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Interaction of Drugs with Z-DNA: Cooperative Binding of Actinomycin D or Actinomine to the Left-Handed Forms of Poly(dG-dC)·Poly(dG-dC) and Poly(dG-m^{5}dC)·Poly(dG-m^{5}dC) Reverses the Conformation of the Helix†

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ABSTRACT: The interaction of actinomycin D and actinomine with poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under B- and Z-form conditions has been investigated by optical and phase partition techniques. Circular dichroism data show that the conformation at the binding site is right-handed, even though adjacent regions of the polymer have a left-handed conformation. Actinomycin D binds in a cooperative manner to poly(dG-dC)·poly(dG-dC) under both B-form and Z-form conditions. Analysis of the circular dichroism data shows that 5 ± 1 base pairs of left-handed poly(dG-dC)·poly(dG-dC) in 4.4 M NaCl switch to a right-handed conformation for each bound actinomycin D. When the left-handed form of poly(dG-dC)·poly(dG-dC) is stabilized by the presence of 40 μM $[Co(NH_3)_6]Cl_3$, 25 ± 5 base pairs switch from a left-handed to a right-handed conformation for each bound actinomycin D. Actinomine binds cooperatively to left-handed poly(dG-dC)·poly(dG-dC) in 40 μM $[Co(NH_3)_6]Cl_3$ and to left-handed poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 2 mM $MgCl_2$. Actinomine does not bind to left-handed poly(dG-dC)·poly(dG-dC) in 4.4 M NaCl at concentrations as high as 100 μM. Each bound actinomine converts 11 ± 3 base pairs of left-handed poly(dG-dC)·poly(dG-dC) in 40 μM $[Co(NH_3)_6]Cl_3$ and 7 ± 2 base pairs of left-handed poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 2 mM $MgCl_2$. The binding isotherm data also indicate that the binding site has a right-handed conformation. The actinomycin D–poly(dG-dC)·poly(dG-dC) binding isotherm in 4.4 M NaCl may be fit by an allosteric binding model, which also indicates that the conformation of poly(dG-dC)·poly(dG-dC) is altered only in the vicinity of the bound ligands and that intercalation into a right-handed binding site is much more favorable than intercalation into a left-handed helix. The conversion of the polymer in 4.4 M NaCl from a left-handed to a right-handed conformation occurs in a sequential manner, forming regions of right-handed poly(dG-dC)·poly(dG-dC) that are nearly saturated with actinomycin D. The important factors controlling the binding are the charge of the drug and the forces stabilizing the left-handed conformation. The biological implications of the simultaneous existence of B and Z forms by these polymers are discussed.

In the preceding paper (Walker et al., 1985) we presented results on the interaction of ethidium with left-handed (Z) forms of poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in various buffers [see the preceding paper (Walker et al., 1985) and Rich et al. (1984) for a review of left-handed (Z) DNA]. Experiments also were performed with actinomycin D, an important antitumor drug, and actinomine in order to discern whether the highly cooperative binding of ethidium to left-handed poly(dG-dC)·poly(dG-dC) in 4.4 M NaCl (Pohl et al., 1972; Walker et al., 1985) is unique or is observed with other drugs as well.

The unusually slow dissociation of actinomycin D from DNA correlates directly with the ability to inhibit DNA-dependent RNA polymerase (Muller & Crothers, 1968; Rosenberg et al., 1982). Mirau & Kearns (1983) have shown that actinomycin D is an effective inhibitor of the salt-induced B–Z transition of poly(dG-dC)·poly(dG-dC) and have correlated this with the slow association and dissociation kinetics, while van de Sande & Jovin (1982) have demonstrated that actinomycin D will bind to a condensed form of Z-DNA in a $MgCl_2$–ethanol solution. The pentapeptide side chains of actinomycin D are responsible in part for its high DNA binding affinity and site-exclusion parameter of approximately four base pairs per drug (Muller & Crothers, 1968; Wells & Larson, 1970; Sobell et al., 1971). Actinomine lacks the pentapeptide side chains of actinomycin D, has a 2+ charge at neutral pH (Muller & Crothers, 1968) (Figure 1), and presents the opportunity of studying the influence of the pentapeptide side chains on the reversal of left-handed DNA, although the 2+ charge of actinomine plays an important role in its binding characteristics.

In this report, we correlate circular dichroism (CD) results with equilibrium binding isotherms obtained from optical titration and phase partition methods to characterize the binding of actinomycin D and actinomine to poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under B- and Z-form conditions. The equilibrium binding and CD data are con-

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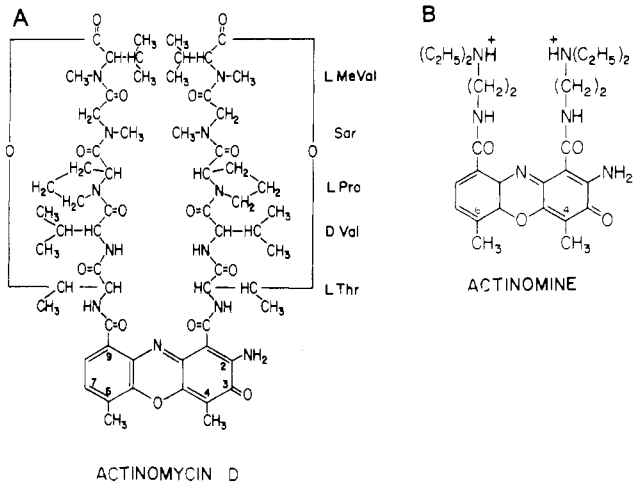

FIGURE 1: Molecular structures of actinomycin D and actinomine. Abbreviations: Thr = threonine; Val = valine; Pro = proline; Sar = sarcosine; MeVal = methylvaline. The tertiary nitrogens of actinomine are protonated at pH 7.

sidered in terms of the distribution of bound drugs along the helix, the distance over which drug-induced conformational alterations are propagated, the conformation of the complexes, and the possible biological significance of these observations.

