Stefano, J. E., & Gralla, J. D. (1980) *J. Biol. Chem. 255*, 10423–10430.
Stefano, J. E., & Gralla, J. D. (1982) *J. Biol. Chem. 257*, 13924–13929.
Talkington, C., & Pero, J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1185–1189.
von Hippel, P. H., Bear, D. B., Winter, R. B., & Berg, O. G. (1982) *Promoters: Structure and Function* (Rodriguez, R. L., & Chamberlin, M. J., Eds.) pp 3–33, Praeger, New York.
Williamson, V. M., & Doi, R. H. (1978) *Mol. Gen. Genet. 161*, 135–141.

Covalent Attachment of an Alkylamine Prevents the B to Z Transition in Poly(dG-dC)†

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ABSTRACT: Covalent complexes of *n*-butylamine and double-stranded poly(dG-dC) were prepared by coupling the amine to exocyclic amino groups of guanine bases with CH_2O. Neither the absorption spectrum above 230 nm nor the $s^0_{20,w}$ of the complexes in low to moderate ionic strength solvents, freed of excess unreacted reagents, differs significantly from that of unreacted poly(dG-dC) or a control which had been exposed only to CH_2O. In contrast, the CD spectra are profoundly altered. The minimum at 252 nm becomes more negative, and the rotational strength of the positive band above 260 nm is reduced as a linear function of the extent of amine attachment. At 0.22 mol of amine per mole of nucleotide, the transformation is similar to that observed by others in poly(dG-dC) when complexed to core histones in reconstituted core particles or in concentrated LiCl solvents at temperatures below the B → Z transition. Sedimentation studies reveal that these changes in the circular dichroism (CD) spectra reflect secondary structural effects rather than the formation of aggregates or ψ type structures. Raman spectra reveal, however, that these secondary structural changes must occur within the B family as the amine complex retains B backbone geometry. The conformation produced by the attachment of the amine is probably a higher winding angle (overwound) B variant. If the substitution level is above 0.06 mol of amine per mole of nucleotide at 27 °C, exposure of the complex to 4 M NaCl does not appreciably alter the absorbance spectrum and only causes a further depression of the positive band and an increase in negative rotational strength of the negative band of the CD spectrum, indicating a failure to transform to the Z structure. These spectral properties are unchanged by heating at 60 °C for 1 h. In fact, the solution of derivative in 4 M NaCl must be heated above 80 °C, with consequent total loss of covalently bound amine, before transformation to the Z form occurs. Examination of a series of such derivatives of different amine content between pH 7 and 10.7 in 4 M NaCl reveals that it is the charge on the amine rather than its steric properties which prevents transformation to the Z form. The B → Z transformation will not ensue until the *charged* amine content has been reduced to an *average* of ca. 0.05 mol of positive charge per mole of nucleotide, or ca. 1 in 20 bases. Control samples poly(dG-dC) exposed only to CH_2O in the reaction mixture show the typical B → Z transformation in CD and absorption spectra in going from 20 mM to 4 M NaCl. The stabilization of the B form of poly(dG-dC) by the attachment of a positively charged amine in the minor groove thus appears to be a thermodynamic rather than a kinetic phenomenon. These derivatives should be useful in elucidating the role of elelctrostatic factors in the B → Z transition.

We have previously found that primary amines can be covalently attached to bases in linear (Chen et al., 1981) and covalently closed duplex DNA (Kilkuskie, 1982; Kilkuskie et al., 1982) by coupling with CH_2O at concentrations of the latter insufficient to cause significant denaturation or cross-linking of bases during the time course of the reaction. Although exhaustive dialysis (which removes the excess amine and formaldehyde) will result in some reversal of the reaction, a significant fraction of bases retains amine and formaldehyde in a mole ratio of 1:1 of amine/formaldehyde. Derivatization appears to have the same conformational effect on random sequence DNA as do increases in the electrolyte content of the aqueous solvent or complexion with core histones. This effect has been shown by our laboratory (Chen et al., 1981) to be due to a positive charge on the derivatized base product rather than the steric properties of the formaldehyde/amine adduct. Since the linear charge density and the ion content of the grooves of duplex DNA can be varied by controlling the extent of amine attachment to the final product, these derivatives can be used to elucidate the role of electrostatics in modulating the conformational characteristics of DNA. We have correspondingly undertaken an extensive series of investigations of their properties. The results of our studies with random sequence DNAs form the background of this current study. Because of their relevance, these are summarized below.

A large number of amines will undergo this reaction, although the major share of the characterization work has been

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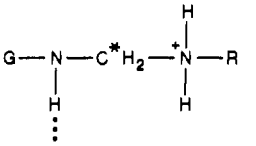
conducted on the stable adduct formed with *n*-butylamine (BuA),[1] isolated by exhaustive dialysis. The extent of the retention of amine by the dialyzed DNA sample is dependent on the concentration of all reaction components and the time of exposure to the reaction conditions (Chen et al., 1981) as well as the GC content of the DNA (Maibenco et al., 1984). Under standard reaction conditions, the extent of derivatization is linearly dependent on GC content, with little or no reaction evidenced by poly(dA-dT) and maximal reaction with poly(dG-dC). Although the cytosines in poly(dI-dC) will react in the presence of excess formaldehyde and amine, there is complete reversal and loss of all amine label upon dialysis of the reaction product. DNAs that have been reacted with reagents which have a preference for the exocyclic amino group of G will result in a stable product with a lesser amount of attached amine compared to an unreacted control (Maibenco and Hanlon, unpublished results). These results have identified G as the stable reaction site that *survives dialysis*. This is in agreement with conclusions arrived at by Semin et al. (1981), who have conducted somewhat different studies with a similar derivative prepared with glycine and formaldehyde.

The properties of the DNA–amine adduct, freed of excess formaldehyde and amine, reveal that its secondary structure is base stacked and duplex in character. In fact, the stability of the attachment is dependent on this duplex secondary structure as conversion to the single-stranded form or digestion to mononucleotides will result in rapid loss of the amine. The duplex form, however, shows little or no loss of amine at pH 7 and room temperature (ca. 25 °C) over a 2-week period. Its extinction coefficient and hyperchromic increase upon cooperative melting are negligibly different from those of unreacted DNA. The T_m's of the derivatives, however, are shifted to higher temperatures. Hydrodynamic properties of the derivatives and their controls are comparable, with no evidence of collapse or aggregation of the DNA adducts. If anything, the latter appear to have a marginally higher persistence length in solution (Chen et al., 1981).

Raman spectroscopy and X-ray diffraction studies reveal that the backbone geometry of the calf thymus DNA adduct corresponds to a member of the B family (Chen et al., 1983; Fish et al., 1983). Studies with closed circular DNA derivatives (PM-2 DNA), however, suggest that the winding angle of the derivative is increased relative to the unreacted control treated only with formaldehyde (Kilkuskie, 1982; Kilkuskie et al., 1982). The characteristics of the CD spectra are also changed by derivatization in a manner reminiscent of the effects seen in solvents containing high concentrations of electrolytes. The rotational strength of the positive band in the CD spectra decreases with increasing amounts of amine bound. At 0.12 mol of *n*-butylamine per mole of nucleotide in aqueous solvents of modest ionic strength, for instance, the rotational strength of this band is ca. zero, and the spectrum resembles that of DNA in solvents such as 7 *m* LiCl or 8 *m* NH_4Cl or in complex with core histones at low ionic strengths. Furthermore, the correlation between winding angle changes and the lowering of the rotational strength of the positive band induced by attaching the amine is identical with that correlation between these properties when the changes have been induced by increasing the electrolyte content of the aqueous environment (Kilkuskie, 1982; Kilkuskie et al., 1982). Thus, the secondary structure of the random sequence DNA adduct is a B-form variant similar to that found in aqueous solutions at high ionic strength.

