Biology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205

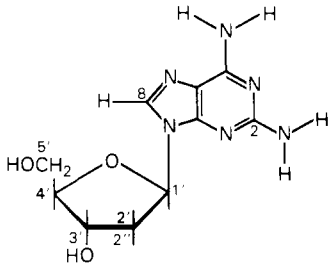
Received May 13, 1985

ABSTRACT: Poly(d2NH$_2$A-dT) forms a structure in high salt that is clearly distinct from the B form present in low salt. Two-dimensional nuclear Overhauser effect (2D NOE) NMR spectra establish that the conformation of the high-salt form is not Z. Correlations of observed cross peaks in the 2D NOE spectra and estimated interproton distances of the common DNA conformations are consistent only with an A form or closely related structure. This interpretation is also consistent with the negative circular dichroic band observed in the high-salt form of poly(d2NH$_2$A-dT) and in A-form ribohomopolymer helices containing 2NH$_2$A.

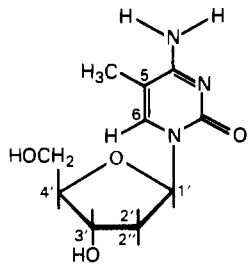
The conformational equilibrium between B and Z DNA has been extensively studied, though reasons for the interconversion are still not well understood. Several studies suggest that the Z form may be biologically important, possibly through a control function [Klysik et al., 1981; Nordheim et al., 1981; Hamada et al., 1982; for a review, see Zimmerman (1982)]. The fact that (dG-dC)$_n$ undergoes the B to Z transition while (dA-dT)$_n$ does not suggests a possible role of the third hydrogen bond present in GC pairs. In assessing this possibility it is important to examine a base pair having this property but

[‡] National Cancer Institute.
[§] National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

different in other respects from GC. 2NH$_2$A (I) forms three



I



II

hydrogen bonds with T, and its oligomers and polymers have therefore been investigated. Preliminary results were interpreted to support a Z conformation for the form present in high salt (Gaffney et al., 1982; Jovin et al., 1983; Howard & Miles, 1983; Howard et al., 1984). 2NH$_2$A has been found to replace all A's in the DNA of SL-2 cyanophage (Kirnos et al., 1977), but the biological implications of this change have not been investigated. The question of whether or not this substitution facilitates a B to Z transition is clearly relevant to its role in vivo.

Perhaps the most suggestive evidence for the Z form of poly(d2NH$_2$A-dT) in high salt was the sign inversion from positive to negative of the first circular dichroic (CD) extremum in high salt, resembling that seen in the B–Z conversion of poly(dG-dC) (Pohl & Jovin, 1972). More recent work, however, has shown that a series of A-form homopolymer helices containing 2NH$_2$A all have negative first extrema, whereas the corresponding B-form deoxyribopolymer helices have positive bands at the same wavelengths (Howard & Miles, 1984).

In order to obtain more definitive evidence on the conformation of the high-salt form of the polymer we have carried out two-dimensional nuclear Overhauser effect (2D NOE) NMR spectroscopy at 270 MHz on solutions of sonicated poly(d2NH$_2$A-dT). We compared the results with 2D NOE spectra of poly(dG-d5MeC), which contains 5-methyl-C (II) and which gives a Z form in high salt, and poly(dA-dT), which remains in the B form. On conversion from a B to a Z form, poly(dG-d5MeC) shows major changes in its 2D NOE contour plot, as seen, for example, in the presence of an intense GH8–GH1′ cross peak, which arises from the *syn*-G base in the Z form (Borah et al., 1985). The pattern of cross peaks of poly(d2NH$_2$A-dT) in low salt is essentially the same as that of the other B-form copolymers. In high salt (4 M NaCl), however, the pattern of the 2D NOE contour plot corresponds to neither the B nor Z form. We interpret this spectrum to indicate that the high-salt form of poly(d2NH$_2$A-dT) has an A-form structure. This assignment is fully consistent with the negative CD band observed in A-form ribohomopolymer helices containing 2NH$_2$A. Although several investigators have reported B to A conversions of DNA in the presence of ethanol [cf. Ivanov et al. (1974) and Gray & Ratliff (1975)], this appears to be the first evidence of this transition in solution promoted by high salt. The ability to define the conformation of a DNA copolymer in solution by analysis of the pattern of the 2D NOE contour plots enhances our capacity to elucidate the structural possibilities in vivo.

Materials and Methods

Materials. A solution of 6.85×10^{-3} mmol (polymer P) of (d2NH$_2$A-dT)$_n$ (Howard et al., 1984) in 2.5 mL of 4×10^{-3} M sodium phosphate buffer, pH 7.2, was sonicated at 10% power output for 3.8 h while temperature was maintained at 5 °C. The solution was passed through a Millipore filter (0.45 μm) and freeze-dried. The residue was dissolved in 0.5 mL of water and centrifuged at 10000 rpm for 30 min. After 0.05 mL of 1 M NaCl was added, the supernatant was freeze-dried 3 times from D_2O (99.96 atom %) and the residue dissolved in 0.5 mL of D_2O. UV analysis showed that the solution contained 7.07×10^{-3} M polymer P. For measurements at high Na^+ concentration the sample was freeze-dried and dissolved in 0.4 mL of 3.9 M NaCl in D_2O. The pH was also determined from the ^{31}P chemical shift of the P_i peak relative to Me_3PO_4 in ^{31}P NMR spectra.