Materials and Methods

Actinomycin D (NSC-3053), a gift of Merck Sharp & Dohme, was checked for purity by silica gel thin-layer chromatography (TLC) (9:1 v/v ethyl acetate/methanol) and C-18 reverse-phase high-performance liquid chromatography (HPLC) (0–100% linear gradient of acetonitrile over 10 min in conjunction with 0.01 M ethylenediamineacetate, pH 7, at a flow rate of 1.5 mL/min). Actinomine, a gift of Dr. Sisir Sengupta of Children's Cancer Foundation, Boston, MA, was checked for purity by HPLC as described above for actinomycin D. It should be noted that buffer solutions of actinomine, which were stored in the dark at 0 °C, decomposed over a period of a month as evidenced by changes in the visible absorption spectrum. Poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) were purchased from P-L Biochemicals/Pharmacia and were used without further purification. A buffer consisting of 50 mM NaCl and 5 mM $H_2NC(CH_2OH)_3$ [tris(hydroxymethyl)aminomethane], pH 8, will be referred to as the 50 mM sodium buffer. For certain experiments, this buffer was used with the addition of $MgCl_2$ or $[Co(NH_3)_6]Cl_3$; these buffers will be correspondingly referred to as either the 0.2 mM magnesium buffer, the 2 mM magnesium buffer, or the 40 μM cobalt hexaammine buffer. The buffer used for the high-salt experiments consisted of 4.4 M NaCl, 10 mM Na_2HPO_4, and 10 mM ethylenediaminetetraacetic acid disodium salt, pH 7, and will be referred to as the 4.4 M sodium buffer. All buffers were passed through either a 0.22- or 0.45-μm Millipore filter to remove particulate matter. The molar extinction coefficients for the compounds are given in the supplementary material (see paragraph at end of paper regarding supplementary material). Unless otherwise noted, all molar extinction coefficients for the polynucleotides and free drugs were determined from a plot of absorbance vs. concentration for data obtained from dilution of a concentrated stock solution of the compound (dissolved in 50 mM sodium buffer) into the indicated solution. All polynucleotide concentrations are expressed in base pairs. Preparation of the left-handed forms of the polymers is described in Walker et al. (1985).

Optical Titrations. Optical titrations were performed on a Varian Cary 219 spectrophotometer with digital display. The presence of the Z form of the polynucleotides was confirmed by CD spectra recorded prior to equilibration with drug. For the actinomine titrations, aliquots from a concentrated actinomine solution were pipetted at 15-min intervals into a polynucleotide solution contained in either a 5- or 10-cm pathlength cell. The final actinomine concentrations were kept well below that at which aggregation is observable from a Beer–Lambert analysis in the absence of DNA. In the case of actinomycin D it was not feasible to measure the entire binding isotherm in 4.4 M sodium buffer by the addition of a concentrated actinomycin D solution into a solution of poly(dG-dC)·poly(dG-dC) because actinomycin D exhibits extremely low solubility in this buffer, producing aggregation and/or surface adsorption complication. Therefore, the following optical titration method was used to measure the binding isotherm for actinomycin D with poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer. A dilute solution of actinomycin D in 4.4 M sodium buffer was allowed to equilibrate in a 10-cm cell for 12 h at 0 °C. Thereafter, aliquots of a concentrated solution of poly(dG-dC)·poly(dG-dC) in 50 mM sodium buffer were added to the actinomycin D solution, and absorbance values were recorded after allowing the solution to equilibrate for at least 12 h at 0 °C. Dilution of the initial 4.4 M NaCl concentration through the addition of DNA was less than 4%. The titration was performed at 0 °C because actinomycin D exhibits an enhanced solubility at lower temperatures. The long equilibration times were necessary to allow the poly(dG-dC)·poly(dG-dC) to establish B–Z equilibrium after introduction into the 4.4 M sodium buffer and to allow for the slow equilibration of actinomycin D and DNA at these low temperatures. Note that this is a sequential titration which required a total of 8 days; thus, at the end of the titration (low r values) all but the last few additions of poly(dG-dC)·poly(dG-dC) had been equilibrating for several days at 0 °C. The actinomycin D concentration inside the cell was well below that at which aggregation is observable at 0 °C according to Beer–Lambert analysis in the absence of DNA. Actinomycin D titrations of poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer were initially performed in the usual manner (i.e., addition of a concentrated actinomycin D solution into a DNA solution), which produced results in agreement with those presented, but with unacceptably large random error due to the uncertainties in the values of the free drug concentration (C_f).

Bound and free actinomycin D concentrations were calculated according to eq 1 and 2 [in the supplementary material of Walker et al. (1985)] from the absorbance measured at either 440 or 425 nm, wavelengths at which actinomycin D displays a large change in absorbance upon intercalation. Bound and free actinomine concentrations were likewise calculated from the absorbance at either 445 or 430 nm. The molar extinction coefficients of actinomycin D and actinomine bound to DNA were obtained according to the method of Walker et al. (1985). From titrations under both B-form and Z-form conditions, molar extinction coefficients of 10 600 and 10 100 M^{-1} cm^{-1} were estimated at 430 nm for actinomine bound to poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC), respectively. A molar extinction coefficient of 10 100 M^{-1} cm^{-1} at 425 nm was estimated for actinomycin D bound to poly(dG-dC)·poly(dG-dC) from titrations under B-form conditions. This value was used for the poly(dG-dC)·poly(dG-dC) titration of actinomycin D in 4.4 M sodium buffer because it was not possible to estimate a bound molar

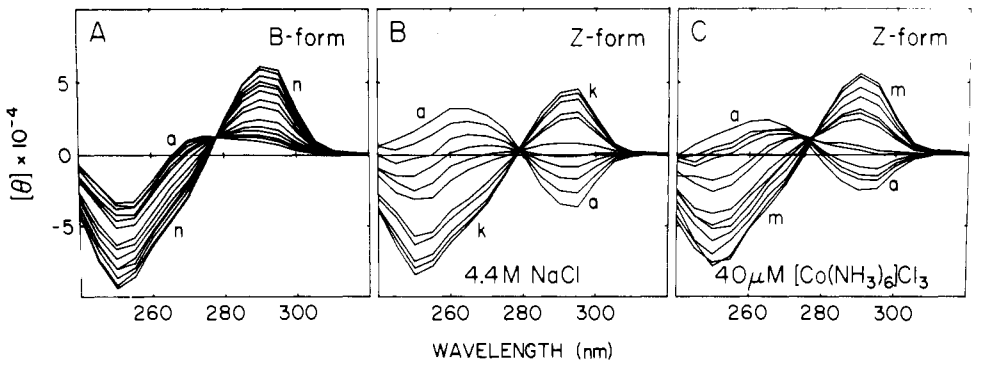

FIGURE 3: Circular dichrosim spectra of actinomycin D: poly(dG-dC)·poly(dG-dC) solutions under B-form (panel A) and Z-form (panels B and C) conditions. In each panel, the initial spectrum of poly(dG-dC)·poly(dG-dC) in the absence of actinomycin D has been denoted with an "a", while the final spectrum of the titration, which corresponds to the largest [actinomycin D]/[base pairs] ratio, has been denoted alphabetically corresponding to its ordinal position in the titration. The buffer conditions were 50 mM sodium buffer (A), 4.4 M sodium buffer (B), and 40 μM cobalt hexaammine buffer (C). The initial base pair concentrations were 19.1 (A), 17.7 (B), and 15.4 μM (C). The $[\theta]$ values take into consideration dilution of the polynucleotide (<20%) during the titration.

