Binding of ethidium and bis(methidium)spermine to Z DNA by intercalation

Richard H. Shafer, Stephen C. Brown, Alain Delbarre* and Donald Wade

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94143, USA

Received 2 March 1984; Revised and Accepted 15 May 1984

ABSTRACT

The interaction of ethidium bromide, a DNA intercalating drug, and bis(methidium)spermine, a DNA bis-intercalating compound, with the left-handed Z form of poly(dG-dC) has been studied in 4.4 M NaCl. Spectrophotometric analysis using absorption, fluorescence and circular dichroism indicates that the complex formed between ethidium and Z DNA resembles very closely that formed with B DNA. This suggests that ethidium binds to Z DNA by intercalation. ³¹P NMR spectra are presented showing both the conversion of the Z form to the B form with increasing amounts of drug and the typical Z form spectrum at low binding densities. Data are also presented which show that the bifunctional intercalator bis(methidium)spermine binds to Z DNA in a manner similar to its binding to B DNA, i.e., by bis-intercalation. These results are important for our understanding the behavior of Z DNA and its biological significance.

INTRODUCTION

Left-handed, or Z, DNA has become a subject of great interest in the past several years. Pohl and Jovin (1) provided the first indication of this form of DNA in solution with CD studies of poly(dG-dC). The X-ray crystallographic studies of Wang et al. (2,3) and Drew et al. (4) confirmed the existence of left-handed DNA in oligonucleotide structures. Subsequently, a variety of synthetic alternating purine-pyrimidine polynucleotides have been shown to exhibit a left-handed conformation under appropriate conditions, e.g., poly(dG-m⁵dC) (5), brominated poly(dG-dC) (6), poly(dG-br⁵dC) (7), poly(dA-dC) poly(dG-dT) (8,9) and poly(dA-m⁵dC) poly(dG-dT) (10).

In addition to such studies characterizing the cooperative B $\leftrightarrow$ Z transition in these synthetic polynucleotide systems, reports have appeared focusing on the presence of the Z conformation under physiological conditions, such as in plasmid DNA (11,12) and in eucaryotic cells using antibodies raised against Z DNA (13-15). Furthermore, Z DNA binding proteins have been described (16).

Relatively few studies, however, have been reported describing the drug binding properties of Z DNA. Pohl et al. (17) initially demonstrated that addition of sufficient amounts of ethidium bromide to the high salt, Z form of poly(dG-dC) resulted in a highly cooperative transition to the right-handed, B conformation. They proposed that this transition was due to the very strong preference for binding of ethidium to the right-handed form of poly(dG-dC) over binding to the left-handed form. Based on this work, it has been inferred that Z DNA does not bind ethidium (18,19). Mirau and Kearns (20) have shown that intercalating drugs inhibit the salt-induced B $\rightarrow$ Z transition in poly(dG-dC) in a fashion correlated with their binding kinetics, but the effect of Z DNA on the drugs was not examined.

A different left-handed form of poly(dG-dC), dubbed Z^{*} DNA, induced by combinations of Mg⁺⁺ and ethanol, has been described by van de Sande and Jovin (18). They showed that this form of poly(dG-dC) can bind ethidium as well as several other drugs, although the nature of the drug-Z^{*} DNA complexes was not explored in any detail. The ability to bind intercalators was listed as one of the differences between Z^{*} and Z forms of poly(dG-dC). More recently, Rio and Lang (21) have studied the binding of ethidium to poly(dG-br⁵dC) poly(dG-br⁵dC) and concluded that the drug also converts this polynucleotide from the Z to the B conformation, even at low salt.

In the experiments described below, we present evidence indicating that the high salt, Z form of poly(dG-dC) can not only bind ethidium by intercalation but also can support bis-intercalation of bis(methidium)spermine (BMSp). This latter compound is a bifunctional intercalator consisting of two ethidium-like chromophores linked by a spermine chain (22,23). This is the first report characterizing drug-Z DNA complexes at low binding levels. Our results are important for understanding both the physical chemical properties of Z DNA and its possible biological functions.