The CD changes can be reversed by titrating to alkaline pH's. At pH 10.5, ca. half the original rotational strength of the positive band has been recovered. If the pH is returned to 7 rapidly, there is little amine loss and the original spectrum of the derivative is obtained. The conformational consequences of the amine attachment, as reflected in the CD properties, are thus attributed to a positive protonic charge that has an approximate pK_a of 10.5 (Chen et al., 1981). These data taken together with what is known about the propensity of formaldehyde to form gem amines (Feldman, 1973) suggest that the stable derivative has the structure



where N–H··· indicates an exocyclic amino group (N2) of G with its Watson–Crick hydrogen bond intact and C* represents the C from formaldehyde. The amine modification thus places a formal positive charge in the minor groove of the helix.[2]

Since the amine attachment appears to effect the same conformational transformation in random sequence DNA as does concentrated electrolyte solvents or complexion with core histones, it was of interest to investigate its effects on the conformation of poly(dG-dC). Although there are some unexplained temperature effects, increases in the electrolyte content of the environment at room temperature generally result in the conversion to the left-handed Z conformation (Wang et al., 1979; Pohl & Jovin, 1972; Thamann et al., 1981). Complexion of the B form of poly(dG-dG) with core histones in reconstituted core particles, on the other hand, effects a transformation to a form that appears to be a B variant as judged by the change in CD properties (Simpson & Kunzler, 1979; Simpson & Shindo, 1980; Nickol et al., 1982). This spectrum is similar to that observed in 3.8 M LiCl (Pohl, 1976) at temperatures below the B → Z transition temperature in that solvent (Behe et al., 1985).

Attachment of amine to poly(dG-dC) can have one of these effects but not both. That is, it could simulate the usual effects of high ionic strength (i.e., 4 M NaCl), thus resulting in a stabilized Z conformation under conditions of low ionic strength. Alternatively, it could have the same influence as Li ions or core histones in converting the polymer to a conformational variant that appears to retain B backbone geometry. We undertook the present study in order to establish which of these two conformational possibilities occurs upon covalent attachment of *n*-butylamine to poly(dG-dC) in 20 mM NaCl and 10 mM Na_2HPO_4/NaH_2PO_4 buffer at pH 7. For convenience, this latter solvent has been designated as SSP.[1]

Experimental Procedures

Poly(dG-dC) was a product of P-L Biochemicals, lot no. 693/63. For these experiments we further purified it by exhaustive dialysis (120 mM NaCl, 10 mM NaH_2PO_4/

[1] Abbreviations: CD, circular dichroism; BuA, *n*-butylamine; poly(dG-dC)–BuA, the covalent complex of *n*-butylamine, CH_2O, and poly(dG-dC). SSP refers to a standard low ionic strength aqueous solvent consisting of 20 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 buffer at pH 7.

[2] In this structure, we have placed the positive charge on the attached amine. We are currently examining the possibility that the proton can be transferred to the N3 of guanine. Resonance effects of the six-membered ring of G coupled with possible proton transfer to the cytosine should result in substantial delocalization of the positive charge. The N7 of guanine will not be involved at all, and the major share of these forms will involve buried nitrogens and the nitrogens of the minor groove.

Na_2HPO_4, pH 7, followed by SSP) in order to remove contaminating amines. Its median sedimentation coefficient, $(s^0_{20,w})_{50}$, was 11.3 ± 0.3 S in solvents in which it was in the B form. By use of the relationship between $s^0_{20,w}$ and molecular weight established for unique sequence DNA by Eigner and Doty (1965), this corresponds to an approximate weight-average molecular weight of 2×10^6. The polymer in the final SSP dialysis solvent was mixed with a stock solution of BuA at room temperature, and an aliquot of 37% CH_2O at pH 7 was then added to start the reaction. The reaction mixture was ca. 0.2 mM in nucleotide, 7 mM in BuA, and 1.9% in CH_2O. The absorption spectrum and the changes in the CD spectrum of the poly(dG-dC) solutions during the reaction were followed in 1-cm CD cells with the appropriate instruments (Cary 14 absorption spectrophotometer and a Cary 60/6001 dichrograph). These and other CD and absorbance spectroscopic measurements were conducted as described in Chen et al. (1981). Except where indicated in the text, the absorbance spectral data were obtained at 25 °C and the CD data at 27 °C. The absorbance data at elevated temperatures were corrected for volume expansion of the solution. Temperatures of the cell contents were measured with a Yellowstone bridge and thermistor assembly (Chen et al., 1981). The reaction was allowed to continue until further changes in the CD spectrum were minimal (ca. 2.5–3 h). The absorbance at the maximum, 256 nm, was monitored continuously, and the reaction was stopped by exhaustive dialysis at 4 °C against SSP when A_{256} had increased by no more than 2%. The pH of the reaction mixture was also monitored during the reaction and found to be ca. pH 7 at all times. A control containing poly(dG-dC) and CH_2O, but no BuA, was treated in an identical fashion. The CD spectral data of the dialyzed products, freed of excess CH_2O and BuA, were reduced to mean residue ellipticities, $[\Theta]_\lambda$, at wavelength λ, by using concentrations calculated from the absorbance data. A value of 7100 M^{-1} cm^{-1} at 256 nm was employed for those calculations. All concentrations of polynucleotide are reported in moles of nucleotide per liter. Spectral measurements were routinely made at 0.15–0.2 mM.

Raman spectra were obtained on a unreacted control and a dialyzed derivative containing 0.22 mol of amine per mole of nucleotide, in 20 mM NaCl and 16 mM sodium cacodylate/cacodylic acid, pH 7, at 40 mg/mL, with a Spex Model 1401 spectrometer interfaced to a Data General Nova 2 minicomputer. Instrumental settings were similar to those used in the study by Fish et al. (1983).

The amount of *n*-butylamine covalently attached to poly(dG-dC) was determined by reacting the polymer with BuA-*1*-^{14}C, specific activity 36.3 mCi/mol, and CH_2O under reaction conditions identical with that employed for unlabeled samples. The labeled reaction mixture was also dialyzed exhaustively against SSP. Two controls consisting of poly(dG-dC) with BuA-*1*-^{14}C but no CH_2O and poly(dG-dC) with CH_2O but no BuA-*1*-^{14}C were simultaneously prepared and dialyzed in separate vessels. The former control had no more than 0.005 mol of BuA bound per mole of nucleotide. Aliquots of the dialyzed solutions (0.85 mL) were counted in a Beckman LS9000 scintillation counter, and the counts were corrected for the efficiency of counting, a background count provided by an equal volume of dialysis fluid, and the small amount of label retained by the control prepared with BuA-*1*-^{14}C but no CH_2O. The amine content is reported in terms of a ratio, *R*, of moles of *n*-butylamine per mole of nucleotide. The amine content of unlabeled samples was determined by reference to the linear relationship between the *R* values of the labeled samples and the value of their mean residue ellipticity in SSP, $[\Theta]^{SSP}_{275}$, at 275 nm, as described in the text.