extinction coefficient directly from the poly(dG-dC)·poly(dG-dC) titration of actinomycin D, due to the cooperative binding of actinomycin D under these high-salt Z-form conditions, the left- to right-handed conversion which accompanies binding, and the manner in which the titration was performed. A plot of the apparent molar extinction coefficient of actinomycin D at 425 nm (ϵ_{app}^{425}) vs. the total actinomycin D to DNA base pair ratio ([actinomycin D]/[base pairs]) for the poly(dG-dC)·poly(dG-dC) titration of actinomycin D in 4.4 M sodium buffer is shown in Figure 2 (in the supplementary material). The estimated error in the reported bound extinction coefficients is approximately 10%; this uncertainty affects the magnitude of the r and r/C_f values in the binding isotherms, but the overall shape of the Scatchard plot (i.e., cooperative or noncooperative) is not significantly affected (Walker et al., 1985).

Phase Partition Studies. The [^{3}H]actinomycin D–poly(dG-dC)·poly(dG-dC) binding isotherms in 50 mM sodium buffer and 40 μM cobalt hexaammine buffer were measured by phase partition techniques using a 1:1 (v/v) 1-chloroheptane (Kodak)–cyclohexane (Mallinckrodt) organic solvent system. [^{3}H]Actinomycin D was purchased from Amersham and purified by preparative C-18 reverse-phase HPLC as described for unlabeled actinomycin D. The aqueous and organic phases were prequilibrated before sample preparation. The samples were equilibrated by vigorous shaking on a wrist-action shaker at 22 °C for 24 h in microcentrifuge tubes which had been pretreated with a 5% solution of dichlorodimethylsilane (Sigma) in chloroform (Mallinckrodt). After equilibration, the two phases were separated by centrifugation (16000g for 10 s), 50-μL aliquots of each phase were added to 10 mL of scintillant (Amersham ACS), and the actinomycin D concentration in each phase was determined by liquid scintillation counting. Typical values for the partition coefficient, which is defined as the ratio of the actinomycin D concentration in the organic phase to that in the aqueous phase in the absence of DNA, were 3.3 and 8.3 for the 50 mM sodium and 40 μM cobalt hexaammine buffers, respectively. Error bars have been included in the binding isotherms obtained by this method as an estimate of the random error associated with phase partition analysis. For a review of phase partition studies see Albertsson (1971), Waring et al. (1975), Davanloo & Crothers (1976), Krugh et al. (1981), Graves & Krugh (1983a), and references cited therein.

Circular Dichroism (CD) Spectroscopy. CD spectra were recorded on a JASCO J-40 spectropolarimeter interfaced to a Digital PDP 11/34 computer. The signal-averaged spectra were recorded at ambient temperature ($\simeq$23 °C) from 320 to 240 nm at 5-nm intervals, base line corrected, and transferred to a Tektronix 4051 graphics terminal and Tektronix 4662 plotter for analysis and plotting. Molar ellipticity, $[\theta]$, values were calculated in terms of the polynucleotide base pair concentration.

The CD titrations with actinomine were performed concurrently with the optical titrations. Hence, sample preparation was as previously described, and the value of r corresponding to each spectrum was obtained directly. The slow binding kinetics of actinomycin D required the preparation of individual samples for each CD spectrum. For the experiments performed in the 50 mM sodium buffer and the 40 μM cobalt hexaammine buffer, individual samples containing varying actinomycin D to poly(dG-dC)·poly(dG-dC) concentration ratios were equilibrated 24 h at 22 °C. The samples in 4.4 M sodium buffer were equilibrated at 0 °C in order to increase drug solubility. The CD spectra were subsequently recorded in 1-cm path-length cells. Direct determination of r values was deemed impractical due to the nature of the sample preparation. Consequently, the value of r corresponding to each spectrum was estimated from data obtained by equilibrium binding experiments and is believed to be reliable to within ±5%.

RESULTS

Circular Dichroism Spectra of Actinomycin D Complexes. The CD spectra of poly(dG-dC)·poly(dG-dC) with various concentrations of actinomycin D in 50 mM sodium, 4.4 M sodium, and 40 μM cobalt hexaammine buffers are shown in Figure 3. The presence of either 4.4 M NaCl or 40 μM cobalt hexaammine buffer induces a left-handed conformation in poly(dG-dC)·poly(dG-dC), as evidenced by the approximate inversion in the CD spectrum of the polymer. The shapes of the CD spectra for the B and Z forms of poly(dG-dC)·poly(dG-dC) [curves a (Figure 3A–C)] agree with previously published data (Pohl & Jovin, 1972; Behe & Felsenfeld, 1981; Walker et al., 1985). Poly(dG-dC)·poly(dG-dC) solutions containing saturating concentrations of actinomycin D [curve n (Figure 3A), curve k (Figure 3B), and curve m (Figure 3C)] exhibit very similar CD spectra, regardless of whether the polynucleotide was initially in a left- or right-handed conformation. This is clear evidence that the binding of actinomycin D results in the conversion of the left-handed helix to a right-handed helix, which is consistent with the observation that actinomycin D is effective in preventing the salt-induced B–Z conversion (Mireau & Kearns, 1983; G. T. Walker and

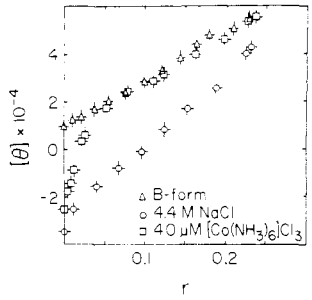

FIGURE 4: Molar ellipticity values as a function of r for actinomycin D titrations with poly(dG-dC)·poly(dG-dC) under B- and Z-form conditions. The data from Figure 3 are plotted at 290 nm for the B form in 50 mM sodium buffer (△), Z form in 4.4 M sodium buffer (○), and Z form in 40 μM cobalt hexaammine buffer (□).

T. R. Krugh, unpublished results). The conformation of the actinomycin D saturated poly(dG-dC)·poly(dG-dC) appears to be relatively insensitive to the presence of either 4.4 M sodium buffer or 40 μM cobalt hexaammine buffer.