NMR Methods. Proton 2D NOE experiments were performed at 270 MHz on a Nicolet (GE-NMR) spectrometer with a Bruker superconducting magnet, interfaced with a Nicolet 1280 computer and 293C pulse programmer. The 2D NOE spectra were collected by using a sequence of three nonselective pulses (Kumar et al., 1980). The free induction decay (FID) pattern consisted of 1024 data points in the t_2 dimension with a sweep width (SW) of 2500 Hz and 64 t_1 values incremented by the dwell time (SW/2). Data were collected in the pure absorption mode by using a phase-cycling routine that allowed spectra to be recorded in quadrature mode. The FIDs were apodized by a Gaussian function with the 10–20-Hz line broadening in both dimensions. The resulting data matrix was symmetrized, but all the cross peaks discussed were also present in the unsymmetrized data. ^{31}P NMR spectra were recorded at 160 MHz on a Varian XL-400 NMR spectrometer at 22 °C, with Me_3PO_4 added as an internal chemical shift standard.

Interatomic Distances. Interatomic distances and structures were obtained with the XRAY computer program developed by Richard Feldmann, Division of Computer Research & Technology, NIH. Coordinates are stored on disk, and the sources of the structures are listed in the *Atlas of Macromolecular Structure on Microfiche* (Feldmann, 1976) except for more recent structures, which are also cited here.

Results

2D NOE Spectrum of Poly(d2NH$_2$A-dT) in Low Salt. The contour plot of 2D NOE spectra of double-stranded poly(d2NH$_2$A-dT) in 0.1 M NaCl solution using a 50-ms mixing time is shown in Figure 1A. Spectra on the two axes, which are equivalent to the one-dimensional spectra, are projections of the diagonal peaks that represent spin magnetization that does not cross-relax during the mixing time. The assignment of resonances relevant to the discussion are labeled and are based on previous assignments of analogous sequences (Borah et al., 1985). At short mixing times cross peaks are observed only for protons that are separated by less than ca. 3.5 Å. Those that are observed in Figure 1A indicate cross-relaxation interactions between base protons–(H2′,H2″), base protons–T(Me), H1′–(H2′,H2″), and H3′–(H2′,H2″).

2D NOE Spectrum of Poly(d2NH$_2$A-dT) in High Salt. Addition of 4 M NaCl to poly(d2NH$_2$A-dT) causes changes in the aromatic proton chemical shifts. Figure 2 shows one-dimensional spectra of poly(d2NH$_2$A-dT) in the aromatic region in low- and high-salt solutions. The 2NH$_2$A H8 and T H6 protons occurring at 7.72 and 7.35 ppm, respectively, in low-salt solution are both shifted in the high-salt solution and overlap at 7.82 ppm, as shown by the areas measured. The H1′ resonances of both of the sugar residues also undergo a downfield shift of ca. 0.2 ppm in the high-salt solution.

Figure 1B shows the 2D NOE contour plots of this polymer in 4 M NaCl with a 50-ms mixing time. Because of the lack of resolution of the base protons, the cross peaks related to both H8 and H6 protons with sugar (H2′,H2″) and T(Me)