MATERIALS AND METHODS

Poly(dG-dC) was obtained from P-L Biochemicals, ethidium bromide was obtained from Calbiochem and calf thymus DNA (type I) was obtained from Sigma. BMSp was a gift from Professor Peter B. Dervan. Stock solutions of DNA were made up in low salt buffer (0.025 M Na₂PO₄, 0.025 M NaHPO₄, 0.1 mM Na₂EDTA, pH 6.9), sonicated to an average length of 250 $\pm$ 100 base pairs, clarified by centrifugation and dialyzed against low or high salt buffer (see below). Poly(dG-dC) was sonicated to a length of 150 $\pm$ 60 base pairs

for NMR studies. For spectrophotometric experiments, high salt buffer was made up by adding solid NaCl to the low salt buffer above to reach a concentration of 4.4 M (final pH=6.2). For NMR experiments, high salt buffer consisted of 10 mM Na-cacodylate, 4 M NaCl, 0.5 mM Na₂EGTA₂, 10% D₂O, 1.2 mM trimethyl phosphate, pH adjusted to 7.2 by addition of NaOH.

Absorbance measurements were made on a Cary 118 and Gilford 2600 spectrophotometer. Fluorescence measurements were carried out using a Perkin-Elmer MPF-2A fluorimeter and CD spectra were obtained on a Jasco 500A spectropolarimeter. Concentrations were determined spectrophotometrically, using the following extinction coefficients in low salt buffer: ϵ_{254} (per mole base pair) = 14,200 for poly(dG-dC), ϵ_{480} = 5600 for ethidium and ϵ_{502} = 9850 for BMSp. DNA to drug ratios (BP/D) are reported in terms of total DNA base pair and total drug concentrations.

³¹P NMR experiments were carried out using a locally constructed Fourier transform spectrometer operating at 97.2 MHz for phosphorous, attached to a Nicolet 1180 computer system. Poly(dG-dC) concentration was approximately 0.9 mM in base pairs and the temperature was maintained at 38±2°C in 10 mm tubes. 15,000-25,000 transients were acquired using a 75° pulse, 1.69 s delay, 0.81 s acquisition time, a spectral width of ± 630 Hz and broad band proton decoupling. Chemical shifts are reported upfield from internal trimethyl phosphate.

RESULTS

Absorbance Studies: The effect of adding an excess of either calf thymus DNA or poly(dG-dC) to ethidium bromide in high salt buffer (containing 4.4 M NaCl) on the drug's visible spectrum is shown in Figure 1A. Comparison of these spectra indicates that the spectrum of ethidium is very similar whether bound to B or Z DNA. Addition of either form of DNA results in a shift of the absorption band to higher wavelengths, and a decrease in the maximum. This is typical for the binding of many drugs to DNA. The CD spectrum of the high salt ethidium-poly(dG-dC) complex is that corresponding to Z DNA (see below).

In Figure 1B, data are presented showing the effect of increasing amounts of calf thymus DNA and poly(dG-dC) on the absorbance at 474 nm of a fixed concentration of ethidium at high salt. These titration results indicate that calf thymus DNA binds ethidium more strongly than does poly(dG-dC). The smooth curves in Figure 1B show the best fit to the data using equation 10 of McGhee and von Hippel (25) with *n*, the number of base

1. Effect of poly(dG-dC) or calf thymus DNA on the visible absorption spectrum of ethidium bromide and BMSp in high salt (4.4 M NaCl) buffer: A, ethidium (96 μ M) free (a), + poly(dG-dC), BP/D=76 (b), + DNA, BP/D=76 (c); B, titration of ethidium (30 μ M) with poly(dG-dC) ($\bullet$) and DNA ($\circ$); solid lines represent best fit using McGhee-Von Hippel analysis with site size fixed at 2 base pairs. C, BMSp (6.4 μ M) free (a), + poly(dG-dC), BP/D=48 (b), + DNA, BP/D=48 (c).