Only 4–6 Base Pairs of Left-Handed Poly(dG-dC)·Poly(dG-dC) in 4.4 M NaCl Switch to a Right-Handed Form for Each Bound Actinomycin D. The left- and right-handed forms of the polynucleotides exhibit large differences in ellipticity at 290 nm, providing a convenient wavelength for monitoring conformation. Molar ellipticity data, $[\theta]$, at 290 nm is shown as a function of r in Figure 4 for experiments in 50 mM sodium, 4.4 M sodium, and 40 μM cobalt hexaammine buffers. The B-form (50 mM sodium buffer) data at 290 nm change smoothly as actinomycin D saturates the polynucleotide. The molar ellipticity data at 290 nm for the actinomycin D titration of poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer exhibit a smooth change from negative to positive values as a function of r and converge with the B-form data at $r \approx 0.25$, implying that only four to six base pairs of the left-handed polynucleotide switch to a right-handed conformation for each bound actinomycin D. Analysis of the CD data at other wavelengths in the 250–290-nm region produced identical conclusions for the actinomycin D experiments. Since the neighbor exclusion range for actinomycin D is four base pairs (Muller & Crothers, 1968; Winkle & Krugh, 1981), it appears that the left-handed structure is not altered much beyond the binding site. The helix must be nearly saturated with actinomycin D before the left-handed conformation is completely converted to a (drug-saturated) right-handed form. The CD spectra change in a systematic manner as a function of the bound drug, suggesting that the drug-induced left- to right-handed conversion proceeds without formation of alternate conformations, although it should be noted that the CD spectra would not detect a minor component.

Approximately 25 Base Pairs of Left-Handed Poly(dG-dC)·Poly(dG-dC) in 40 μM $[Co(NH_3)_6]Cl_3$ Switch to a Right-Handed Form for Each Bound Actinomycin D. In the titration in 40 μM cobalt hexaammine buffer the molar ellipticity data change rapidly from the negative values characteristic of a left-handed form and converge with the B-form titration data at an r value of 0.04–0.05 (Figure 4). Within experimental error, the molar ellipticities in these two titrations are the same above an r value of 0.05. The reciprocal of the r value for the convergence of the left-handed and right-handed molar ellipticity data is interpreted in terms of the number of base pairs that adopt a right-handed conformation for each bound actinomycin D; the 0.04–0.05 r value indicates that 25 ±5 base pairs of left-handed poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer switch to a right-handed form for each bound actinomycin D. In other words, once one actinomycin D is bound for every 25 ±5 base pairs, the CD data indicate that the polynucleotide exists in a right-handed form. The equilibrium binding isotherm data presented below support this interpretation.

Isoelliptical Points. The overlaid CD spectra from the actinomycin D titration of poly(dG-dC)·poly(dG-dC) under B-form conditions (50 mM sodium buffer) show a clearly defined isoelliptical point at ~277 nm (Figure 3A). (To facilitate the time averaging used to record these spectra, the data were collected at 5-nm intervals; the uncertainty in the wavelength of an isoelliptical point is estimated to be ±3 nm.) The appearance of an isoelliptical point is consistent with the interpretation that the observed CD spectra arise from weighted contributions of drug-bound and drug-free regions of poly(dG-dC)·poly(dG-dC).

An isoelliptical point is observed at ~279 nm in the CD data for the interaction of actinomycin D with the left-handed Z form of poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer (Figure 3B), also suggesting a two-state model in which the observed CD spectrum is a weighted average of drug-bound (right-handed) and drug-free (left-handed) regions of the polynucleotide. Since an isoelliptical point is observed at ~277 nm in the absence of drug between the spectra of the B form and the 4.4 M NaCl Z form of poly(dG-dC)·poly(dG-dC) (data not shown), the appearance of an isoelliptical point at ~279 nm in the high-salt titration data would be expected even if extended stretches of the left-handed polynucleotide were converted to a right-handed conformation for each bound drug. However, the CD data at 290 nm and the shape of the corresponding equilibrium binding isotherm (as discussed below) rule out the possibility of a drug-induced long-range conversion of the left-handed polynucleotide in 4.4 M sodium buffer.

The CD spectra for the titration of actinomycin D with left-handed poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer (Figure 3C) do not have a true isoelliptical point in the 270–280-nm region. However, close inspection of the data at low r values ($r < 0.06$) reveals a smooth shift in the point of intersection from ~273 to ~277 nm. The initial position of the intersection point at ~273 nm approximates that observed in the absence of actinomycin D between the B form and the Z form (40 μM cobalt hexaammine buffer) of poly(dG-dC)·poly(dG-dC) (data not shown). The intersection point at ~277 nm, which is observed for all r values greater than 0.06, is characteristic of the isoelliptical point observed in the titration under B-form conditions. The shift in the intersection point from ~273 to ~277 nm occurs before the r value reaches 0.06 and is consistent with actinomycin D converting regions of the left-handed polynucleotide outside the binding site to a right-handed conformation in the 40 μM cobalt hexaammine buffer. The shift in the intersection point supports the analysis of the CD data at 290 nm (which has been presented) and the equilibrium binding data (as follows).

Actinomycin D Equilibrium Binding Isotherms. The equilibrium binding isotherms for the interaction of actinomycin D with poly(dG-dC)·poly(dG-dC) under B- and Z-form conditions are shown in the form of Scatchard (1949) plots in Figure 5. While all three binding isotherms exhibit cooperative binding (i.e., a positive slope at low r values), the binding isotherm for the interaction of actinomycin D with left-handed poly(dG-dC)·poly(dG-dC) in 4.4 M NaCl is unique because the r/C_f values extrapolate to zero as r approaches zero (Figure 5B). A plot of r vs. C_f for the 4.4 M NaCl data (Figure 6A) shows that, within experimental error, the concentration of free actinomycin D remains nearly con-

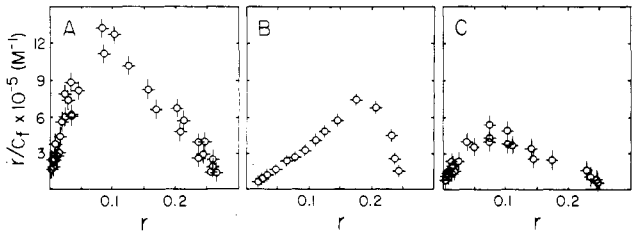

FIGURE 5: Scatchard analyses of actinomycin D binding to poly(dG-dC)·poly(dG-dC) under B-form (panel A) and Z-form (panels B and C) conditions. (A) Data obtained at 22 °C by phase partition methods in 50 mM sodium buffer for which the base pair concentration range was 3–75 μM. (B) Data obtained at 0 °C by optical titration methods in 4.4 M sodium buffer as described in the text. (C) Data obtained at 22 °C by phase partition methods in 40 μM cobalt hexaammine buffer for which the base pair concentration range was 11–12 μM.