Samples with lesser amounts of amine were prepared from the more heavily derivatized ones described above by exposure to elevated pH's and temperatures followed by prolonged dialysis. These factors generally lead to some loss of the amine adduct. The amount of amine remaining after these operations was assessed by counting the ^{14}C-labeled samples at *R* values of 0.07 or above. The controls for the samples of reduced amine content were the original matching CH_2O controls treated in an identical fashion. The spectral characteristics of these secondary controls did not differ from those of the original controls.

Sedimentation coefficients of polynucleotide samples were determined at 25 °C and concentrations of 0.1 mM with a Spinco Model E ultracentrifuge equipped with absorption optics and a scanner. The ionic strength of the low-salt solvent, SSP, was supplemented by added NaCl to the extent of 100 mM, in order to avoid electrostatic anomalies. The boundary of sedimenting material was followed at 265 nm in a double-sector 12-mm cell. The concentration of polymer upon reaching operating speed (48 000–52 000 rpm) was uniformly found to be ±4% of what had been loaded, as calculated from the value of A^{12mm}_{265} of the solution, prior to centrifugation. Thus, no high molecular weight material was lost from the cell by pelleting out on the way up to speed. The median sedimentation coefficients, $(s^0_{20,w})_{50}$, were corrected to the conventional standard conditions (Svedberg & Pedersen, 1940), although the buoyant density correction for the values in 120 mM NaCl were negligible. The values in 4 M NaCl were corrected for preferential hydration as well by using an assumed value of 0.54 cm^3/g for the partial specific volume of poly(dG-dC) and an approximate preferential hydration of between 6 and 7 water molecules per mole of nucleotide. These values were based on the available literature data for random sequence DNAs in 4 M NaCl (Hearst & Vinograd, 1961; Cohen & Eisenberg, 1968) with adjustment of the preferential hydration value for the lower degree of hydration of high GC content polymers (Tunis & Hearst, 1968). (No adjustments were made in the partial specific volume for the associated amine.) There have been suggestions that the hydration of the B and the Z structures differs (Dickerson et al., 1982; Behe et al., 1985). Unless the hydration properties of the Z forms and the poly(dG-dC)–BuA complex in solution are really bizarre, however, errors introduced in the correction factors by the inappropriateness of the model chosen (B-form random sequence DNA) should not lead to errors in the corrected sedimentation coefficients greater than the expected experimental accuracy.

Results

The character of the changes in the CD spectral properties of poly(dG-dC) in the presence of CH_2O and amine in solvents of modest electrolyte content was similar (although more highly exaggerated) to that previously observed for calf thymus DNA. As shown in Figure 1, a small alteration was observed during the course of the reaction in the solutions containing CH_2O alone. When the reaction mixture contained amine, however, the changes were dramatic. The positive band above 260 nm became increasingly more negative with time, the negative band at 250 nm decreased in magnitude, and that at 220–230 nm increased in positive amplitude. The reaction rate decreased significantly by 2 h, and it was terminated at 2.5 h by dialysis.

The nature of the conformation at the end of the allotted reaction period is unclear. The spectrum conforms to no

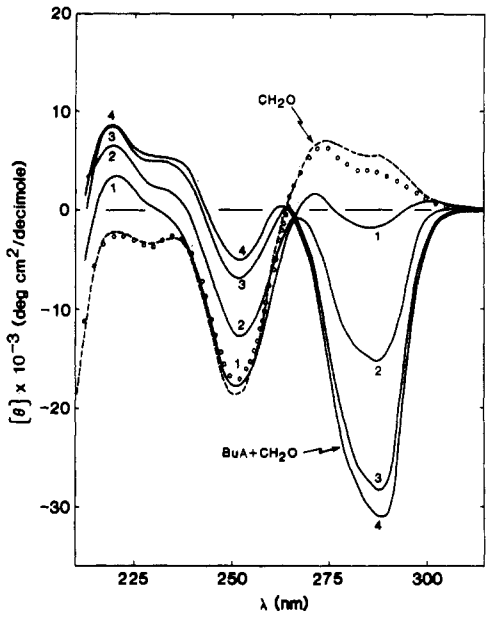


FIGURE 1: Transformation of the CD spectrum of poly(dG-dC) in reaction mixtures containing CH_2O and *n*-butylamine: (---) unreacted sample in SSP, pH 7; (O) the sample in 1.9% CH_2O plus SSP at 2.5 h; (—) poly(dG-dC) in a reaction mixture of 7 mM BuA and 1.9% CH_2O plus SSP at (1) 0.15, (2) 0.45, (3) 1.27, and (4) 1.65 h.

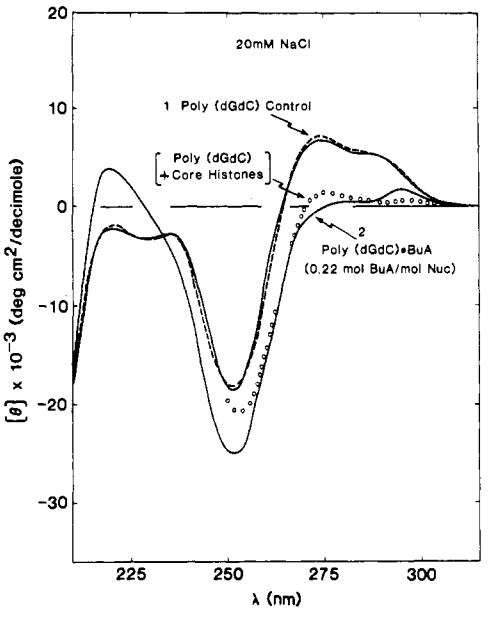


FIGURE 2: CD spectra of the dialyzed reaction products of poly(dG-dC) in SSP. Spectra in set 1 are poly(dG-dC) samples with no BuA attached: (---) poly(dG-dC) that had not been exposed to either CH_2O or BuA; (—) reaction control that had been exposed to 1.9% CH_2O for 2.5 h and then dialyzed. Spectrum 2 (—) is a dialyzed reaction product with 0.22 mol of BuA per mole of nucleotide. All samples are in SSP. The spectrum of [poly(dG-dC) + core histones] (O) was taken from Simpson and Shindo (1980).

known conformational end point or mixtures of conformational species such as a B and a Z form. Nor can it reflect the conversion to a base unstacked single-strand form since the A_{256} increased by no more than 2% over the entire reaction period (2–2.5 h). There is some resemblance to the CD spectral properties of poly(dA-dT) in concentrated CsFl or solvents containing CsCl that is thought to reflect the presence of a new type of secondary structure called X-DNA (Vorlickova et al., 1984). The Raman spectrum of poly(dA-dT) in the CsFl solvent obtained by Fodor et al. (1985) suggests that it is a B structure with some conformational adjustments of the bases. Thus, the poly(dG-dC) in the reaction solvent may be a GC variant of the poly(dA-dT) structure in CsFl.