pairs per binding site, fixed at 2. Data for poly(dG-dC) were obtained with the smallest BP/D ratio > 20 to avoid the drug-induced Z $\rightarrow$ B transition. The resulting binding constants are 6×10^4 for calf thymus DNA and 6×10^3 for poly(dG-dC), with base pairs as the measure of DNA concentration. A similar titration in 1M NaCl showed superimposable curves for calf thymus DNA and poly(dG-dC). Thus, the differences in Figure 1B most likely reflect different nucleic acid conformations at high salt.

In Figure 1C we present similar data for the DNA bis-intercalator BMSp (22,23). Here, as in the case of ethidium, the visible absorption spectrum of BMSp in the presence of excess Z DNA resembles that found in the presence of excess B DNA.

2. Effect of poly(dG-dC) or calf thymus DNA on the fluorescence emission of ethidium bromide and BMSp in high salt (4.4 M NaCl) buffer excitation at 484 nm: A, ethidium (96 μ M) free (a), + poly(dG-dC), BP/D=76 (b), + DNA, BP/D=76 (c); B, BMSp (6.4 μ M) free (a), + poly(dG-dC), BP/D=48 (b), + DNA, BP/D=48 (c).

Fluorescence Studies: Addition of either right-handed calf thymus DNA or left-handed poly(dG-dC) results in similar changes in the fluorescence behavior of ethidium and BMSp, as shown in Figure 2. A large increase in fluorescence quantum yield upon binding occurs for both Z and B forms of DNA. The emission spectrum of bound ethidium is almost the same for complexes formed with both DNA's, as seen in Figure 2A. In Figure 2B it can be seen that excess amounts of either B or Z DNA have the same effect on the fluorescence emission spectrum of the BMSp dimer as well.

Circular Dichroism Studies: The original work of Pohl and Jovin (1) demonstrated that drastic changes occur in the CD spectrum of poly(dG-dC) upon increasing the NaCl concentration from 1 M to 4.4 M. At high BP/D, as indicated in Figures 1 and 2, e.g. > 75 , the CD spectrum of the complex is essentially that of the Z form of the polynucleotide alone (data not shown). Thus, binding a small amount of ligand per nucleotide does not alter the overall conformation of poly(dG-dC).

One interesting characteristic of the CD properties of the ethidium-DNA complex is the exciton band at 308 nm whose intensity varies with the degree of binding, vanishing in the presence of excess DNA. This band is thought to arise from interactions among bound ligands (26-28). Kuhlmann

3. A. Variation of CD intensity at 314 nm with D/BP ratio for BMSp complexes with poly(dG-dC) (O) and calf thymus DNA ($\square$) in high salt buffer, with BMSp concentration = 3.8 μ M; B, UV region of CD spectrum of poly(dG-dC) (47 μ M base pairs) alone ($\square$), plus 1 μ M BMSp (Δ), plus 2 μ M BMSp (O).

et al. (29) have exploited this behavior to demonstrate binding by bis-intercalation for a dimer of ethidium linked by a methylene chain. In that case the CD intensity reached a non-zero limiting value with increasing amounts of DNA added to a fixed amount of drug.

We have examined the concentration dependence of the CD intensity in this wavelength region for BMSp binding to both calf thymus DNA and poly(dG-dC) at values of D/BP ranging from 0 to 0.15. These data are presented in Figure 3A, where it can be seen that the limiting value of $\Delta\epsilon$ at low binding levels is non-zero for both polymers. This behavior is consistent with the notion that BMSp binds to Z DNA, as well as to B DNA, by bis-intercalation. It is of interest to note that BMSp exhibits a considerably larger residual value of $\Delta\epsilon$ when bound to poly(dG-dC) than when bound to calf thymus DNA. When measured in 1 M NaCl, there is still a significant difference between the effect of the two DNA's, although not as large as that found in Figure 3A. Thus this large difference cannot be attributed solely to a change in poly(dG-dC) conformation. The CD in the region below 300 nm was essentially the same for these complexes as for the poly(dG-dC) or calf thymus DNA alone. In Figure 3B we show CD spectra of BMSp-poly(dG-dC) complexes indicative of the Z conformation. Furthermore, at values of BP/D as low as 4 there was no indication that binding of BMSp has converted poly(dG-dC) back to the B form.

