Elsamicin A Can Convert the Z-Form of Poly[d(G-C)] and Poly[d(G-m^{5}C)] Back to B-Form DNA†

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ABSTRACT: The interaction of poly[(G-C)] and poly[d(G-m^{5}C)] with the antitumor antibiotic elsamicin A, which binds to alternating guanine + cytosine tracts in DNA, has been studied under the B and Z conformations. Both the rate and the extent of the B-to-Z transition are diminished by the antibiotic, as inferred by spectroscopic methods under ionic conditions that otherwise favor the left-handed conformation of the polynucleotides. Moreover, elsamicin converts the Z-form DNA back to the B-form. The circular dichroism data indicate that elsamicin binds to poly[d(G-C)] and poly[d(G-m^{5}C)] to form a right-handed bound elsamicin region(s). The transition can be followed by changes of the molar ellipticity at 250 nm, thus providing a convenient wavelength to monitor the Z-to-B conformational change of the polymers as elsamicin is added. The elsamicin A effect might be explained by a model in which the antibiotic binds preferently to a B-form DNA, playing a role as an allosteric effector on the equilibrium between the B and Z conformations, thus favoring the right-handed one.

Z-DNA is a left-handed, double-helical molecule whose structural properties differ significantly from those of the classic B-form DNA (Pohl & Jovin, 1972; Wang et al., 1979). Formation of Z-DNA has been observed in some alternating purine–pyrimidine polymers such as poly[d(G-C)] (Pohl & Jovin, 1972; Wang et al., 1979) and poly[(dT-dG)·(dC.dA)] (Haniford & Pulleyblank, 1983)). It is favored, in poly[d(G-C)], when the cytosines are methylated at their 5-position (Behe & Felsenfeld, 1981), and in recombinant plasmids subjected to negative torsional stress (Singleton et al., 1982; Zacharias et al., 1988). Both the chemistry and biology of Z-DNA are a matter of current interest, in part to understand the possible role of Z-DNA in gene expression. Evidence for the existence of Z-DNA in vivo has been provided. It has been described that right-handed B and left-handed Z conformations coexist in equilibrium in plasmids in *Escherichia coli* (Zacharias et al., 1988), and they have been detected in chromosomes from *Drosophila* (Nordheim et al., 1981) and in metabolically-active mammalian cells (Wittig et al., 1991). Moreover, sequences that may adopt the Z conformation are found in the enhancer regions of the SV40 minichromosome (Nordheim & Rich, 1983; Casasnovas et al., 1989) and between two transcription units alternatively expressed during the development of *Drosophila hydei* (Jiménez-Ruiz et al., 1991). Nuclear proteins that interact specifically with DNA have been isolated from a variety of sources (Azorin & Rich, 1985; Lafer et al., 1985). On the other hand, the exact role of Z-DNA in gene expression still remains undefined. Indeed, Z-DNA may play a positive or negative control over gene transcription depending on its location in genomic DNA, since the changes in supercoiling generated during gene transcription should suffice to generate B-to-Z-form transitions (or Z-to-B) (Zacharias et al., 1988; Tsao et al., 1989).

Gene expression depends upon interaction between nucleic acids and regulatory proteins. These interactions are characterized by both high affinity and high specificity (Steitz, 1990). Ideally, substances like antitumor drugs that may interfere with genetic expression should present these properties. However, the binding specificity of antitumor antibiotics to DNA is low in comparison to that of protein–DNA interactions. Nevertheless, it is noteworthy that at least some drugs might effectively interfere with DNA–protein interactions and therefore, play an important role in modulating gene expression (Bartkowiak et al., 1989; Chaires, 1990). Many such drugs appear to have a remarkable effect on the B-to-Z transition (Mirau & Kearns, 1983; Chaires, 1983, 1985, 1986; Walker et al., 1985a,b; Krugh et al., 1987; Gilbert et al., 1991), but the precise characteristics of the mechanisms involved are not fully understood. DNA in B conformation and Z-DNA form an equilibrium system, in which proteins can help to stabilize one of the conformations, e.g., the Z-form (Azorín & Rich, 1985; Lafer et al., 1985), but other factors, such as monovalent and divalent cations (Pohl & Jovin, 1972; Wang et al., 1979) and, in principle, many intercalating drugs, may also be involved (Mirau & Kearns, 1983; Chaires, 1983; Walker et al., 1985a). Studies on the effects of intercalating drugs on the rate of the B-to-Z transition show that actinomycin D is more efficient than ethidium as an inhibitor of the transition (Mirau & Kearns, 1983; Gilbert et al., 1991), while the relative efficiency of ethidium and daunomycin in inhibiting such conformational change is identical (Chaires, 1986). The existing data have been reevaluated (Gilbert et al., 1991) to propose a mechanism in which the primary effect of intercalators is on the nucleation event leading to the B-to-Z transition. Ethidium binding to Z-DNA results in regions of right-handed DNA (a B-DNA–drug complex), whereas circular dichroism spectra show that the majority of the polymer is still in a left-handed conformation (Krugh et al., 1987). For daunomycin it has been reported (Chaires, 1986) that the energetically unfavorable Z-to-B transition, at high salt, is driven by coupling to the energetically favorable binding of daunomycin to the B conformation.