For the control in the reaction mixture containing only CH_2O, removal of excess unreacted CH_2O by dialysis resulted in the reversal of the effects observed. In contrast, significantly altered CD spectral properties persisted in the case of the experimental solution containing both CH_2O and amine. This is demonstrated by the spectra shown in Figure 2. The spectrum of the dialyzed control (set 1, solid line) is essentially indistinguishable from that of the unreacted poly(dG-dC) control (set 1, dashed line). The transformed spectrum exhibited by the BuA adduct (set 2, solid line) at this level of derivatization (0.22 mol of amine/mol of nucleotide) is similar to that of poly(dG-dC) in 3.8 M LiCl (Pohl, 1976) and that of poly(dG-dC) complexed to core histones. A spectrum of the latter, taken from Simpson and Shindo (1980), is displayed as the beaded curve in Figure 2. It is also very close in shape to the spectrum of a form identified by Goto (1984) as the first rapidly formed intermediate (B*) in the NaCl-induced B → Z transition of poly(dG-dC).

An examination of the sedimentation properties indicates that these CD spectral effects reflect an alteration of secondary structure rather than the collapse or condensation of the polynucleotide. The values of the median sedimentation coefficient $(s^0_{20,w})_{50}$, in a solvent of modest electrolyte content are reported in column 3 of Table I, for the unreacted control, the CH_2O control, and the amine-derivatized polymer whoe spectra are shown in Figure 2. These values differ insignificantly from one another.

Table I: Sedimentation Coefficients of Poly(dG-dC) Samples at pH 7 in Moderate and High Ionic Strength Solvents

sample	$R \pm 0.01$ (mol of BuA/mol of nucleotide)	$(s^0_{20,w})_{50} \pm 0.3^a$ (S) 120 mM NaCl	4 M NaCl
poly(dG-dC) unreacted	0	11.3	10.1[b]
poly(dG-dC) CH_2O control	0	11.7	9.8[b]
poly(dG-dC)–BuA	0.22	11.7	11.9[b]

[a] Mass lost by pelleting on the way up to speed was no greater than 4% of mass loaded at 0 rpm. [b] Value corrected for a preferential hydration of 6.5 mol of H_2O per mole of nucleotide (0.34 g/g) and a partial specific volume of poly(dG-dC) of 0.54 cm^3/g.

Despite the differences in the CD spectra between the control and the derivative, the Raman spectra revealed that the latter still retained B geometry. The intensities of the major lines whose characteristics distinguish B forms from A and Z (Benevides & Thomas, 1983; Thamann et al., 1981) are given in Table II. Both the intensities and the normalized intensities (relative to the PO_2^- stretching vibration at 1095 cm^{-1}) of these lines in the spectrum of the derivative differed very little from that of the underivatized control sample, and there was no evidence of shifts or decreases in intensity corresponding to conversions to A or Z structures.[3] The CD alterations seen in the derivative were thus a reflection of an altered B secondary structure. In view of the similarity in behavior to random sequence DNA, the altered B secondary structure is probably a higher winding angle variant.

Examination of derivatives of lower amine content revealed a family of CD spectra intermediate between the control and the $R = 0.22$ sample. A plot of the rotational strength or the

[3] It might be noted that differences were found in the positions and intensities of some of the lines associated with the base vibrational modes. These are currently being examined more closely in an effort to clarify the mode of interactions of the amine with the G residue. In no instance are these shifts interpretable in terms of the conversion of a significant fraction of base pairs to an alternate backbone geometry of the duplex.

Table II: Characteristics of the Conformationally Sensitive Lines in the Poly(dG-dC)–BuA Derivative

$\nu \pm 2$ (cm^{-1})	assignment[a]	poly(dG-dC)–BuA (0.22 mol of amine/mol of nucleotide) I_ν[b] ± 0.2	I_ν/I_{1095}	poly(dG-dC) $I_\nu \pm 0.2$	I_ν/I_{1095}
684	G (in B form)	5.2	0.74	5.5	0.77
784	sym [R–O–P–O–R]; C	15.2	2.17	15.3	2.15
833	asym [R–O–P–O–R]	6.00	0.86	5.1	0.72
1095	sym PO_2^-	7.00	1.00	7.10	1.00
1423	CH_2 deformation (in B form)	3.50	0.50	3.90	0.55

[a] Taken from Benevides and Thomas (1983) and Thamann et al. (1981). [b] Contributions of the CH_2O and BuA moieties to the intensity data are negligible for the data reported. [See Fish et al. (1983).]

value of the mean residue ellipticity, $[\Theta]_\lambda$, at a specific wavelength, λ, exhibited a linear dependence on the value of the amine content, R, in terms of moles of BuA per mole of nucleotide. This permitted an evaluation of the amine content for the unlabeled samples or for those where direct measurements were not feasible. This relationship between R and $[\Theta]$ at 275 nm in SSP at 27 °C was

$$R = 0.24 - [(3.3 \times 10^{-5})[\Theta]^{SSP}_{275}] \quad (1)$$

Nickol et al. (1982) have reported that poly(dG-m^{5}dC) in core particles reconstituted with the B form of this polynucleotide will not assume the Z form under ionic conditions that stabilize this form in the protein-free polymer. It was of interest, therefore, to ascertain whether the BuA derivative exhibited similar behavior. Solutions of the experimental sample and its CH_2O control, whose CD spectra are shown in Figure 2 (solid lines), were made 4 M in NaCl by the addition of solid NaCl. The CD spectra of these high-salt solutions are shown in Figure 3. Their absorption spectra at room temperature were essentially identical with those displayed in the lower part of Figure 4 that are of a CH_2O control (spectrum 5) and a derivative of somewhat lower BuA content (R = 0.17) (spectrum 4) in 4 M NaCl. The absorption spectrum 5 of the amine-free control was typical of the Z form. In contrast, the absorption spectral properties of the derivative (spectrum 4) are those usually assigned to a B form. The characteristics of the CD spectrum of the derivative [shown in Figure 3 (curve 2)] would imply that the latter has an even higher winding angle than that of the B variant at lower ionic strength in SSP. [We have also found similar behavior in a BuA derivative of the methylated polymer poly(dG-dm^{5}C) where the B → Z transition can normally be induced at millimolar concentrations of Mg^{2+} (Maibenco and Hanlon, unpublished results).]

Conformational differences between the control and the derivatized samples in 4 M NaCl are also reflected in the sedimentation properties shown in column 4 of Table I. Although the corrections for the preferential hydration of the polymer are only approximate (as described under Experimental Procedures), the differences observed in $(s^0_{20,w})_{50}$ are too large to be ascribed to errors in the correction terms.[4] The

[4] If the true sedimentation coefficients of these samples in 4 M NaCl were indeed all the same, differences of the magnitude and direction reported in Table I for 4 M NaCl would require that the Z form controls have a preferential hydration twice that of B form random sequence DNA and B form poly(dG-dC) in this solvent or that the BuA derivative preferentially associates NaCl. Both these possibilities are extremely unlikely.