^{31}P NMR: ^{31}P NMR spectra for poly(dG-dC) in 4 M NaCl alone and in the presence of ethidium are shown in Figure 4. In the absence of ethidium, two well resolved peaks are seen about 1.4 ppm apart from each other,

4. ^{31}P NMR spectra showing ethidium induced Z + B transition in poly(dG-dC) - 0.9 mM base pairs - in high salt (4 M NaCl) buffer (see text for details).

similar to reports of others (19). At BP/D=30, the spectrum still consists of two peaks at essentially the same position. At the lower ratios of BP/D, more and more amplitude appears in the region between the two peaks at -4.2 and -2.8 ppm. This absorption at intermediate frequencies is most likely due to the increasing amounts of B form and B-Z junctions. CD spectra of the same solutions obtained in cells of 0.1 cm path length show intensity changes consistent with this interpretation. That is, the CD of the complex appears as the sum of B and Z components, with increasingly larger contributions from the former as BP/D values decrease.

DISCUSSION

The data we have presented show that when poly(dG-dC) is added to ethidium under high salt solution conditions the spectroscopic properties of the drug undergo changes that indicate complex formation. The various spectra of the bound drug are qualitatively the same as those of the drug bound to calf thymus DNA under similar conditions. Thus, it appears that the prototype Z DNA can bind ethidium. This result is different from the implications of the data reported by others (17). In that work, measurements of the UV absorption at 284 nm of high salt solutions of ethidium and

poly(dG-dC) at various ratios of base pairs to drug (BP/D) were described. No indication of interaction was observed at high values BP/D, while at lower values changes were seen indicative of a cooperative drug-induced transition in the polynucleotide conformation. However, as demonstrated by the data shown above, evidence for binding at high BP/D values is obtained when measurements are made in the visible region of the drug's absorption spectrum, where the polynucleotide does not absorb.

The changes observed for the various spectral properties of ethidium bound to Z DNA not only demonstrate that a complex is formed, but also provide some insight into the structure of the complex. The overall similarity in the spectral behavior of the complex with Z and B DNA suggests that the drug's chromophore is in a similar environment in both complexes. In particular we note the large increase in fluorescence intensity seen in Figure 2 for the drug bound to Z DNA as well as to B DNA. A detailed discussion of the fluorescence enhancement of ethidium upon DNA intercalation has been presented by Olmsted and Kearns (30). They proposed a mechanism for quenching of the drug's fluorescence via proton transfer from the chromophore amino groups to the solvent in the excited state, thereby leading to a reduced fluorescence for free drug. In the DNA complex, solvent accessibility of the drug amino groups is severely reduced with the ability to transfer protons to the solvent diminished correspondingly. Hence, the drug exhibits a strong fluorescence when bound to DNA. The extent to which the fluorescence enhancement of ethidium upon binding to Z DNA is similar to that observed upon binding to B DNA is a strong indication that the drug's amino groups are protected from solvent in both complexes. Viscosity experiments (24) also showed similar results for calf thymus DNA and poly(dG-dC) in high salt (data not shown), although it was difficult to obtain data at the relatively high concentrations required without initiating the drug-induced Z $\rightarrow$ B transition. These findings are consistent with an intercalative mechanism for both complexes, and argues against outside binding to Z DNA.