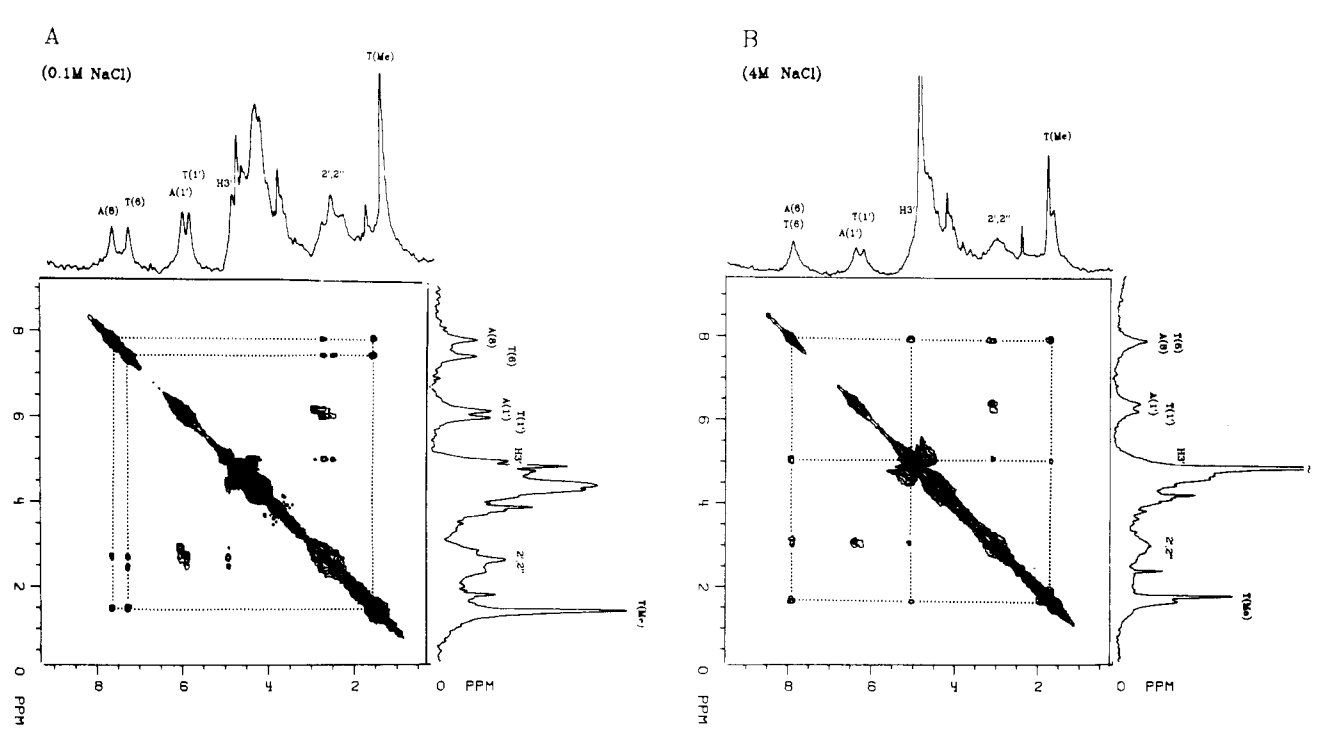


FIGURE 1: Contour plots of the 2D NOE spectra of double-stranded poly(d2NH$_2$A-dT) in (A) 0.1 M NaCl and (B) 4 M NaCl. The mixing time in both cases was 50 ms. Cross peaks for pairs of interacting protons are identified by joining resonances by horizontal and vertical lines on either side of the diagonal as illustrated.

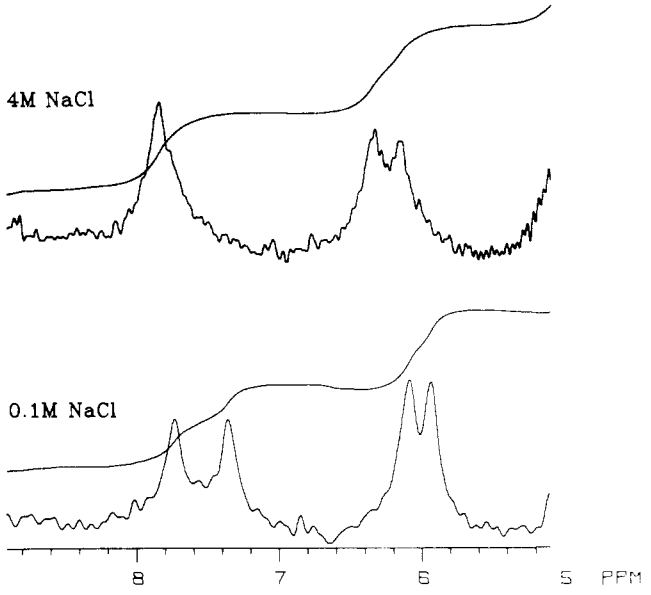


FIGURE 2: NMR spectra at 270 MHz of poly(d2NH$_2$A-dT) showing the chemical shifts of the base and H1′ protons in low- and high-salt solutions.

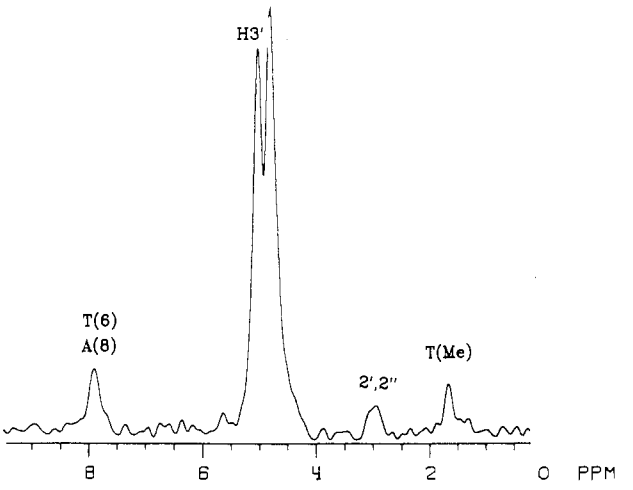


FIGURE 3: Cross section of the absorption mode 2D NOE spectra of poly(d2NH$_2$A-dT) in 4 M NaCl, showing the cross peaks for intranucleotide H3′–H8,H6 and H3′–H2′, and internucleotide H3′–T(Me) interactions.

also overlap. The most significant effect in the high-salt solution is the appearance of two new cross peaks, one corresponding to base proton–H3′ and the other to H3′–T(Me) interactions. Neither of these features is present in the low-salt-solution spectrum. Figure 3 shows a cross section of the 2D NOE spectrum in high salt illustrating the base proton–H3′, H3′–(H2′,H2″), and H3′–T(Me) interactions. Figure 4 shows a section of the 2D NOE contour plot of poly(d2NH$_2$A-dT) obtained with a 25-ms mixing time. This exhibits a cross-peak pattern similar to that observed for a 50-ms mixing time but with some suggestive changes in relative intensities.

2D NOE Spectrum of Poly(dA-dT) in High Salt. The pattern of cross peaks observed in 2D NOE spectra of poly(dA-dT) in 4 M NaCl (Figure 5A) is closely similar to that previously found in both low-salt and high-CsF concentrations (Borah et al., 1985). There is also a basic similarity of the cross peak pattern to that observed in the low-salt form of poly(2NH$_2$A-dT). We also reproduce (Borah et al., 1985) the 2D NOE contour plot of poly(dG-d5MeC) in 3 mM $MgCl_2$ (Figure 5B) for comparison with the spectra of the other copolymers.