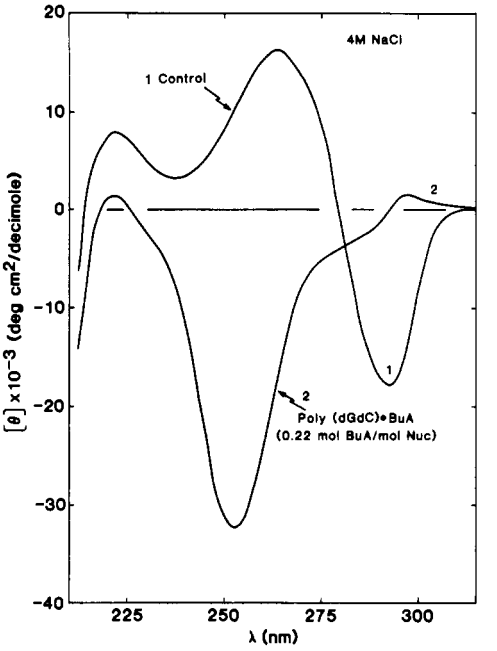


FIGURE 3: CD spectra of poly(dG-dC) reaction control (spectrum 1) and poly(dG-dC)–BuA derivative at 0.22 mol of BuA per mole of nucleotide (spectrum 2) in 4 M NaCl and 10 mM NaH_2PO_4/Na_2HPO_4, pH 6.

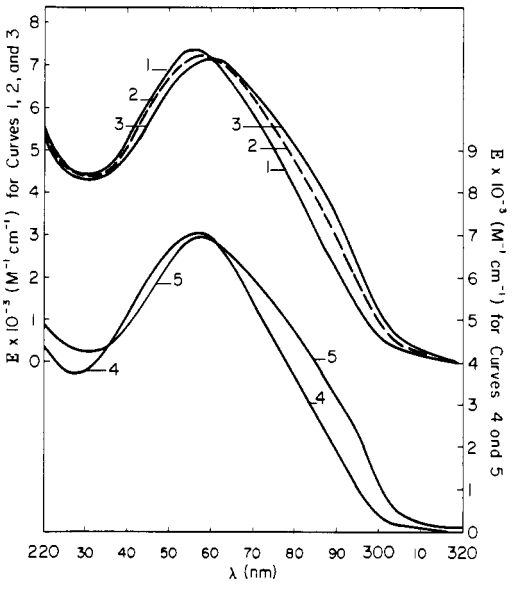


FIGURE 4: Absorption spectra of derivatized poly(dG-dC). Poly(dG-dC) in 4 M NaCl–10 mM NaH_2PO_4/Na_2HPO_4, pH 6, as a function of temperature. Spectrum 1 was obtained at 82.5 °C, spectrum 2 at 92.3 °C, and spectrum 3 at 97 °C. Cooling to 25 from 97 °C gave spectrum 5, which was identical with that of the reaction control containing no BuA in the same solvent. The derivatized sample with an original amine content of 0.17 mol of BuA per mole of nucleotide gave spectrum 4 at 25 °C prior to heating. The extinction coefficient scale for the upper spectra (1–3) are on the left ordinate. That for the lower spectra (4 and 5) is on the right ordinate.

similarity between the values of the derivative in 4 M NaCl and in 120 mM NaCl implies that the hydrodynamic characteristics—and hence the conformation—are the same in both solvents. In contrast, the lower values of the two controls in 4 M NaCl compared to those in 120 mM NaCl probably reflect the greater stiffness of the Z form, which has been previously observed by Thomas and Bloomfield (1983). The most important aspect of these results for our study is that they reveal no evidence for the aggregation and/or condensation effects observed for poly(dG-m^{5}dC) by Thomas and

Bloomfield (1985) in solvents that favor the Z state. Thus, the characteristics of the CD spectra reflect secondary structural differences *only* and cannot be attributed to the formation of ψ type structures or other aggregated forms that distort the CD spectra.

Since Goto (1984) had identified the form whose CD spectral characteristics are similar to those of our BuA derivative as an intermediate along the conformational pathway leading from the B to the Z, it was always possible that the derivative was an unstable trapped form, rather than a true thermodynamically stable structure, under our solvent conditions at room temperature. In order to evaluate this possibility, we examined the effects of temperature on the CD and absorption spectra. In the first experiment we followed the absorption spectra up to 97 °C of a derivative with 0.17 mol of amine/mol of nucleotide. There was only a small hyperchromic increase in this temperature range (ca. 2% at 82.5 °C). Between 80 and 97 °C, however, there was an apparent B → Z transformation, as shown by the data in the upper part of Figure 4. Curve 1 was the spectrum observed at 82.5 °C, which was essentially identical, except for the small (2%) hyperchromic increase at 256 nm, with the one obtained at room temperature (25 °C) before the temperature was raised (curve 4). Curve 2 represents the spectrum at 92.3 °C, and curve 3, which shows the typical full Z shift (Pohl & Jovin, 1972), was obtained at 97 °C. When the sample had cooled to room temperature, the Z spectrum persisted and was indistinguishable from that of the underivatized control (curve 5). This transition, however, was accompanied by a loss of the amine adduct. After removal of NaCl by dialysis, the sample was recounted and found to contain no labeled amine. Another aliquot of the derivative in 4 M NaCl maintained at room temperature for the time period of this experiment and subsequently dialyzed showed negligible loss of amine. From this, we have concluded that the B → Z transformation above 80 °C reflects the effects of loss of amine. The B form of the derivative and the amine attachment would thus appear to be stable at temperatures at least as high as 80 °C.

There are two possibilities that can account for the amine loss in this experiment: In random sequence DNA, amine is lost during the thermally driven helix → coil transition because the attachment to the single-strand form is unstable under the conditions of the denaturation experiment. The existence of a small hyperchromic effect at the elevated temperatures, indicative of increased fluctuational opening of the base pairs, suggests that the same mechanism might be operative in this current case. On the other hand, the derivative might be approaching the temperature range for a thermally driven B → Z transition and lose its amine adduct as it converts to the Z form, either because of loss from the transition state or because the Z-form adduct is unstable. This latter set of possibilities is suggested by the observation that a minimally derivatized sample of poly(dG-dm^{5}C), predominantly in the B form, will dissociate its amine as the temperature is raised to favor the Z form. The underivatized methylated polymer in the Z form fails to react with the amine/formaldehyde reagent even though the B form reacts readily (Maibenco and Hanlon, unpublished results). At the present time, we are unable to distinguish between these two options.