The binding of BMSp, a bifunctional DNA intercalator, is also of interest. The spectroscopic data shown above provide evidence that its complex formed with Z DNA is very similar to that formed with B DNA. The strongest evidence for actual bis-intercalation in the binding to Z DNA lies in the CD results. In contrast to the ethidium monomer, BMSp exhibits a non-vanishing CD intensity in the exciton band at infinite dilution of bound molecules on the DNA lattice in both calf thymus DNA and poly(dG-

dC). This behavior is expected for a bis-intercalated species (29).

Experiments aimed at examining the effect of ethidium on the ^{31}P NMR spectrum of poly(dG-dC) in high salt were undertaken in an effort to observe a manifestation of the drug interaction through a property peculiar to the left-handed form of DNA. Wilson and co-workers have demonstrated a downfield shift in the ^{31}P NMR spectrum of sonicated calf thymus DNA upon addition of several intercalators, including ethidium (31-33). The ^{31}P NMR data displayed in Figure 4 show that at high values of BP/D little effect is observed on the Z DNA peaks. This may be expected due to the low level of drug binding. At lower values of BP/D, however, the changes in the ^{31}P NMR spectrum appear to reflect mainly the gradual conversion of the polynucleotide conformation from Z to B, although there is a slight broadening of the peak at -4.2 ppm. Thus it may be that binding levels sufficiently high to affect the chemical shift of the Z form are not possible under these conditions because they result in conversion to the B form.

One important aspect of the ethidium-poly(dG-dC) interaction in high salt is apparent in the ^{31}P NMR titration, however. In each of the NMR samples at the various BP/D values, the drug was found to be essentially completely bound as determined by optical measurements. The change in conformation from Z to B, as monitored by ^{31}P NMR, occurs gradually as increasingly greater amounts of drug are added. This behavior is consistent with other data presented here indicating that ethidium has a binding affinity which is not drastically different for the two forms of poly(dG-dC). As noted above, analysis of the absorbance titration data in Figure 1 based on the assumption that the ethidium binding site size is two base pairs for both B and Z forms of DNA results in a tenfold lower affinity for the latter. While this is only an estimate, it supports the notion, as do the ^{31}P NMR data, that the Z $\rightarrow$ B transition does not appear to be a highly cooperative process, as indicated previously (17). A relatively small degree of cooperativity was also reported for the binding of another intercalator, adriamycin, to poly(dG-m⁵dC) (38).

While all the data obtained on the interaction of ethidium and BMSp with poly(dG-dC) in high salt strongly argue that Z DNA can bind these drugs by intercalation, one might suppose that the local polynucleotide conformation at the binding site has been altered to that of B DNA. That this is not the case is supported by the following considerations. Pohl and Jovin (1) have shown that the kinetics of the B $\leftrightarrow$ Z transition are slow, occurring on a time scale of minutes. We have carried out

preliminary stopped-flow measurements mixing high salt solutions of ethidium and poly(dG-dC), BP/D=50. Results of these experiments yield an association rate constant of $4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ and a dissociation rate constant of 22 s^{-1} . Both the time scale and bi-molecular nature of the kinetics suggest that a complex is formed directly with the left handed polynucleotide. In addition, the ratio of these rate constants give a binding constant of $1.8 \times 10^3 \text{ M}$, in fair agreement with our estimate from fitting UV titration data.

As noted above, we found no evidence that increasing amounts of BMSp added to high salt solutions of poly(dG-dC) convert the polynucleotide conformation from Z to B. Thus, in this case, there is not much reason to assume that the local conformation has changed at the binding site. We also note that recent theoretical studies have suggested that Z DNA can bind intercalators (37).