^{31}P NMR Results. ^{31}P spectra of poly(d2NH$_2$A-dT) in 4 M NaCl exhibit a doublet of broad peaks at −3.73 and −4.45 ppm relative to Me_3PO_4. This result is consistent with that reported previously for this polymer in 5 M CsF solution (Howard et al., 1984).

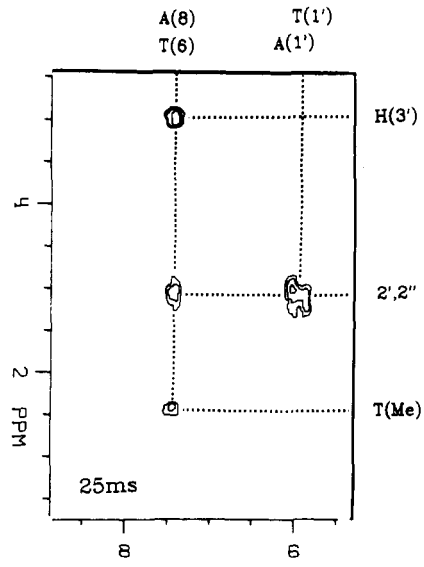


FIGURE 4: A portion of the contour plots of the absorption mode 2D NOE spectra of poly(d2NH$_2$A-dT) in 4 M NaCl using a mixing time of 25 ms.

Discussion

Recent work in this laboratory and elsewhere has revealed that two-dimensional NOE spectroscopy provides a unique method for detailed conformational analysis of polydeoxynucleotides in solution (Assa-Munt & Kearns, 1984; Borah et al., 1985). By use of this method structural information is obtained on the basis of spatial connectivities between nuclei coupled by dipolar interaction. Important structural features such as the handedness, the nature of the sugar puckering, and syn- vs. anti-nucleotide conformation are revealed by this technique. The elucidation of such structural features of poly(d2NH$_2$A-dT) will be illustrated by the application of 2D NOE spectroscopy.

Detailed analysis of the 2D NOE spectra of poly(dA-dT) and poly(dG-d5MeC) has shown that in low-salt solution these copolymers exist as right-handed B forms (Assa-Munt & Kearns, 1984; Borah et al., 1985). In low salt (0.1 M NaCl) the contour plots of 2D NOE spectra of poly(d2NH$_2$A-dT) are very similar to those of the former two copolymers. In all of the experiments the mixing times used were comparable (25–50 ms). For the chain lengths used in these studies, the cross-peak intensities increase approximately linearly with mixing times in this range, and spin diffusion and second-order NOE effects are negligible (Borah et al., 1985). The pattern of cross peaks observed for poly(d2NH$_2$A-dT) in low salt (Figure 1A) and the cross sections of the 2D NOE spectra (data not shown) can be interpreted on the basis of a right-handed B conformation. For example, cross-relaxation interactions for intranucleotide H8 (or H6)–H2′, intranucleotide H1′–H2″, and internucleotide H8 (or H6)–H2″, which are observed in this case, are typical of the B conformation. Similarly, the interbase H8–T(Me) has intensity comparable to that of the intrabase H6–T(Me), indicating that the interproton distances between these pairs of protons are also comparable (ca. 2.5 Å) to those found in the B form (Arnott & Hukins, 1972; Zimmerman, 1982).

In assigning the poly(d2NH$_2$A-dT) structure in low salt to a right-handed B form we specifically exclude the left-handed B structure. For a left-handed B form, the calculated intranucleotide distances between H8 (or H6) and H2′ are 4.2 Å, which would predict little or no cross-relaxation interaction between these protons (Dhingra et al., 1983). However, strong cross peaks corresponding to these interactions are seen in this spectrum.

Analysis of the 2D NOE spectra of poly(dA-dT) in high salt shows that this copolymer retains its B-DNA structure. In the previous studies CsF was used because it was reported to cause less aggregation and precipitation than NaCl (Vorlickova et al., 1980), but there were minor differences in the CD spectra and the ^{31}P NMR spectra (Chen & Cohen, 1983) in the presence of these two salts. The cross-peak pattern now