Another type of experiment in which the CD properties of this derivative and its control were monitored after exposure for a longer time to elevated but less extreme temperatures is shown in Figure 5. Panel A demonstrates the results for the control and panel B those obtained for the complex. The CD spectra shown as the solid line set 1 curves in both panels

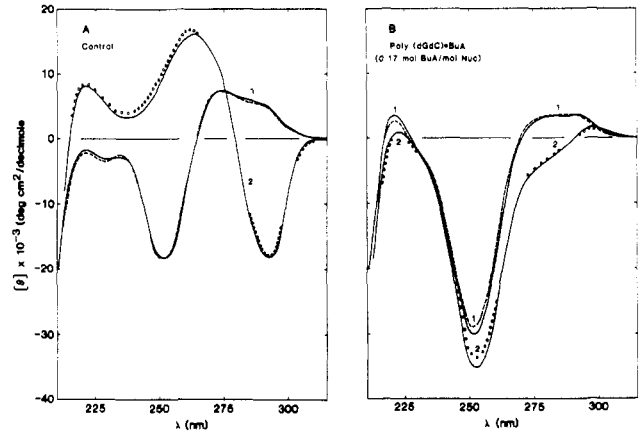


FIGURE 5: Comparison of low- and high-salt CD spectra of the poly(dG-dC)–BuA complex at 0.17 mol of BuA/mol of nucleotide (panel B) and its reaction control (panel A). In both panels, the spectra are coded as follows: spectrum 1 (—) is the sample in SSP before the salt content of the solution is increased; spectrum 2 (—) is the sample in 4 M NaCl–10 mM NaH_2PO_4/Na_2HPO_4, pH 6, before heating; (O) is the sample in 4 M NaCl after heating at 60 °C for 1 h; and (---) is the heated sample after exhaustive dialysis against SSP to remove excess NaCl.

were first obtained in SSP at 27 °C. The two solutions were then made 4 M in NaCl, and the CD spectra shown as the solid line set 2 were obtained. Heating these solutions for 1 h at 60 °C followed by cooling to 27 °C (open beaded curves) had trivial effects on the CD properties, as demonstrated by the correspondence to the solid line spectra of set 2. The high concentration of NaCl was then replaced by the moderate ionic strength SSP solvent by dialysis, and the set of spectra shown on the dashed lines of set 1 were obtained. The data shown in panel B demonstrate that little or no amine was lost in the heating period. This was confirmed by a sample count that gave an R value of 0.17 for this heated and redialyzed sample. These results reveal that the B form of the derivative is indeed the thermodynamically stable form in solvents that induce the formation of the Z form in the control.

It was of interest to ascertain what was the minimal average level of amine required to prohibit the B → Z transition at room temperature in 4 M NaCl. To this end, the CD spectral properties of a set of derivatives whose level of amine substitution varied from 0.23 to 0.06 were examined after standing 1 h at 27 °C in 4 M NaCl at pH 7. In this range of R values, the spectral differences were a linear function of this amine content. At 275 nm, the relation between $[\Theta]_{275}^{4\,M\,NaCl}$ and R was

$$R = -0.14 - [(6.6 \times 10^{-5})[\Theta]_{275}^{4\,M\,NaCl}] \qquad (2)$$

and the spectra could be accounted for in terms of two independent components. At 0.06 mol of amine/mol of nucleotide, however, small shape distortions and an isoelliptic point appeared at ca. 285 nm, suggesting the appearance of at least one additional component. Although this component had some characteristics similar to that of the Z form above 250 nm, this was not consistently the case over the entire wavelength range. The spectral distortions were very similar to that exhibited by the dashed spectrum and the solid-line spectrum 1 shown in Figure 6A (which will be discussed below). They probably reflect the presence of a small amount of Z structure coupled with an increase in the fractional content of B/Z junctions. If the entire deviation were attributed to Z structure, the amount of the Z form present at an R value of 0.06 in 4 M NaCl could amount to ca. 5% of the total base pairs. We thus conclude that at this average amine content of 0.06,

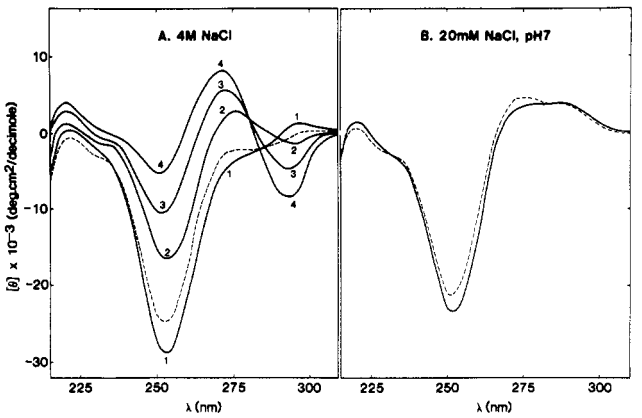


FIGURE 6: Effects of pH on the CD spectrum of a poly(dG-dC)–BuA derivative. (Panel A) Spectra of a poly(dG-dC) derivative at 0.12 mol of BuA/mol of nucleotide in 4 M NaCl–10 mM NaH_2PO_4/Na_2HPO_4 at pH 7 (curve 1) and pH 10.7 at 5 min (curve 2), 16 min (curve 3), and 26 min (curve 4) and after a rapid return to pH 7 (---). (Panel B) Spectra of the same derivative in SSP before the increase in pH (—) and after the return to pH 7 and subsequent dialysis against SSP (---).

approximately 1 derivitized base per 15–20 is marginally able to prevent the B → Z transition in 4 M NaCl.

Previous pH titration studies with calf thymus DNA had demonstrated that it was the positive charge of the amine rather than the steric properties of the attachment that stabilized the altered CD spectral properties (Chen et al., 1981). As is demonstrated in the experiments described below, this was also found to be true for the inhibitory effect of amine on the B → Z transition. Several samples in 4 M NaCl at *R* levels of 0.06–0.22 were titrated quickly to elevated pH's and maintained there for ca. 30 min. The pH was then quickly lowered to pH 7 and the CD spectrum obtained again in order to establish reversibility. After removal of the excess NaCl by dialysis against the SSP solvent, the sample was recounted to assess amine loss at the elevated pH and the CD spectral properties were reexamined. At *R* values of 0.22, the positive band increased in magnitude but there was no evidence of a Z component. Comparison with control samples in the same solvent at the same pH revealed that the CD spectra of the Z form CH_2O controls at ca. pH 10.5 and those of the B form experimental samples in which the positive charge of the amine had been reduced crossed over at 278 nm at a positive ellipticity of ca. 1.5×10^3 deg cm^2 dmol^{-1}.

At lower levels of amine substitution, however, the formation of the Z form at elevated pH was clearly apparent. The results of one of these experiments in which a definite B → Z transition occurred is shown in Figure 6. A sample with an amine content of 0.11 in 4 M NaCl plus SSP, pH 7 (curve 1), was brought to pH 10.7 by the addition of NaOH, and its CD properties were observed as a function of time up to 27 min. During that period, curves 2–4 were obtained, exhibiting a common isoelliptic point of 280 nm, somewhat red shifted but characteristic of the partially deprotonated amine-substituted B form → Z transition in this solvent. After 35 min, the pH was returned to 7, and the spectrum shown as the dashed curve was obtained. This particular spectrum remained stable over a 24-h period. As previously discussed, the differences between this latter spectrum and the solid line spectrum 1 is probably attributable to the creation of a small amount of Z form and B/Z junctions due to amine loss at pH 10.7.

Removal of the excess NaCl by dialysis against SSP gave the dashed curve shown in panel B whose characteristics, compared to those of the original solution in this solvent (solid line curve), indicated little loss of amine. Recounting the sample after this procedure gave an amine content of 0.09. When a pK_a of 10.5 was used and this uncertainty in amine content was taken into account, the positive charge at which the B → Z transformation in 4 M NaCl became significant was evaluated as 0.05–0.04 mol per mole of nucleotide. This is in agreement with the results of the determination using solutions at pH 7 with different amine contents and confirms the fact that it is the positive charge which is effecting the stability of the B form and not the steric presence of the amine itself.