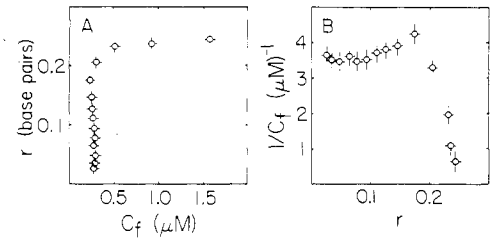

FIGURE 6: (A) Plot of r vs. the free actinomycin D concentration (C_f) for the data described in Figure 5B. (B) Plot of $1/C_f$ vs. r for the data described in Figure 5B.

stant at 0.3 ± 0.1 μM until the helix is approximately 70% saturated ($r \sim 0.18$). At higher r values, the free actinomycin D concentration increases rapidly, in concert with the rapid falloff in the r/C_f values in the Scatchard plot (Figure 5B). Another interesting feature of the 4.4 M NaCl data is that the concentration of free actinomycin D must reach approximately 0.3 μM before significant binding is observed. The effect of C_f on the shape of the binding isotherm in Figure 5B is illustrated by plotting $1/C_f$ as a function of r, as shown in Figure 6B. The slight fluctuation in the value of $1/C_f$ below $r = 0.18$ is not considered to be significant because the slow kinetics in this experiment meant that 8 days was required for the collection of data. The rapid falloff in the value of $1/C_f$ for $r > 0.18$ results in the rapid decrease in the r/C_f values in the Scatchard plot (Figure 5B).

If actinomycin D induced a long-range allosteric conversion in the conformation of the polymer from a left-handed to a right-handed form, then the binding isotherm would be expected to reach a maximum well below the observed value of $r = 0.18$. The experimental binding isotherm in 4.4 M sodium buffer (Figure 5B) was fit with the allosteric model of Crothers and co-workers (Bresloff & Crothers, 1981; Dattagupta et al., 1980) using the following set of parameters, where the subscripts represent forms 1 and 2, the left- and right-handed forms of the polynucleotide, respectively: $K_2/K_1 = 1000$, $\tau_1 = 1$, $\tau_2 = 3$, $S = 0.56$, and $\sigma = 0.013$. The model calculates the r values for binding to each of the two forms (r_1, r_2). Over a range of $r = 0.005$–0.2, r_2 was constant at a value 0.2 ± 10% while r_1 was equal to 0.002 ± 10%. The values of S and σ are identical with those used in the fit of the corresponding ethidium data (Walker et al., 1985) as might be expected since these parameters represent the equilibrium between the two forms of DNA—an equilibrium which should be independent of drug. A plot of the calculated binding isotherm and a plot of the fraction of form 2 vs. r, as well as r_1 and r_2 vs. r plots, are in the supplementary material. The main conclusions drawn from fitting the binding data are that actinomycin D

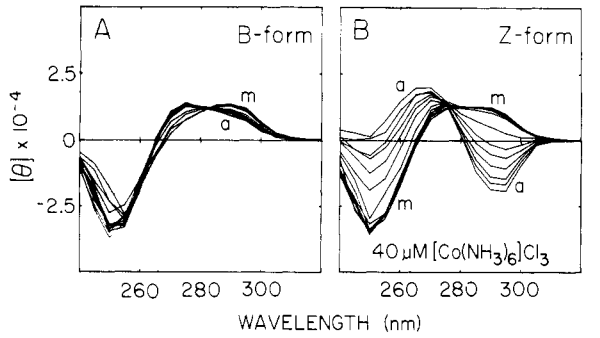

FIGURE 7: Circular dichroism spectra of actinomine: poly(dG-dC)·poly(dG-dC) solutions under B-form (panel A) and Z-form (panel B) conditions. In each panel, the initial spectrum of poly(dG-dC)·poly(dG-dC) in the absence of actinomine has been denoted with an "a", while the final spectrum of the titration, which corresponds to the largest [actinomine]/[base pairs] ratio, has been denoted with an "m". The buffer conditions were 50 mM sodium buffer (A) and 40 μM cobalt hexaammine buffer (B). The initial base pair concentrations were 12.2 (A) and 15.3 μM (B). The $[\theta]$ values take into consideration dilution of the polynucleotide (<25%) during the titration.

exhibits a much greater binding affinity to the right-handed form of the polynucleotide and that the left-handed form is sequentially converted to a right-handed form to which one actinomycin D is bound per five base pairs, even at the lowest levels of binding (i.e., actinomycin D binds in clusters).

The binding isotherm for the interaction of actinomycin D with right-handed poly(dG-dC)·poly(dG-dC) in the 50 mM sodium buffer is shown in Figure 5A. The initial positive slope in the binding isotherm is indicative of a cooperative binding process. These data are consistent with our previously published results (Winkle & Krugh, 1981), with slight differences attributable to a variation in the Na^+ ion concentration and the lower temperature (5 °C) used in the previous studies. The equilibrium binding of actinomycin D to poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer also exhibits a cooperative binding isotherm (Figure 5C). The site exclusion parameter is approximately four base pairs per bound drug for all titrations.

Effect of Actinomycin D on Ethidium Binding. Ethidium does not bind efficiently to poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer until the concentration of free ethidium reaches 20 μM (Pohl et al., 1972; Walker et al., 1985). It was of interest to determine whether bound actinomycin D might drastically reduce or eliminate this minimum ethidium concentration. Addition of actinomycin D to an 18 μM solution of left-handed poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer containing 5 μM ethidium does not result in an increase in fluorescence intensity of the solution (data not shown). Since an increase in fluorescence intensity normally accompanies ethidium binding, we conclude that the binding of actinomycin D does not induce the binding of ethidium under the conditions studied. These data lend further support to the conclusion that actinomycin D does not convert extended regions of left-handed poly(dG-dC)·poly(dG-dC) in 4.4 M NaCl to a right-handed form because ethidium would be expected to bind to regions of right-handed DNA.

Actinomine Experiments: Circular Dichroism Spectra. The CD spectra for actinomine titrations with poly(dG-dC)·poly(dG-dC) and poly(dG-m^5dC)·poly(dG-m^5dC) under both B-form and Z-form conditions are shown in Figures 7 and 8, respectively. The CD spectra of Z-form poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer [curve a (Figure 7B)] and Z-form poly(dG-m^5dC)·poly(dG-m^5dC) in 2 mM magnesium buffer [curve a (Figure 8B)] are in agreement with

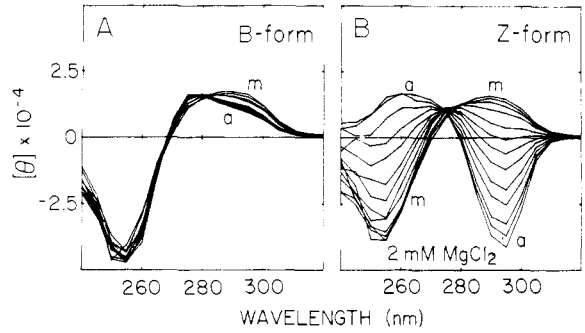

FIGURE 8: Circular dichroism spectra of actinomine: poly(dG-m^{5}dC)·poly(dG-m^{5}dC) solutions under B-form (panel A) and Z-form (panel B) conditions. In each panel, the initial spectrum of poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in the absence of actinomine has been denoted with an "a", while the final spectrum of the titration, which corresponds to the largest [actinomine]/[base pairs] ratio, has been denoted with an "m". The buffer conditions were 50 mM sodium buffer (A) and 2 mM magnesium buffer (B). The initial base pair concentrations were 10.1 (A) and 20.4 μM (B). The $[\theta]$ values take into consideration dilution of the polynucleotide (<10%) during the titration.