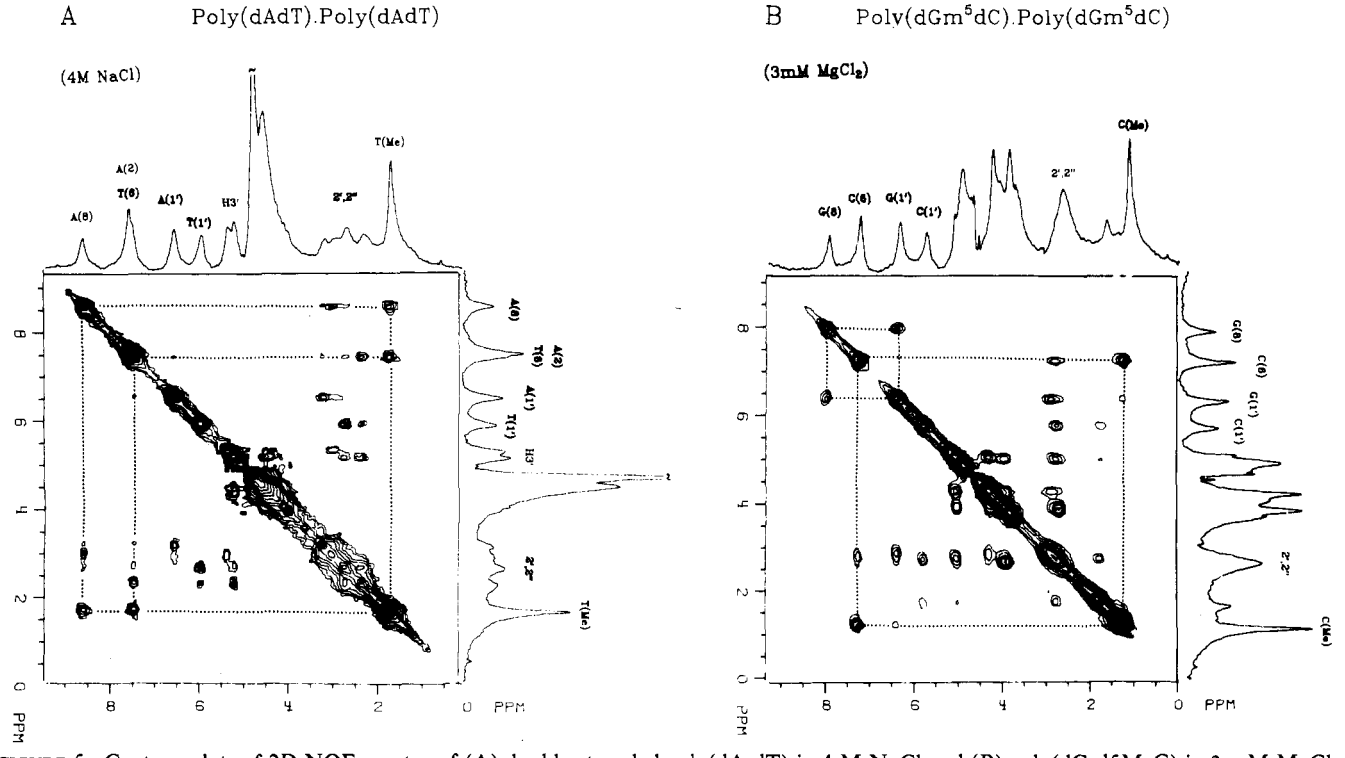


FIGURE 5: Contour plots of 2D NOE spectra of (A) double-stranded poly(dA-dT) in 4 M NaCl and (B) poly(dG-d5MeC) in 3 mM $MgCl_2$ (Borah et al., 1985). Mixing time was 50 ms in both cases.

Table I: Interproton Distances (Å) Computed for Different DNA Models[a]

model	purine intranucleotide proton interaction			pyrimidine intranucleotide proton interaction			internucleotide interaction
	H8–H3′	H8–H1′	H8–H2′	H6–H3′	H6–H1′	H6–H2′	H3′–Me
A DNA	2.84	3.63	3.77	2.68	3.51	3.42	2.38
B DNA	4.10	3.69	2.14	3.83	3.58	1.92	4.54
alt-B DNA	3.69	3.59	2.99	3.69	3.51	1.85	3.68
C DNA	4.10	3.69	2.14	4.10	3.57	1.91	4.07
D DNA	4.13	3.70	2.15	4.13	3.57	1.90	3.98
Z DNA	5.26	2.32	3.98	4.64	3.54	3.27	

[a] These distances were obtained by using the XRAY program from the coordinates contained in the NIH library of structures.

reported in the presence of 4 M NaCl (Figure 5A) is closely similar to that observed previously in high CsF concentrations. All the features of the 2D NOE spectra of poly(dA-dT) in 4 M NaCl also conform only to a right-handed B form.

Unlike poly(dA-dT), double-stranded poly(d2NH_2A-dT) shows interesting new features in its 2D NOE contour plot in high salt (Figures 1B, 3, and 4). Although the cross peaks for the interactions of base protons with (H2′,H2″) and with T(Me) now overlap, because of the loss of resolution between the resonances of the purine H8 and pyrimidine H6 protons (Figure 2), two further distinct features are noteworthy: (1) a strong cross peak for cross relaxation between base protons and H3′ and (2) a cross peak of medium intensity for cross relaxation between H3′ and T(Me). A cross section through the 2D NOE spectrum illustrating these interactions is seen in Figure 3. It may be noted that the cross-peak intensity for the base proton–H3′ interaction is nearly double that of the H3′–T(Me) cross peak due to the overlap of the two base proton resonances.

That the high-salt form of poly(d2NH_2A-dT) is not a Z structure is evident from the fact that no cross peak for the H8–H1′ interaction is seen. By contrast, the Z form of poly(dG-d5MeC) shows a very strong cross peak for this interaction (Figure 5B). Consequently, we can conclude that while the G base is in the syn conformation in poly(dG-d5MeC), both 2NH_2A and T are in the anti conformation in the high-salt form of their copolymer.

The presence of a strong base proton–H3′ interaction indicates a C3′-endo conformation for this copolymer. We have surveyed the H3′ noncovalent bond distances in most of the known DNA models, including A, B, alt-B, C, D, and Z forms [see Zimmerman (1982)]. Some of the distances relevant to this discussion are included in Table I. We note that only the A form with a 3′-endo sugar pucker has short enough H8–H3′ and H6–H3′ distances (2.84 and 2.68 Å, respectively) to give rise to such a cross peak (Figure 6). For the other conformations the base proton–H3′ distance is larger than 3.5 Å, and we expect to see no cross peak at the short mixing times used in these experiments.