It is intriguing to speculate about the structure of the ethidium-Z DNA complex. As binding by intercalation lengthens the DNA helix, the polynucleotide conformation near the binding site must be altered. When ligands intercalate into B DNA, the base pairs undergo an unwinding, from an angle of 36° to one of 10° in the case of ethidium. Changes in the sugar-phosphate backbone accompany this unwinding to yield an increase in the helical pitch. In the Z form of DNA, successive base pairs are at an average angle of -30° (2) although the basic repeating unit is a dinucleotide. It is possible that, upon intercalation in Z DNA, the angle of the bases shifts to a smaller negative number, i.e., the drug forces the base pairs to wind rather than unwind relative to each other, in order for the helix to lengthen. In this case, one might expect the Z-intercalated drug complex to resemble locally, the B-intercalated drug complex, as indicated by the data presented above. Furthermore, the dinucleotide repeating unit of Z DNA may result in a marked preference for a pyrimidine-purine, or purine-pyrimidine intercalation site.

The fact that Z DNA may support binding by both intercalation and bis-intercalation has implications regarding the flexibility and dynamics of Z DNA. Structural (3), hydrogen exchange (34), and hydrodynamic (35) evidence indicates that the Z form of DNA is less flexible than the B form. However, it appears that the Z form can still deform in order to accommodate intercalating ligands. Sobell (36) has proposed that drug intercalation requires a particular deformation of the DNA helix, such as formation of a kink. While Z DNA may be more rigid than B DNA, it appears

capable of such a deformation on a similar time scale as B DNA, should this effect govern drug binding.

That Z DNA can bind intercalators is also important in assessing the biological significance of this DNA form. One can expect high values of BP/D under typical in vivo conditions, where the Z form appears to support complex formation without changing conformation. Thus, a drug may still be able to bind to left-handed regions of DNA and exert an effect. Of course it remains to be seen if the biological effect of drug binding is similar for the various forms of DNA. On the other hand, the difference in binding affinity for certain drugs with left- and right-handed DNA may be very extreme, as originally envisaged for ethidium. In this regard, we note that preliminary experiments we have carried out indicate that actinomycin D, another DNA intercalating drug, appears capable of binding to Z DNA. Further studies are under way to characterize in greater detail the interaction of ethidium with Z DNA, as well as examining the binding of other drugs.

ACKNOWLEDGMENTS

It is a pleasure to thank Prof. P.B. Dervan for a sample of BMSp, Prof. J.T. Yang for allowing us to use his CD instrument and Dr. N. Pattabiraman for many valuable discussions. This work was supported by U.S. Public Health Service Grant CA 27343 awarded by the National Cancer Institute, DHHS.

*Permanent address: Departement de Chimie Organique, ERA613 (CNRS), SC 21 (INSERM), UER des Sciences Pharmaceutiques et Biologiques, 4 Avenue de l'Observatoire, 75006 Paris, France

REFERENCES

1. Pohl, F.M. and Jovin, T.M. (1972) *J. Mol. Biol.* **67**, 375-396.
2. Wang, A.H.-J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., van Boom, J.H., van der Marel, G., and Rich, A. (1979) *Nature* **282**, 680-686.
3. Wang, A.H.-J., Quigley, G.J., Kolpak, F.J., van der Marel, G., van Boom, J.H., and Rich, A. (1981) *Science* **211**, 171-176.
4. Drew, H., Takano, T., Tanaka, S., Itakura, K., and Dickerson, R.E. (1980) *Nature* **286**, 567-573.
5. Behe, M. and Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1619-1623.
6. Lafer, E.M., Möller, A., Nordheim, A., Stollar, B.D., and Rich, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3546-3550.
7. Malfroy, B., Rousseau, N., and Leng, M. (1982) *Biochemistry* **21**, 5463-5467.
8. Vorlickova, M., Kypr, J., Kleinwachter, V., and Palecek, E. (1982) *Nucleic Acids Res.* **10**, 1071-1080.