Recently, we (Salas & Portugal, 1991; Párraga & Portugal, 1992; Párraga et al., 1992), and others (Uesugi et al., 1991), have become interested in the antibiotic elsamicin A (Figure 1), a new fermentation product (Konishi et al., 1986), which

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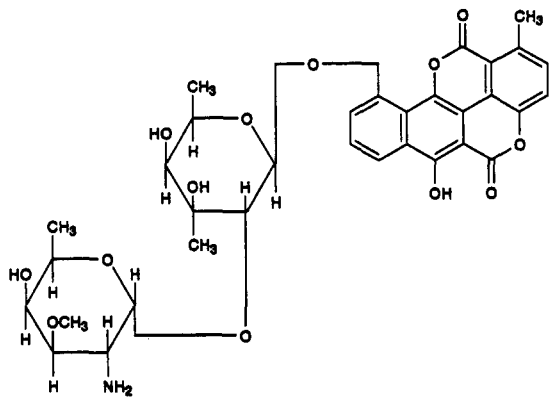

FIGURE 1: Chemical structure of elsamicin A.

exhibits strong inhibitory activity against various tumors (Konishi et al., 1986; Shuring et al, 1988) and is presently undergoing Phase I clinical trials (Shuring et al., 1988; Gaver et al., 1989). Elsamicin A contains a lactone chromophore, chartarin, in common with some related antibiotics like chartreusin (Takai et al., 1980), but differs in the novel amino sugar (2-amino-2,6-dideoxy-3-*O*-methyl-D-galactose) in its disaccharide moiety; see Figure 1. Elsamicin appears to recognize and bind to DNA regions containing the triplet GCG (Salas & Portugal, 1991; Parraga & Portugal, 1992), while in a reducing environment it produces DNA strand scission (Uesugi et al., 1991; Parraga et al., 1992).

Since alternating CG sequences are among those required to obtain a Z conformation (Wang et al., 1979), we tentatively consider that preferential binding to such sequences could be part of the mechanism used to inhibit cell processes like replication or transcription. In this article, we show, using several experimental approaches (changes in the ultraviolet spectra, DNAse I digestion, and circular dichroism), that elsamicin A inhibits both the rate and extent of the B-to-Z transition in poly[d(G-C)] and poly[d(G-m^{5}C)] and can convert the Z-form of both polymers back to a right-handed DNA close to the B-form. These results agree with preferential binding of elsamicin to DNA in the B conformation.

MATERIALS AND METHODS

Elsamicin A (BMY-28090) was kindly provided by Bristol-Myers Squibb, Wallingford (U.S.A.). A working stock solution (500 μM) was freshly prepared in water. Poly[d(G-C)] and poly[d(G-m^{5}C)] were purchased from Pharmacia-Iberica and used without further purification. Polynucleotides were dissolved in an appropriate buffer (see below) by rotational mixing at 4 °C for about 16 h and then dialyzing the sample overnight against a large volume of the same buffer. The following buffers were used to dissolve the polynucleotides: PE buffer (for the unmethylated polymer) containing 25 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), pH 7.0, 50 mM NaCl, and 0.2 mM EDTA; P buffer (for the methylated sample) containing 25 mM PIPES, pH 7.0, and 50 mM NaCl. For all experiments, buffered solutions of suitable NaCl concentrations were used. Polynucleotide concentrations were determined using an extinction coefficient of 16 800 M^{-1} cm^{-1} at 254 nm for both polymers.

Kinetics of the B-to-Z Transition in Poly[d(G-C)]. Kinetic studies were performed in a Shimadzu UV-160A spectrophotometer, at 25 °C. The final polymer concentration was 50 μM (bp). The B-to-Z transition for poly[d(G-C)] was monitored by recording the difference in absorbance at 295 nm, as described previously (Chaires, 1983).