The other distance of interest, also included in Table I, is the internucleotide AH3′–T(Me) distance, which is short for the A form (2.38 Å) and long (>3.5 Å) for the other conformations (taking the nearest distance of approach in each case). Thus, only in the A form can cross relaxation arise for this internucleotide interaction, and the occurrence of such a cross peak confirms the assignment of this structure to an A form.

Haasnoot et al. (1983) have analyzed the conformation of a hybrid DNA–RNA oligonucleotide, where the A-form RNA was characterized mainly by sugar proton coupling constants. In addition, they have recently used resolved base proton–H2′ (but not H3′) cross peaks in 2D NOE spectra of ribo- and deoxyribonucleotides to differentiate between A and B conformations (Haasnoot et al., 1984). However, chemical shift differences between the sugar protons in the ribo and deoxy series and line-width differences between oligomers and polymers make their analysis inappropriate in the case of a polydeoxynucleotide.

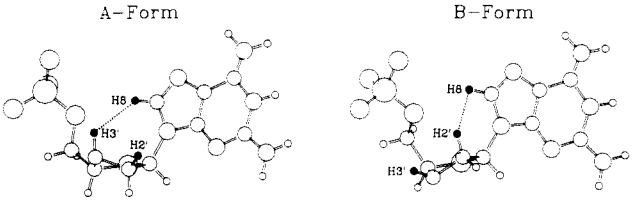


FIGURE 6: Structure of d2NH_2A in A and B forms of DNA.

The B to A conversion reported here is relevant to the spine of hydration identified in crystallographic studies by Dickerson and colleagues (Drew & Dickerson, 1981). In B DNA a regular pattern of hydrogen-bonded water molecules exists in the minor groove: water molecules bridge the C2O and N3 positions in successive base pairs, and the oxygens of these water molecules are in turn bonded by a second layer of water molecules. Dickerson had suggested that the spine of hydration is one of the main stabilizing elements of B DNA in relation to A or Z. A recent study of homopolymers of 2NH_2A (Howard & Miles, 1984) permits an estimate of ca. 15 °C in T_m stabilization of B DNA and roughly 3–4 kcal of enthalpy attributable to this spine of hydration. Though one may thus anticipate that the 2-NH_2 substitution will be relatively destabilizing to the B form, there is at present no way to predict whether the favored alternative form will be A or Z. The classic example of poly(dG-dC), with a spine of hydration precluded by the 2-NH_2 group as with 2NH_2A, is converted to the Z rather than the A form. The reason for the effect of 4 M NaCl on Z DNAs that lack a spine of hydration is also by no means clear at present.

It should be noted, as seen in Figure 3, that the cross-peak intensity for the intranucleotide base proton–H3′ interaction is nearly twice the intensity of the cross peak for the internucleotide T(Me)–AH3′ interaction, although the interproton distances are of comparable magnitude (ca. 2.7 and 2.4 Å, respectively). This may be explained by the fact that two different intranucleotide interactions are contributing to the cross peak, and they are not resolvable owing to overlap in the chemical shift of both H3′s and base protons. The doubled intensity presumably means that the sugar pucker of both the 2NH_2A and T nucleotides is 3′-endo. However, because of the overlap of these resonances it would probably be impossible to carry out quantitative distance determinations by the 2D NOE method, which requires accurate intensity measurements

of cross peaks at several mixing times.

Observation of two equal ^{31}P resonances in Z-form DNA demonstrates that the P atoms of the polymer occur in two equal populations that are nonequivalent in their chemical or physical environments. The structure in Z DNA clearly provides two equal populations in the alternating dimer repeat that account for this observation. Other regularly repeating non-Z DNA copolymers may also exhibit two ^{31}P resonances either in low salt or when ionic strength is increased to several molar. The low-salt forms are usually assigned an alternating B DNA structure (Klug et al., 1979; Shindo et al., 1979), although evidence for the specific conformation of this model in solution has been inconclusive. Failure to observe (H6,H8) to H3′ cross peaks (Assa-Munt & Kearns, 1984; Borah et al., 1985) would not rule out this structure because the distance calculated from the model (Klug et al., 1979) is greater than 3.5 Å (Table I). The evidence, on the other hand, does not establish that this structure is correct. Whether or not the detailed structure can be identified, however, observation of two ^{31}P resonances does require that the P atoms exist in two distinct environments. In fact, all repeating purine–pyrimidine polymers do have two chemically distinct environments for P, either pu-p-py or py-p-pu. The bases are rather far from P to exert much influence on the chemical shift, however, and some alternating copolymers exhibit only a single resonance (Cohen et al., 1981) either for this reason or possibly because of coincidental overlap.

A method of conformational analysis derived from the crystallographic study of a B-form dodecamer (Dickerson & Drew, 1981) and proposed by Calladine [1982; cf. Dickerson (1983)] is relevant to DNA containing regularly alternating pu-py sequences. Here the steric clash between purine residues in opposite strands, upon which the analysis primarily depends, occurs at every step of the helix, thus maximizing the need for steric relief by the four strategies outlined by Calladine. A basic prediction of the proposal is therefore that all DNAs composed of pu-py repeating sequences should have alternating structures, incorporating to some extent the following features: (1) an alternation in the local helix twist or rotation angle between values approximately 5° greater and smaller than the mean value of 36°; (2) an alternating opening of the roll angle θ_R to the major and minor grooves; (3) a sliding of succeeding base pairs out of the stack in opposite directions, with a resulting increase in the main chain torsion angle δ at the purines and a corresponding decrease at the pyrimidines; (4) a flattening of the propeller twist angle of all base pairs.