9. Zimmer, C., Tymen, S., Marck, C., and Guschlbauer, W. (1982) *Nucleic Acids Res.* 10, 1081-1091.
10. McIntosh, L.P., Grieger, I., Eckstein, F., Zarling, D.A., van de Sande, J.H., and Jovin, T.M. (1983) *Nature* 304, 83-86.
11. Klysik, J., Stirdivant, S.M., Larson, J.E., Hart, P.A., and Wells, R.D. (1981) *Nature* 290, 672-677.
12. Singleton, C.K., Klysik, J., Stirdivant, S.M., and Wells, R.D. (1982) *Nature* 299, 312-316.
13. Nordheim, A., Pardue, M.L., Lafer, E.M., Möller, A., Stollar, D.B., and Rich, A. (1981) *Nature* 294, 417-422.
14. Lemeunier, F., Derbin, C., Malfoy, B., Leng, M., and Taillandier, E. (1982) *Expt. Cell Res.* 141, 508-513.
15. Morgenegg, G., Celio, M.R., Malfoy, B., Leng, M., and Kuenzle, C.C. (1983) *Nature* 303, 540-543.
16. Nordheim, A., Tesser, P., Azorin, F., Kwon, Y.H., Möller, A., Rich, A. (1982) *Proc. Natl. Acad. Sci. USA* 79, 7729-7733.
17. Pohl, F.M., Jovin, T.M., Baehr, W., and Holbrook, J.J. (1972) *Proc. Natl. Acad. Sci. USA* 69, 3805-3809.
18. Van de Sande, J.H. and Jovin, T.M. (1982) *EMBO J.* 1, 115-120.
19. Patel, D.J., Kozlowski, S., Nordheim, A., and Rich, A. (1982) *Proc. Natl. Acad. Sci. USA* 79, 1413-1417.
20. Mirau, P.A. and Kearns, D.R. (1983) *Nucleic Acids Res.* 11, 1931-1941.
21. Rio, P. and Leng, M. (1983) *Nucl. Acids Res.* 11, 4947-4956.
22. Dervan, P.B. and Becker, M.M. (1978) *J. Amer. Chem. Soc.* 100, 1968-1970.
23. Becker, M.M. and Dervan, P.B. (1979) *J. Amer. Chem. Soc.* 101, 3664-3666.
24. Jones, R.L., Lanier, A.L., Keel, R.A., and Wilson, W.D. (1980) *Nucl. Acids Res.* 8, 1613-1624.
25. McGhee, J.D. and Von Hippel (1974) *J. Mol. Biol.* 86, 469-489.
26. Dalglish, D.G., Peacock, A.R., Fey, G., Harvey, C. (1971) *Biopolymers* 10, 1853-1863.
27. Atkipis, S. and Kindelis, A. (1973) *Biochemistry* 12, 1213-1221.
28. Houssier, C., Hardy, B., and Fredericq, E. (1974) *Biopolymers* 13, 1141-1160.
29. Kuhlmann, K.F., Mosher, C.W., and Hammer, R.F. (1980) *Biochem. Biophys. Res. Comm.* 92, 1172-1179.
30. Olmsted, J. and Kearns, D.R. (1977) *Biochemistry* 16, 3647-3654.
31. Jones, R.L. and Wilson, W.D. (1980) *J. Amer. Chem. Soc.* 102, 7776-7778.
32. Wilson, W.D., Keel, R.A., and Mariam, Y.H. (1981) *J. Amer. Chem. Soc.* 103, 6267-6269.
33. Wilson, W.D. and Jones, R.L. (1982) *Nucleic Acids Res.* 10, 1399-1410.
34. Ramstein, J. and Leng, M. (1980) *Nature* 288, 413-414.
35. Thomas, T.J. and Bloomfield, V.A. (1983) *Nucleic Acids Res.* 11, 1919-1930.
36. Sobell, H.M. (1980) in *Nucleic Acid Geometry and Dynamics*, Sarma, R.H., Ed., pp. 289-323, Pergamon Press, New York.
37. Gupta, G., Dhingra, M.M., and Sarma, R.H. (1983) *J. Biomolecular Structure and Dynamics* 1, 97-113.
38. Chen, C., Knop, R.H., and Cohen, J.S. (1983) *Biochemistry* 22, 5468-5471.