We have calculated the mean relaxation time (Bernasconi, 1973) to obtain a normalized initial rate of the transition, because of the length heterogeneity of the polynucleotides used:

$$1/\tau^* = (1/\Delta A^\circ_{295})(\mathrm{d}\,\Delta A_{295}/\mathrm{d}t)_{t\to 0} \qquad (1)$$

where $1/\tau^*$ is the mean relaxation time, ΔA_{295} the absorbance at time t, and ΔA°_{295} the overall change in absorbance between the initial and final states.

Reversal of the B-to-Z Transition in Poly[d(G-C)]. The transition was initiated as described above, but it was interrupted by the addition of elsamicin after 5 min. In all experiments, the same amount of drug was added to the reference solution.

DNase I Digestion Studies. DNase I digestions were monitored in a Beckman DU-70 spectrophotometer at 25 °C. Poly[d(G-C)] solutions were prepared in PE buffer, while solutions of poly[d(G-m^{5}C)] were prepared in P buffer. The same concentration of $MgCl_2$ (3 mM) was present in all solutions, and the final polymer concentration was 40 μM (bp). The reaction was initiated by the addition of DNase I (Sigma) to a final concentration of 9.2 μg/mL. The initial rates were determined from the linear portion of the time course (Chaires, 1985).

Circular Dichroism Measurements. Circular dichroism (CD) measurements were performed in 1-cm path length cells using a Jasco J-720 spectropolarimeter interfaced to a Hewlett-Packard (Vectra 386/25) computer. The temperature was kept constant (25 °C). The wavelengths chosen ranged from 240 to 320 nm. Scans were recorded at 1-nm intervals, and three spectra of each sample were recorded and averaged. A scan of buffer alone was recorded to provide a base line. The concentration of the polymers was 10 μM (in base pairs). Molar ellipticities were calculated in terms of the nucleotide concentration, in base pairs, according to

$$[\theta] = \theta/(10)(cl) \qquad (2)$$

where θ is the measured ellipticity (mdeg), c is the molar concentration of polymer in base pairs, and l is the path length in cm.

Circular dichroism was used to monitor the conformation of poly[d(G-C)] and poly[d(G-m^{5}C)] as a function of the drug added to DNA under B-form and Z-form conditions, following, with minor modifications, the method described elsewhere (Walker et al., 1985a). The fraction of the polynucleotide in the right-handed conformation may be calculated from plots of the molar ellipticity versus the ratio of moles of drug added per mole of polynucleotide (base pairs) using the equation:

$$\alpha = ([\theta]_Z - [\theta]_r)/([\theta]_Z - [\theta]_B) \qquad (3)$$

where $[\theta]_Z$ and $[\theta]_B$ are the molar ellipticities of Z- and B-form poly[d(G-C)] or poly[d(G-m^{5}C)] at 250 nm and $[\theta]_r$ is the apparent ellipticity at a particular ratio of antibiotic added per base pairs of the polynucleotide (r added).

RESULTS

The effect of elsamicin A on the poly[d(G-C)] conformation in the presence of high salt was studied by determining the change in absorbance at 295 nm. Absorbance versus time spectra are displayed in Figure 2. This kinetic analysis shows that both the rate and the extent of the B-form to Z-form transitions are clearly inhibited by elsamicin. Moreover (see Figure 3), the antibiotic converts the Z-form of poly[d(G-C)] back to the B conformation. When small amounts of antibiotic

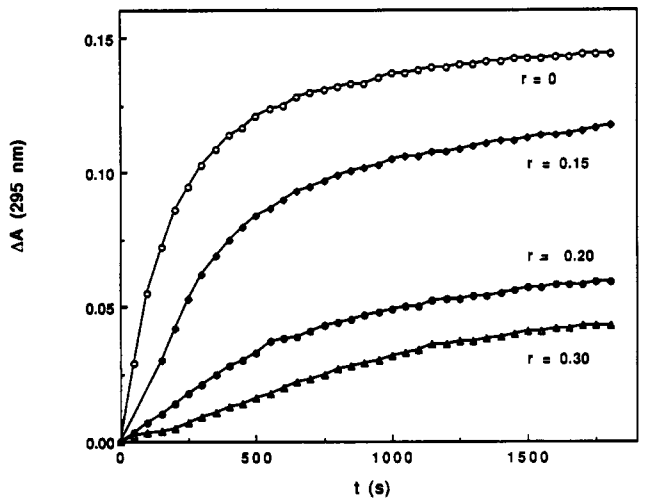

FIGURE 2: Kinetic analysis of the B-to-Z transition in the presence of different amounts of elsamicin A. The change in absorbance at 295 nm of a 50 μM poly[d(G-C)] solution containing 4 M NaCl relative to a reference solution containing 2 M NaCl. The reaction was initiated by adding poly[d(G-C)] to a buffered solution containing 4 M NaCl.