Of the above structural responses to relieve interchain purine clash, the third, with its effect on the torsional angle δ about the C3′–C4′ bond, appears most likely to have interpretable effects on NMR spectra of polymers. The spectra of repeating pu-py polymers in Figures 1A and 5A exhibit aromatic–H2′,H2″ cross peaks characteristic of the C2′-endo conformation, which corresponds to high values of δ. There are no aromatic–H3′ cross peaks to indicate the presence of a C3′-endo pucker, present at low values of δ. As noted above, however, this form is not necessarily ruled out since the relevant distance in the alternating B form model proposed by Klug et al. (1979) is slightly greater (Table I) than that at which significant NOE effects are detectable.

In the high-salt form of poly(d2NH$_2$A-dT) an aromatic–H3′ cross peak indicative of the C3′-endo conformation (and a low value of δ) is observed. In view of the fact that the intensity of the H6,H8–H3′ cross peak is roughly twice that of the other cross peaks in Figure 3, we have suggested that both pu and py residues contribute to it and have C3′-endo pucker. We also observe, however, an aromatic–H2′,H2″ cross peak of moderate intensity in Figures 1B and 4, which may arise from a C2′-endo conformation of one of the two component nucleotides. We think it is more likely that this cross peak is due to the short (1.73 Å) internucleotide TH6–AH2′ distance in the A DNA conformation. The relative intensity of this cross peak was found to increase at a shorter mixing time of 25 ms (Figure 4), indicating that it arises from a very short interproton distance. For reasons noted above, the B, alt-B, C, D, and Z conformations can be eliminated by the present NOE study. An A-form structure, however, is consistent with both the NMR and CD data, although with the possible modification of alternating C2′-endo py residues.

Conclusions

2D NOE spectra show that the high-salt form of poly(d2NH$_2$A-dT) does not have the B or Z conformation and indicate that it is a member of the A family.

Registry No. Poly(d2NH$_2$A-dT), 55711-20-1; poly(dG-d5MeC), 51853-63-5.

References

Arnott, S., & Hukins, D. W. L. (1972) *Biochim. Biophys. Acta 47*, 1504–1509.

Assa-Munt, N., & Kearns, D. R. (1984) *Biochemistry 23*, 791–796.

Borah, B., Cohen, J. S., & Bax, A. (1985) *Biopolymers 24*, 747–765.

Calladine, C. R. (1982) *J. Mol. Biol. 161*, 343–352.

Chen, C.-W., & Cohen, J. S. (1983) *Biopolymers 22*, 879–893.

Cohen, J. S., Wooten, J. B., & Chatterjee, C. L. (1981) *Biochemistry 20*, 3049–3055.

Dhingra, M. M., Sarma, M. H., Gupta, G., & Sarma, R. H. (1983) *J. Biomol. Struct. Dyn. 1*, 417–428.

Dickerson, R. E. (1983) *J. Mol. Biol. 166*, 419–441.

Dickerson, R. E., & Drew, H. R. (1981) *J. Mol. Biol. 149*, 761–786.

Drew, H. R., & Dickerson, R. E. (1981) *J. Mol. Biol. 151*, 535–556.

Feldmann, R. (1976) *Atlas of Macromolecular Structure on Microfiche*, Tracor-Jitco, Rockville, MD.

Gaffney, B. L., Marky, L. A., & Jones, R. A. (1982) *Nucleic Acids Res. 10*, 4351–4361.

Gray, D. M., & Ratliff, R. L. (1975) *Biopolymers 14*, 487–498.

Haasnoot, C. A. G., Westerink, H. P., van der Marel, G. A., & van Boom, J. H. (1983) *J. Biomol. Struct. Dyn. 1*, 131–149.

Haasnoot, C. A. G., Westerink, H. P., van der Marel, G. A., & van Boom, J. H. (1984) *J. Biomol. Struct. Dyn. 2*, 345–360.

Hamada, H., Petrino, M. G., & Kakunage, T. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6465–6469.

Howard, F. B., & Miles, H. T. (1983) *Jerusalem Symposia on Quantum Chemistry and Biochemistry*, pp 511–520, Kluwer, Boston.

Howard, F. B., & Miles, H. T. (1984) *Biochemistry 23*, 6723–6732.

Howard, F. B., Chen, C.-W., Cohen, J. S., & Miles, H. T. (1984) *Biochem. Biophys. Res. Commun. 118*, 848–853.

Ivanov, V. I., Minchenko, L. E., Minyat, E. E., Frank-Kamenetskii, M. D., & Schyolkina, A. K. (1974) *J. Mol. Biol. 87*, 817–833.