CONCLUSIONS

From the results of these experiments, we have concluded that the conformational effects of the butylamine attachment to poly(dG-dC) qualitatively mimics the effects of the association of core histones with the B form of the polymer. Raman spectra confirm that the CD spectral properties of the amine derivative reflect an altered B form rather than a Z. Similar effects observed in studies with the BuA derivatives of random sequence DNAs suggest that this B variant of poly(dG-dC) has a higher winding angle. The stabilization of this B structure, which is attributable to the positive charge on the amine, appears to be truly thermodynamic rather than kinetic. The average minimal amount of positively charged amine required to maintain the altered B form in 4 M NaCl at 27 °C is between 0.04 and 0.06 mol per mole of nucleotide or an average level of derivatization of ca. 1 in 20 bases.

This form is not unique. As noted before, the CD spectrum of the derivative shown in Figure 2 is very similar in shape not only to the polynucleotide complexed to core histones but also to ones previously reported for B-form poly(dG-dC) in certain concentrated aqueous electrolyte solutions, notably the rapidly formed species in 3.4 M NaCl just prior to the transition and the stable form present at room temperature in 3.8 M LiCl. It is interesting that the temperature-driven transition in concentrated LiCl solvents is unusual when compared to the transitions in concentrated NaCl solutions (Behe et al., 1985). The pattern of changes of the transition temperature midpoint in LiCl is complex, but in 3.8 M LiCl that midpoint is above 60 °C and the B form is the stable species at room temperature. Because of the similarity in the spectra, we suggest that Li cations in these solvents are also binding to the B form in the minor groove at the G loci.

It is unclear why placing a positive charge at G in the minor groove should decrease the equilibrium constant of the B → Z transition. The influences that other base modifications exert on the B → Z transition have generally been rationalized in terms of the effects of the modifications on the free energy levels of the B or the Z forms. The explanations for the changes in these levels usually involve steric or structural requirements for better van der Waals interactions in one form or the other, the energetics of syn/anti conversions, the blockade of specific ion binding sites in Z DNA between the O6 and the N7 of guanine and the phosphate backbone (Gessner et al., 1985), electrostatic factors and asymmetric distribution of diffusible ions in the grooves (Pack & Klein, 1984; Pack et al., 1986), and/or hydration effects (Behe et al., 1985; Pack et al., 1986; Dickerson et al., 1982; Zacharias et al., 1982).

In this present instance, the explanation of the effects of this particular modification involves consideration of the pathway governing the transition. This pathway may involve one or more intermediates in equilibrium with the B and the Z forms. Usually the multiplicity of these intermediates is ignored in discussing the B → Z transitions, as it is assumed that the important intermediate is an "open" state through which the

polynucleotide chains pass whose energy level is not prohibitive for the anti → syn transition of the guanine bases (Olson et al., 1983). There is also experimental evidence that documents its existence for unmodified poly(dG-dC) (Mirau & Kearns, 1985). The nature of this open state is uncertain: It could be the usual universal open state similar to that invoked to explain DNA melting phenomena. Alternatively, it could be similar in structure to the elongational opening required for intercalation reactions. The latter would, in fact, permit the B → Z transition to occur with disruption of the Watson–Crick H bonds (Harvey, 1983). Perturbations of the free energy level of the intermediate state would not normally be expected to affect the equilibrium constant for the transition, although it would have a profound effect on the kinetics of the conversion.

In this case, however, it also affects considerations of the energetics of the system, since fluctuational opening of the duplex, either as a melted segment or as an unstacking of the base pair in an elongated intermediate, would probably result in the loss of the adduct from the segments of the polynucleotide chain newly exposed to solvent. Even if it somehow survived in the open state, however, it would be lost from the Z form since this conformation does not form these adducts. (See previous discussion.) The final Z product in either case will have no attached BuA. Thus, the probable pathway that is appropriate for this discussion is

$$[B(BuA)] \rightleftharpoons [O(BuA)] \rightleftharpoons O + BuA + CH_2O \rightleftharpoons Z + BuA + CH_2O$$

where O represents the open-state transient. The overall reaction

$$[B(BuA)] \rightleftharpoons Z + BuA + CH_2O$$

involves the conversion of the B-form adduct, [B(BuA)], to an underivatized Z form. The failure to convert to this Z form at temperatures between 25 and 82.5 °C in 4 M NaCl would thus appear to be due to additional stabilization, or the lowering of the free energy, of the B form with the adduct in place rather than to an increase in the free energy of the final Z form since the latter will be unmodified. This is also congruent with our data on the derivatives of random sequence DNA, whose increases in T_m's reflect enhanced stability of the B-form duplex structure.

Since we can demonstrate that the inhibition is dependent on the positive charge and not on the steric properties of the amine, it is probable that the origin of this free energy decrease for the adduct has to do with the electrostatic effects of placing the positive charge in the minor groove together with, possibly, some charge-induced alterations of the hydration in that locus. The calculations of Pack and Klein (1984) indicate that the minor grooves of both B- and Z-form DNA have the highest local concentrations of diffusible cations. Even though the Z form has a lower linear charge density, the extent of counterion association with this form is greater than for the B form in solvents of modest electrolyte content. This would generally mean an uptake of ions upon converting from the B to the Z form and a negative temperature coefficient for the B → Z transition. This temperature-driven transition, however, generally has a positive coefficient in these solvents, and this has been attributed by Behe et al. (1985) to the release of water of hydration, which contributes a larger positive entropy term to the process than the negative entropy change due to ion uptake. A positively charged amine residue in the minor groove of the B adduct would almost certainly reduce the concentration of diffusible cations in that locus but, more importantly, also interfere with the extensive hydration of the minor groove which characterizes all B-type duplexes (Drew & Dickerson, 1981). The overall uptake of cations in the B → Z transition would probably be the same since the adduct, as one of the compensatory cations, is lost in the transition. The hydration contribution, however, would be significantly reduced and, thus, the unfavorable entropic differences between the B and the Z forms could well be minimized. At the same time, the counterion screening of the phosphate groups (which lowers the free energy by mostly enthalpic effects) would be more effective in the derivatized B form containing a site-bound positive charge. This screening would be lost as the polymer converted to the Z state. The net result would be a decrease in the free energy of the B form containing the adduct compared to that of the underivatized polymer in the temperature range in which the water activity of the solvent was still sufficient to support B-form hydration.

In light of these observations, we suggest that the B* transient produced in concentrated NaCl solution (Goto, 1984) is not a conformational intermediate in the sense that it has characteristics of both B- and Z-type geometry. Rather, it has a structure that the polynucleotide will assume under screening conditions when the backbone is in the B form. The fact that this form is unstable in concentrated NaCl at room temperature, whereas it is the stable form for the polymer in 3.8 M LiCl and for the adduct where the amine is site bound, suggests that the Na ion, in contrast to Li, is not site bound.