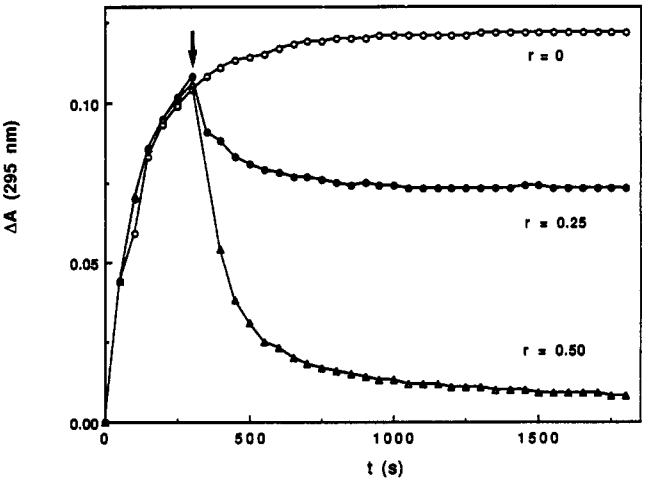

FIGURE 3: Reversal of the B-to-Z transition by elsamicin. The transition was initiated as described in Figure 2 but interrupted by adding different amounts of the antibiotic, at the point indicated by an arrow, using the ratios of added elsamicin (μM) to polynucleotide (μM in base pairs) indicated in the future.

Table I: Rate and Extent of the B-to-Z Transition in the Presence and Absence of Elsamicin A[a]

r added (elsamicin/ polynucleotide base pairs)	$1/\tau^*$ (s^{-1})	extent of relaxation (%)
0	4.16×10^{-3}	100
0.15	1.69×10^{-3}	82
0.20	1.10×10^{-3}	40
0.30	5.80×10^{-4}	30

[a] The mean relaxation time ($1/\tau^*$) for the B-to-Z transition was measured as described in the text. The extent of relaxation was calculated as $(\Delta A^\circ_{295})/(\Delta A^\circ_{295})_{r=0} \times 100$.

are added to the polynucleotide (i.e., low input elsamicin/base pair ratios), the transition is slowed, while as the ratio increases, the B-to-Z transition is completely reversed. Table I and Figure 4 display the effect of elsamicin on the rate of the B-to-Z transition either in the absence or in the presence of different amounts of added antibiotic. Results in Figure 4 are presented in a relative scale, $(1/\tau^*)/(1/\tau^*)_0$, to minimize any difference due to the molecular weight of the polymer samples used in the different experiments. For the sake of

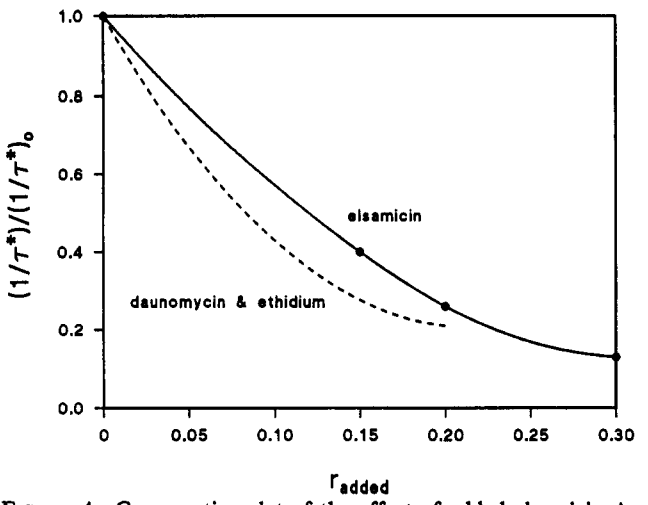

FIGURE 4: Comparative plot of the effect of added elsamicin A, daunomycin, and ethidium on the mean relaxation time ($1/\tau^*$) of the B-to-Z conformational transition as a function of the drug added per base pair of poly[d(G-C)]. For the sake of comparison the figure is presented in a relative scale $(1/\tau^*)/(1/\tau^*)_0$ to minimize any differences due to the molecular weight of the polymer samples used in the different experiments. The data plotted for elsamicin were calculated from Figure 2 and Table I. Data for daunomycin and ethidium are adapted from published values (Chaires, 1983).