Jovin, T. N., McIntosh, L. P., Arndt-Jovin, D. J., Zarling, D. A., Robert-Nicoud, M., van de Sande, J. H., & Jorgenson,

K. F. (1983) *J. Biomol. Struct. Dyn. 1*, 21-57.
Kirnos, M. D., Khudyakov, I. Y., Alexandrushkina, N. I., & Vanyushkin, B. R. (1977) *Nature (London) 270*, 369-370.
Klug, A., Jack, A., Viswamitra, M. A., Kennard, O., Shakked, Z., & Steitz, T. A. (1979) *J. Mol. Biol. 131*, 669-680.
Klysik, J., Stirtivant, S. M., Larson, J. E., Hart, P. A., & Wells, R. D. (1981) *Nature (London) 290*, 672-677.
Kumar, A., Ernst, R. R., & Wüthrich, K. (1980) *Biochem. Biophys. Res. Commun. 95*, 1-6.
Nordheim, A., Pardue, M. L., Lafer, E. M., Moller, A., Stollar, B. D., & Rich, A. (1981) *Nature (London) 294*, 417-422.
Pohl, F. M., & Jovin, T. (1972) *J. Mol. Biol. 67*, 375-396.
Shindo, H., Simpson, R., & Cohen, J. S. (1979) *J. Biol. Chem. 254*, 8125-8129.
Vorlickova, M., Kypr, J., Kleinwachter, V., & Palacek, E. (1980) *Nucleic Acids Res. 8*, 3965-3973.
Zimmerman, S. (1982) *Annu. Rev. Biochem. 51*, 395-427.

Ethidium Binding to Left-Handed (Z) DNAs Results in Regions of Right-Handed DNA at the Intercalation Site†

G. Terrance Walker, Michael P. Stone,‡ and Thomas R. Krugh*
Department of Chemistry, University of Rochester, Rochester, New York 14627
Received December 18, 1984; Revised Manuscript Received May 20, 1985

ABSTRACT: The equilibrium binding of ethidium to the right-handed (B) and left-handed (Z) forms of poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) was investigated by optical and phase partition techniques. Ethidium binds to the polynucleotides in a noncooperative manner under B-form conditions, in sharp contrast to highly cooperative binding under Z-form conditions. Correlation of binding isotherms with circular dichroism (CD) data indicates that the cooperative binding of ethidium under Z-form conditions is associated with a sequential conversion of the polymer from a left-handed to a right-handed conformation. Determination of bound drug concentrations by various titration techniques and the measurement of circular dichroism spectra have enabled us to calculate the number of base pairs of left-handed DNA that adopt a right-handed conformation for each bound drug; 3-4 base pairs of left-handed poly(dG-dC)·poly(dG-dC) in 4.4 M NaCl switch to the right-handed form for each bound ethidium, while approximately 25 and 7 base pairs switch conformations for each bound ethidium in complexes with poly(dG-dC)·poly(dG-dC) in 40 μM $[Co(NH_3)_6]Cl_3$ and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 2 mM $MgCl_2$, respectively. The induced ellipticity at 320 nm for the ethidium–poly(dG-dC)·poly(dG-dC) complex in 4.4 M NaCl indicates that the right-handed regions are nearly saturated with ethidium even though the overall level of saturation is very low. The circular dichroism data indicate that ethidium intercalates to form a right-handed-bound drug region, even at low r values where the CD spectra show that the majority of the polymer is in a left-handed conformation.

The striking observation of the salt-induced cooperative conformational change of poly(dG-dC)·poly(dG-dC) from a right-handed helix to the left-handed (Z) helix (Pohl & Jovin, 1972; Wang et al., 1979) is a clear illustration that deoxyribonucleic acid (DNA)[1] can exist in a variety of conformations and that the structure has a pronounced effect on the function of DNA [for reviews, see Rich et al. (1984) and Wells et al. (1980), and references therein]. Poly(dG-dC)·poly(dG-dC) assumes a left-handed (Z-form) conformation in 4.4 M NaCl, to which ethidium does not bind efficiently until the ethidium concentration reaches approximately 20 μM (Pohl et al., 1972). The intercalation of ethidium is accompanied by a highly cooperative left- to right-handed conformational transition of the polynucleotide, as evidenced by circular dichroism spectroscopy. In subsequent experiments, van de Sande & Jovin (1982) studied the binding of ethidium, actinomycin D, and mithramycin to a condensed form of poly(dG-dC)·poly(dG-dC) in $MgCl_2$–ethanol which was designated as Z*-DNA. All three drugs were found to reverse the sedimentability of Z*-DNA, an observation consistent with the alteration of the conformation of poly(dG-dC)·poly(dG-dC) from a left-handed helix to a right-handed helix (but not a "B" form since the duplex is distorted to accommodate the ligands). Ethidium binding to left-handed poly(br^{8}dG-br^{5}dC)·poly(br^{8}dG-br^{5}dC) and poly(dG-br^{5}dC)·poly(dG-br^{5}dC) also is accompanied by a left- to right-handed transition (Moller et al., 1984; Rio & Leng, 1984). The binding of netropsin induces a left- to right-handed reversal of poly(dG-dC)·poly(dG-dC), whereas distamycin-3 is ineffective (Zimmer et al., 1983). Kinetic studies involving the inhibition of the salt-induced B to Z transition of poly(dG-dC)·poly(dG-dC) by proflavin, ethidium, actinomycin D, and bis(methidium)spermine have been interpreted in terms of inhibition of the nucleation and propagation of the Z form (Mirau & Kearns, 1983). Adriamycin and daunomycin also have been reported to effectively inhibit

† This research was supported by Grants CA-17865 and CA-35251 from the National Cancer Institute.
‡ Present address: Department of Chemistry, Vanderbilt University, Nashville, TN 37235.

[1] Abbreviations: DNA, deoxyribonucleic acid; Me_2SO, dimethyl sulfoxide; CD, circular dichroism.

0006-2960/85/0424-7462$01.50/0