Miller et al. (1985) have recently shown that poly(dG-dm^{5}C) in the Z form can form an altered type of core particle if an assembly factor protein is incorporated in the reconstitution mixture. Since BuA attachment simulates the conformational effects of core histones in core particle B DNA, the stability of the B form of poly(dG-dC)–BuA derivative would argue against there being a significant B → Z conversion of DNA in vivo in nucleosomes as long as the core histones are firmly attached. Indeed, Nickol et al. (1982) were unable to induce a B → Z transition in B-form core particles containing poly(dG-m^{5}dC) under conditions where the protein-free polymer readily assumed the Z conformation. If stretches of Z DNA are important in eukaryotic gene regulation, we suggest that the mechanism involve core histone dissociation prior to the B → Z conversion.

In fact, this increase in the stability of the B form by placing a positive charge at G residues in the minor groove may have even wider significance with respect to conformational mechanisms of gene regulation and enzymatic activity. We have recently found that the adducts of random sequence DNA fail to undergo the usual B → A transition in mixed aqueous–ethanol solvents in which the controls transform normally (Ringquist, 1987). This suggests that the positive charge in the minor groove of the duplex reduces the probability of conversion to alternate non-B forms of any type. This may be an important factor for enzymatic activity and the regulation of gene expression by proteins that interact electrostatically with DNA at minor groove sites.

References

Behe, M., Felsenfeld, G., Szu, S., & Charney, E. (1985) *Biopolymers 24*, 289–300.

Benevides, J., & Thomas, G. J., Jr. (1983) *Nucleic Acids Res. 11*, 5747–5761.

Chen, C., Kilkuskie, R., & Hanlon, S. (1981) *Biochemistry 20*, 4987–4995.

Chen, C., Pheiffer, B., Zimmerman, S. B., & Hanlon, S. (1983) *Biochemistry 22*, 4746–4751.

Cohen, G., & Eisenberg, H. (1968) *Biopolymers 6*, 1077–1100.

Dickerson, D., Drew, H., Connor, B., Wing, R., Frantini, A., & Kopka, M. (1982) *Science (Washington, D.C.) 216*, 475–485.
Eigner, J., & Doty, P. (1965) *J. Mol. Biol. 12*, 549–580.
Fish, S., Chen, C., Thomas, G. J., Jr., & Hanlon, S. (1983) *Biochemistry 22*, 4751–4756.
Fodor, S., Starr, P., & Spiro, T. (1985) *Biopolymers 24*, 1493–1500.
Gessner, R., Quigley, G., Wang, A., van der Marel, G., van Boom, J., & Rich, A. (1985) *Biochemistry 24*, 237–240.
Goto, S. (1984) *Biopolymers 23*, 2211–2222.
Harvey, S. (1983) *Nucleic Acids Res. 11*, 4867–4878.
Hearst, J., & Vinograd, J. (1961) *Proc. Natl. Acad. Sci. U.S.A. 47*, 1005–1014.
Kilkuskie, R. (1982) Ph.D. Dissertation, University of Illinois, Chicago, IL.
Kilkuskie, R., Wood, N., & Hanlon, S. (1982) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 41*, 1023.
Maibenco, D., Chang, S., Chen, C., & Hanlon, S. (1984) *Biochemistry 23*, 3373, Abstract 145.
Miller, F., Dixon, G., Rattner, J., & van der Sande, J. (1985) *Biochemistry 24*, 102–109.
Mirav, P., & Kearns, D. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 1594–1598.
Nickol, J., Behe, M., & Felsenfeld, G. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1771–1775.
Nordheim, A., Hao, W., Wogan, G., & Rich, A. (1983) *Science (Washington, D.C.) 219*, 1434–1436.
Olson, W., Srinivasan, A., Marky, N., & Balaji, N. (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*, 229–241.
Pack, G., & Klein, B. (1984) *Biopolymers 23*, 2801–2823.
Pack, G., Prasad, C., Salatsky, J., & Wong, L. (1986) *Biopolymers 25*, 1697–1715.
Pohl, F. (1976) *Nature (London) 260*, 365–366.
Pohl, F., & Jovin, T. (1972) *J. Mol. Biol. 67*, 375–396.
Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D., & Klug, A. (1984) *Nature (London) 311*, 532–537.
Ringquist, S. (1987) Ph.D. Dissertation, University of Illinois, Chicago, IL.
Semin, Y., Volkov, V., Protasova, I., & Poverennyi, A. (1981) *Mol. Biol. 15*, 1303–1314.
Simpson, R., & Kunzler, P. (1979) *Nucleic Acids Res. 6*, 1387–1415.
Simpson, R., & Shindo, H. (1980) *Nucleic Acids* Res. *8*, 2093–2103.
Svedberg, T., & Pederson, K. D. (1940) *The Ultracentrifuge*, Clarendon, London.
Thamann, T., Lord, R., Wang, A., & Rich, A. (1981) *Nucleic Acids Res. 9*, 5443–5457.
Thomas, T. J., & Bloomfield, V. A. (1983) *Nucleic Acids Res. 11*, 1919–1930.
Thomas, T. J., & Bloomfield, V. A. (1985) *Biochemistry 24*, 713–719.
Tunis, M. J., & Hearst, J. E. (1968) *Biopolymers 6*, 1345–1353.
Vorlickova, M., Minyat, E., & Kypr, J. (1984) *Biopolymers 23*, 1–4.
Wang, A., Quigley, G., Kolpak, F., Crawford, J., van Boom, J., van der Marel, G., & Rich, A. (1979) *Nature (London) 282*, 680–686.

Role of a Bulged A Residue in a Specific RNA–Protein Interaction†

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ABSTRACT: The translational operator of the R17 replicase gene contains a bulged A residue that is essential for the specific binding to R17 coat protein. A large number of operator variants have been synthesized to more precisely examine the role of the bulged A residue on this specific protein–RNA interaction. By use of RNA ligase and transcription of synthetic DNA templates by T7 RNA polymerase, 14 different nucleotides were introduced to the bulged A position of three different coat protein binding fragments. The affinity between coat protein and each fragment was determined by a nitrocellulose filter binding assay. The data indicate that while functional groups on N^1, C^2, C^6, N^7, and 2′OH of the bulged A can be substituted without greatly changing protein binding, bulky substituents cannot be tolerated at these positions. Data from additional fragments that have base-pair changes adjacent to the bulged A suggest that the propensity of the bulged A to intercalate into the helix can affect protein binding.

The coat protein of bacteriophage R17 binds specifically to a hairpin loop in the initiation region of the replicase gene and thereby represses translation of that gene late in phage infection (Bernardi & Spahr, 1972). This highly specific RNA–protein interaction has been extensively characterized by using a synthetic 21-nucleotide fragment corresponding to the −17 to +4 position of the replicase gene that has the same affinity for coat protein as the 3.5-kilobase R17 genomic RNA (Krug et al., 1982; Carey & Uhlenbeck, 1983). By measurement of the affinity of coat protein with more than 50 enzymatically synthesized variants of the 21-nucleotide fragment, the RNA structural requirements for tight protein binding were determined (Carey et al., 1983b; Romaniuk et al., 1987). Among the essential residues for the coat protein binding is an extrahelical or "bulged" A residue at position −10 of the replicase gene. Either deletion of this residue or substitution with a C residue reduced the K_a to coat protein by at least 1000-fold.

Peattie et al. (1981) have proposed a special role for the

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