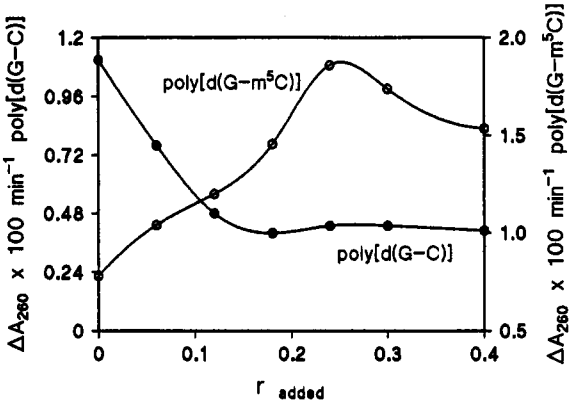

FIGURE 5: Effect of added elsamicin A in the initial rate of the DNase I cleavage of poly[d(G-C)] (closed circles) and poly[d(G-m^5C)] (open circles). The left and right ordinate axes refer to poly[d(G-C)] and poly[d(G-m^5C)], respectively.

comparison, the relative mean relaxation times $(1/\tau^*)/(1/\tau^*)_0$ are plotted as a function of the elsamicin added per base pair (r added), together with the results obtained for other drugs which are known to bind to DNA (Chaires, 1983). These plots might be taken as evidence that the elsamicin effect on the B-to-Z transition is somewhat greater than for daunomycin and ethidium.

Figure 5 displays the susceptibility of poly[d(G-C)] and poly[d(G-m^5C)] to DNase I cleavage as a function of added elsamicin. The rationale of this experiment is that the DNase I cleaving rate is susceptible to changes in the size of the minor groove (Drew & Travers, 1984) and it should not be able to cut DNA in the Z-form, in which the groove is deep and narrow, irrespective of the presence of the drug, except if a conformational change to a more susceptible DNA conformation takes place. While the addition of elsamicin to poly[d(G-C)], in 3.0 mM $MgCl_2$, decreases the susceptibility of the polynucleotide to DNase I cleavage, as it does in footprinting experiments with B-DNA (Salas & Portugal, 1991), the addition of low levels of the antibiotic increases the initial rate of digestion of poly[d(G-m^5C)], but when an r of about 0.25 is reached, the susceptibility of DNase I decreases,

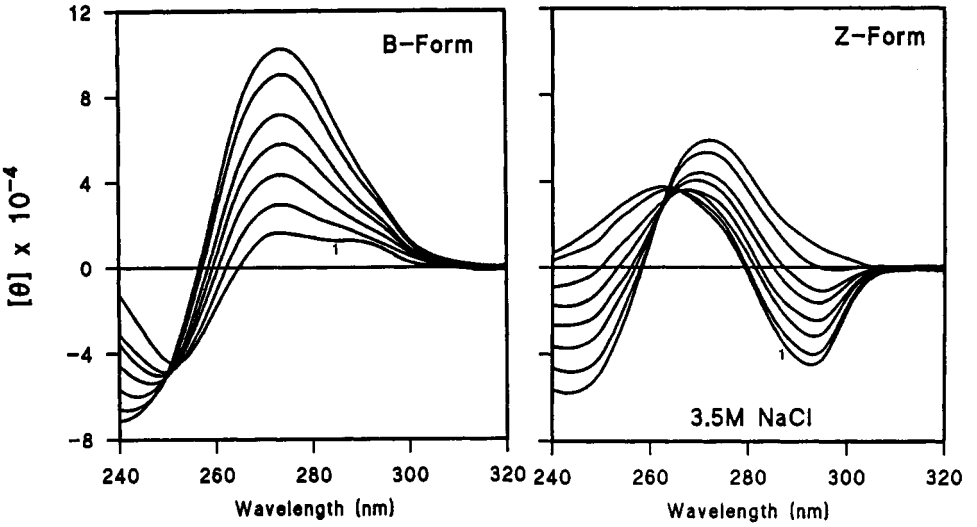

FIGURE 6: Circular dichroism spectra of elsamicin A–poly[d(G-C)] solutions under B-conformation (left panel) and Z-conformation (right panel) conditions. The initial spectrum of the polymer has been indicated (1), while the other spectra depicted correspond to a progressive titration of the polymer with added elsamicin A.