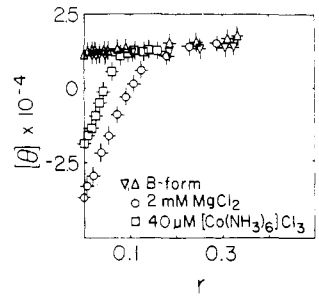

FIGURE 9: Molar ellipticity values as a function of r for actinomine titrations with poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under B- and Z-form conditions. The data from Figures 7 and 8 are plotted at 290 nm. The polynucleotides and buffer conditions were respectively B-form poly(dG-dC)·poly(dG-dC) in 50 mM sodium buffer (▽), B-form poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 0.2 mM magnesium buffer (Δ), Z-form poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer (□), and Z-form poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 2 mM magnesium buffer (○).

previously published data for the left-handed forms of the polymers [e.g., see Behe & Felsenfeld (1981)]. Actinomine saturated polymers display very similar spectra under both B-form and Z-form conditions (compare curves m in panels A and B of Figure 7, respectively, and compare curves m in panels A and B of Figure 8, respectively), which clearly indicates that the binding of actinomine results in the formation of a complex, the conformation of which is relatively insensitive to the Z-form conditions.

Actinomine binding does not result in significant alterations in the CD spectra of the B forms of poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) (Figures 7A and 8A), in contrast to the substantial changes induced by actinomycin D. We ascribe the differences in the CD spectral changes observed under B-form conditions to the effects of the cyclic pentapeptides of actinomycin D on the conformation of the complex [e.g., see Sobell et al. (1971) and Takusagawa et al. (1984) for X-ray crystal structures and discussions]. The CD spectra associated with actinomine titrations of left-handed poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer (Figure 7B) and left-handed poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 2 mM magnesium buffer (Figure 8B) undergo a progressive conversion from the characteristic Z-form spectrum toward that observed for actinomine bound to the respective polynucleotide under B-form conditions. The CD spectra recorded under Z-form conditions change in a systemic manner upon actinomine binding, suggesting that the left- to right-handed conversion of the polynucleotide proceeds without formation of alternate conformations, although minor components would not be detected.

Change in CD as a Function of Bound Actinomine. Plots of molar ellipticity, $[\theta]$, at 290 nm vs. r are shown in Figure 9 for actinomine titrations of poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under both B-form and Z-form conditions. As previously discussed, actinomine titrations of either poly(dG-dC)·poly(dG-dC) or poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under B-form conditions produce only slight changes in the CD spectra of the right-handed polynucleotides, as illustrated in Figure 9. The molar ellipticity data for the titrations performed under Z-form conditions exhibit large changes at 290 nm, proceeding from the negative values characteristic of the left-handed forms of the polynucleotides toward positive values indicative of the B-form titration data. The data for the actinomine titration of left-handed poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer converge with that of the B-form titration of poly(dG-dC)·poly(dG-dC) in 50 mM sodium buffer at $r \sim 0.09$, which indicates that 11 ± 3 base pairs of left-handed poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer switch to a right-handed form for each bound actinomine. The poly(dG-m^{5}dC)·poly(dG-m^{5}dC) data recorded under Z-form conditions (Figure 9) intercept the data from the titration under B-form conditions (0.2 mM magnesium buffer) at $r \sim 0.14$, which indicates that 7 ± 2 base pairs of left-handed poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 2 mM magnesium buffer switch to a right-handed conformation for each bound actinomine.

Isoelliptical Points. In the titration of actinomine with poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer (Z-form conditions, Figure 7B), the point at which the curves intersect in the 260–280-nm region changes for r values from 0 to 0.1. An isoelliptical point exists at ~280 nm for $r > 0.1$ as the CD spectra change only slightly in a manner similar to the corresponding titration under B-form conditions (Figure 7A). Likewise, the Z-form poly(dG-m^{5}dC)·poly(dG-m^{5}dC) data display a similar change in the position of the intersection point from ~274 to ~282 nm for $r < 0.15$ (Figure 8B), which then remains constant at 282 nm for higher r values. The 282-nm isoelliptical point is observed also in the corresponding B-form titration (Figure 8A). The smooth shift in the intersection point for both Z-form titrations suggests that actinomine converts regions of the left-handed polynucleotide (outside the binding site) to a right-handed conformation. Once this left- to right-handed conversion is complete, the CD spectra for these Z-form titrations exhibit an isoelliptical point analogous to the corresponding titrations under B-form conditions.

Actinomine Equilibrium Binding Isotherms. The equilibrium binding isotherms for the interaction of actinomine with the left-handed forms of poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) are shown in panels A and B of Figure 10, respectively. Both binding isotherms are indicative of cooperative binding, as evidenced by a positive slope in the Scatchard plots. The near zero intercept observed for the binding of actinomine to poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under Z-form conditions (Figure 10B) is reminiscent of the type of binding isotherm obtained for actinomycin D (Figure 5B,C) and ethidium binding under Z-form conditions (Walker et al., 1985). A plot of the data from Figure 10B in the form of an r vs. C_f plot shows that very little actinomine binds to poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 2 mM magnesium

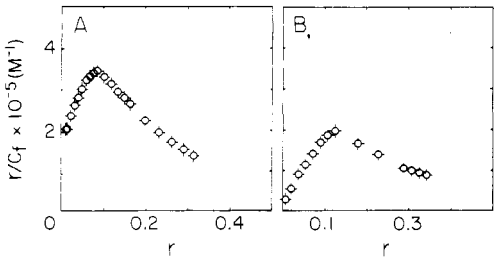

FIGURE 10: Scatchard analyses of actinomine binding to poly(dG-dC)·poly(dG-dC) and poly(dG-m^{5}dC)·poly(dG-m^{5}dC) under Z-form conditions. Panel A is poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer; panel B is poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 2 mM magnesium buffer. The data were obtained by optical titration methods. The initial base pair concentrations were 18.9 (A) and 20.4 μM (B), which decreased by less than 10% during the experiment as a result of dilution.

buffer until the total actinomine concentration reaches 0.4 μM (data not shown).

The binding isotherm for the interaction of actinomine with poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine buffer (Figure 10A) reaches a maximum at $r \approx 0.08$, and for larger r values the binding isotherm represents neighbor exclusion binding. Note that for $r \geq 0.08$ the CD data in Figure 9 indicate that poly(dG-dC)·poly(dG-dC) has a right-handed conformation, and thus for $r > 0.08$ we expect to observe neighbor exclusion binding. The analysis of the isoelliptical points presented above also indicated that the conformational switch was completed below $r = 0.1$. All the data are consistent with a sequential conversion of poly(dG-dC)·poly(dG-dC) in 40 μM cobalt hexaammine from a left-handed to a right-handed duplex, with ~11 base pairs undergoing a transition for each bound actinomine.

Similar arguments may be presented for the binding of actinomine to poly(dG-m^{5}dC)·poly(dG-m^{5}dC) in 2.0 mM magnesium buffer where the maximum in the binding isotherm occurs at $r \approx 0.12$; this corresponds well with the conclusion that 7 ± 2 base pairs of left-handed poly(dG-m^{5}dC)·poly(dG-m^{5}dC) switch to a right-handed form for each bound actinomine. These data are also supported by the analysis of the isoelliptical points, as discussed.

Actinomine Does Not Bind to Poly(dG-dC)·Poly(dG-dC) in 4.4 M NaCl. Addition of 100 μM actinomine to a solution of left-handed poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer does not affect the CD spectrum of the polynucleotide. Likewise, no evidence was obtained for the binding of actinomine to poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer by optical titration methods for actinomine concentrations as high as 40 μM. Higher concentrations of actinomine were not pursued in these experiments. It is apparent that the binding of actinomine, which carries two positive charges at neutral pH, is greatly repressed by the presence of 4.4 M sodium buffer.

Discussion

The Role of Ligand Charge and Cation Concentration in Drug Binding to Z-DNA. Actinomine, which possesses a 2+ charge at neutral pH (Muller & Crothers, 1968), shows no spectroscopic evidence of binding to poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer for concentrations as high as 100 μM which indicates that actinomine has a very low affinity for both the left-handed and the right-handed forms of this polymer in the high-salt buffer. Ethidium, a ligand with a single positive charge, requires a free drug concentration of 20 μM before significant levels of binding are observed to the left-handed form of poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer (Pohl et al., 1972; Walker et al., 1985), while the uncharged actinomycin D is able to bind efficiently at free drug concentrations greater than ≈0.3 μM (Figure 6). The interaction of actinomine (2+), ethidium (1+), and actinomycin D with the high-salt form of poly(dG-dC)·poly(dG-dC) illustrates the effect of ligand charge on binding under Z-form conditions. The relative stability of the B and Z forms is another important factor in determining the shape of the binding isotherms. For example, Krugh et al. (1986) have observed that raising the concentration of magnesium from 2 to 25 mM increases the minimum concentration of free ethidium required before significant binding to poly(dG-m^{5}dC)·poly(dG-m^{5}dC) is observed.

Right-Handed Intercalation Site Is Formed under Z-Form Conditions. The CD spectra associated with either actinomycin D or actinomine titrations of the left-handed forms of the polynucleotides change directly toward that characteristic of a right-handed drug–DNA complex, without any indication of intermediate structures. From the analyses of the CD spectra and equilibrium binding isotherms, we propose that the binding site is a right-handed intercalation site. Formation of alternate binding sites such as a left-handed intercalation site is not required to explain binding under Z-form conditions. However, we note that these experiments probe the equilibrium state of the systems and do not provide insight into the mechanism for attaining the equilibrium conformation. For example, the complex may form via intercalation into residual B-form DNA, or via interacalation into left-handed DNA [as suggested by Gupta et al. (1983)] with subsequent alteration in the handedness of the intercalation site, or perhaps by complex formation with β-DNA (Banerjee & Sobell, 1983; H. M. Sobell, personal communication). Kinetic experiments are required for the determination of the reaction mechanism.

Mireau & Kearns (1983) studied the effect of actinomycin D, ethidium, proflavin, and bis(methidium)spermine on the initial rate of the B to Z transition of poly(dG-dC)·poly(dG-dC) in 3.5 M NaCl and reported that actinomycin D exhibited a measurable decrease at a level of one actinomycin D present per 150 base pairs of the polymer. This was interpreted as inhibition of the propagation step in the B–Z conversion and correlates with the unusually slow dissociation of actinomycin D from poly(dG-dC)·poly(dG-dC) (Krugh et al., 1979) and calf thymus DNA (Muller & Crothers, 1968), in a manner analogous to the effective inhibition of RNA elongation by the actinomycins [e.g., see Muller & Crothers (1968)]. It is worth noting that the dissociation rate of actinomycin D in the high-salt buffer is not known and may differ significantly from the value cited by Mirau & Kearns (1983) for 0.2 M NaCl due to the importance of water in the activation step for dissociation and the change in the water activity in the high-salt solution.

An alternative explanation for the ability of very low levels of actinomycin D to inhibit the B to Z transition arises from a consideration of the cooperative binding of actinomycin D to poly(dG-dC)·poly(dG-dC) observed at low levels of bound drug (Figure 5A). If the structure responsible for the cooperative binding were also one type of nucleation site for the B to Z transition, then the preferential binding of actinomycin D to these sites could have a measurable effect on the initial transition rate at very low r values. This interesting suggestion is under study.

The strong preference for actinomcyin D binding to a right-handed intercalation site may be appreciated from an inspection of the crystal structure of actinomycin D with deoxyguanosine (Sobell, 1973) and the cocrystalline complexes with dG-dC (Takusagawa et al., 1982) where the intercalation

site is an unwound B helix. The intercalation sites in all other cocrystals involving intercalating drugs are also unwound right-handed regions (Berman & Young, 1981; Rich et al., 1984), although there are no simple steric reasons why the intercalators lacking groove binding moieties should not intercalate into Z-DNA [e.g., see Gupta et al. (1983)]. We believe that molecules containing a left-handed pitch for groove binding will be capable of stabilizing left-handed DNA, although none of the molecules currently under study in our laboratory have demonstrated preference for Z-DNA.

Does Actinomycin D Bind in Clusters on Poly(dG-dC)·Poly(dG-dC) in 4.4 M NaCl? In 4.4 M sodium buffer the left-handed form of poly(dG-dC)·poly(dG-dC) is much more stable than the B form of the polymer. The binding of actinomycin D sequentially converts the left-handed polynucleotide, switching only four to six base pairs to a right-handed form per bound actinomycin D. Thus, the regions of right-handed poly(dG-dC)·poly(dG-dC) are nearly saturated with actinomycin D, even at relatively low levels of overall polymer saturation (i.e., low r values), which is similar to the situation for ethidium (Walker et al., 1985). Formation of energetically unfavorable B′–Z interfaces would be minimized if the binding of actinomycin D occurred primarily by extension of regions of previously bound drug, as opposed to random binding along the duplex. Since nearly saturating levels of actinomycin D are necessary for complete conversion of left-handed poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer to a right-handed form (Figure 4), we propose a model in which actinomycin D binds in clusters forming (drug-bound) regions of right-handed DNA. The analysis of the 4.4 M NaCl binding isotherm in terms of an allosteric transition in the DNA structure supports this interpretation.

The interaction of the drugs with the B′–Z interface is suggestive of the possible importance of altered structures in the interaction of ligands with DNA. In a general sense, these experiments suggest that the local conformation of heterogeneous DNA may have a pronounced effect on the binding of ligands. It is interesting to speculate that the cooperative binding observed at low r values in several drug–DNA systems [e.g., see Graves & Krugh (1983b), and references cited therein] may be a result of the affinity of these ligands for a small fraction of the native DNA existing in an altered conformation under physiological conditions. This possibility is the essence of a "two-site" binding model proposed by Rosenberg, Carvlin, and Krugh (unpublished results) in which the DNA is considered to consist of structurally distinct regions, of which one type binds the ligands in a highly cooperative manner while the remaining regions bind the ligands by a neighbor-exclusion process. The cooperative binding sites saturate first, with subsequent binding at higher bound drug to DNA ratios occurring at the remaining sites in a neighbor-exclusion manner. This type of binding is consistent with the humped Scatchard plots observed under B-form conditions [e.g., see Winkle et al. (1982), Winkle & Krugh (1981), Graves & Krugh (1983b), and Walker et al. (1985)], although the binding isotherms may also be reproduced by the allosteric binding model (Hogan et al., 1979; Bresloff & Crothers, 1981).

The fact that we need not invoke an all-or-none model to understand the data could be important in the interpretation of the interactions of Z-DNA with other molecules such as proteins. van de Sande & Jovin (1982), Durand et al. (1983), and Butzow et al. (1984) reported that left-handed DNA supports enzymatic activity which may be interpreted as activity expressed from a left-handed template or, more likely, that the region of the polymer to which the enzyme is bound is in a right-handed conformation, even though the majority of the polymer is in a left-handed conformation. The structural transitions that must accompany movement of the right-handed binding site along a predominantly left-handed polynucleotide would reduce enzymatic activity, as has been observed experimentally for the transcriptional activity of *Escherichia coli* RNA polymerase (Durand et al., 1983; Butzow et al., 1984). A knowledge of the equilibrium and kinetic aspects of these systems is important to our understanding of the relationship between DNA structure and function in vivo.

Supplementary Material Available

Molar extinction coefficients of the compounds, plot of the apparent molar extinction coefficient of actinomycin D vs. the total actinomycin D to DNA base pair ratio (Figure 2), and four figures of a fit of the binding isotherm for actinomycin D and poly(dG-dC)·poly(dG-dC) in 4.4 M sodium buffer according to the allosteric transition model (6 pages). Ordering information is given on any current masthead page.

Registry No. Poly(dG-dC), 36786-90-0; poly(dG-m^{5}dC), 51853-63-5; actinomycin D, 50-76-0; actinomine, 23604-87-7.

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Long-Range Allosteric Effects on the B to Z Equilibrium by Daunomycin†

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ABSTRACT: Spectroscopic and fluorometric methods were used to study the binding of the anticancer drug daunomycin to poly[d(G-C)] and poly[d(G-m^{5}C)] under a variety of solution conditions. Under high-salt conditions that favor the left-handed Z conformation, binding isotherms for the interaction of the drug with poly[d(G-C)] are sigmoidal, indicative of a cooperative binding process. Both the onset and extent of the cooperative binding are strongly dependent upon the ionic strength. The binding data may be explained by a model in which the drug preferentially binds to B-form DNA and acts as an allosteric effector on the B to Z equilibrium. At 2.4 M NaCl, binding of as little as one drug molecule per 20 base pairs (bp) results in the conversion of poly[d(G-C)] from the Z form entirely to the B form, as inferred from binding data and demonstrated directly by circular dichroism measurements. Similar results are obtained for poly[d-(G-m^{5}C)] in 50 mM NaCl and 1.25 mM $MgCl_2$. Under these solution conditions, it is possible to demonstrate the Z to B structural transition in poly[d(G-m^{5}C)] as a function of bound drug by the additional methods of sedimentation velocity and susceptibility to DNase I digestion. The transmission of allosteric effects over 20 bp is well beyond the range of the drug's binding site of 3 bp. Since daunomycin preferentially binds to alternating purine–pyrimidine sequences, which are the only sequences capable of the B to Z transition, the allosteric effects described here may be of importance toward understanding the mechanism by which the drug inhibits DNA replicative events. The results described are of more general interest as an illustration of long-range allosteric effects on DNA conformation, of perhaps general importance in the regulation of gene expression.

The transition of DNA from the right-handed B form to the left-handed Z form is a topic of intense current interest. Since the early observations of the Z structure in solution (Pohl & Jovin, 1972) and in the crystalline state (Wang et al., 1979), Z DNA has been shown to exist (or to be inducible) in polytene chromosomes (Nordheim et al., 1981; Hill & Stollar, 1983; Robert-Nicoud et al., 1984) and to exist in vivo (Lipps et al., 1983). Proteins that will bind specifically to Z DNA have been isolated (Nordheim et al., 1982). Models invoking Z DNA as a potential mediator of gene expression have followed these observations (Rich, 1983), although direct experimental verification of the involvement of Z DNA in the regulation of gene expression is still lacking. The chemistry and biology of Z DNA have recently been reviewed in detail (Rich et al., 1984).

Various aspects of the involvement of small molecules in the B to Z transition are under active investigation in many laboratories. Metal ions are, in general, promoters of the B to Z transition, but metals differ, in often striking ways, in the concentration required for the formation of Z DNA (Pohl & Jovin, 1972; Behe & Felsenfeld, 1981; van de Sande et al., 1982; Chen et al., 1984). In contrast, a number of compounds are inhibitors of the B to Z transition. Many of these are of medical or clinical interest and include intercalators (Pohl et al., 1972; Mirau & Kearns, 1983), carcinogens (Nordheim et al., 1983; Rio & Leng, 1983), and other compounds that bind to DNA (Zimmer et al., 1983). Studies of the effects

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