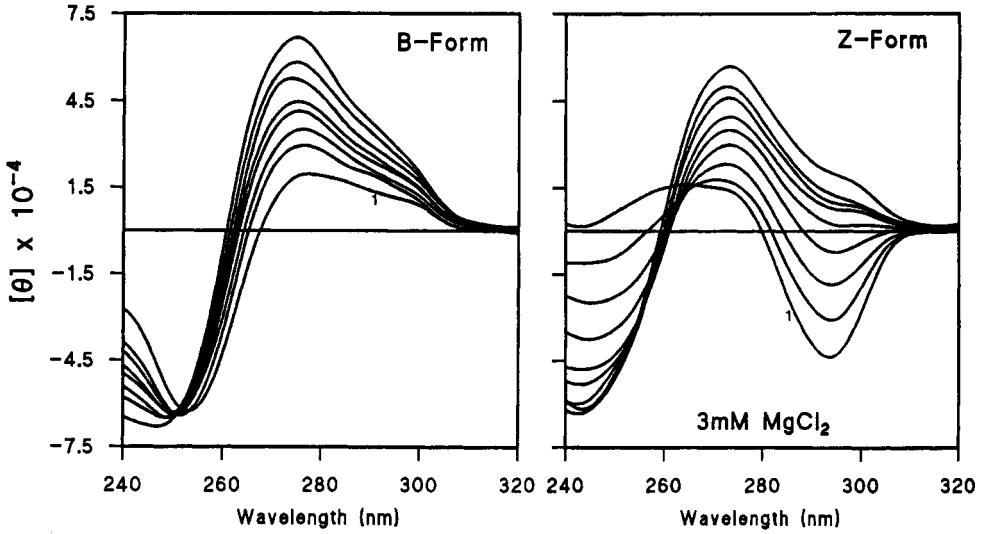

FIGURE 7: Circular dichroism spectra of elsamicin A–poly[d(G-m^{5}C)] solutions under B-conformation (left panel) and Z-conformation (right panel) conditions. In each panel, the initial spectrum of the polynucleotide has been indicated (1). Other details as in legend to Figure 6.

thus reflecting that a change in the minor groove (tentatively through a Z–B transition) is taking place, rendering the polynucleotide more susceptible to the enzyme, which is followed by the elsamicin-binding to the B-form and a subsequent protection. These results are fully consistent with those obtained with daunomycin under similar experimental conditions (Chaires, 1985) although some quantitative differences are evident, possibly due to the different binding affinities of daunomycin and elsamicin for the polynucleotides.

Unfortunately, the binding constants and the binding ratio of elsamicin to poly[d(G-C)] or poly[d(G-m^{5}C)] could not be determined unambiguously in either B-form or Z-form, since in fluorimetric titrations an erratic behavior of the complex was observed characterized by an abrupt decrease in the fluorescence emission followed by a moderate increase in the experimental values. These puzzling results may be related to microaggregation of the drug, in the absence of DNA, at antibiotic concentrations about 1 μM or lower. The presence of such microaggregation is already detected spectrophotometrically, thus preventing the correct determination of the free drug in the solution and, therefore, the ratio of bound elsamicin to DNA. Using poly[d(G-C)], Uesugi et al. have reported an apparent binding constant of about 9×10^4 M^{-1} (Uesugi et al., 1992). Because insufficient experimental details on data collection were given, the applicability of such a value for our polymers or/and experimental conditions is unknown.

To support our interpretation of the changes in polynucleotide conformation after drug binding, we performed circular dichroism experiments. The CD spectra obtained from poly[d(G-C)] titrations with elsamicin, under both B- and Z-form experimental conditions, are displayed in Figure 6, and those pertaining to poly[d(G-m^{5}C)] are in Figure 7. All undergo large conformational changes when the polynucleotides switch from Z- to B-form. The similarity of the elsamicin-saturated polynucleotide CD spectra regardless of the initial polymer conformation of the complexes is clearly evident, with some small differences reflecting small variation in the conformations of the antibiotic–DNA complexes possibly due to differences in buffer conditions. When elsamicin A was added to poly[d(G-C)] in P buffer containing 3.5 M NaCl, total B-to-Z reversion was only observed when r added reached about 0.6 (Figure 6), giving a spectrum which is analogous to the B conformation of the polynucleotide in the presence of antibiotic (cf. Figures 6 and 7).

The CD spectra show clear isoelliptic points at 250 nm upon antibiotic binding, providing a convenient wavelength

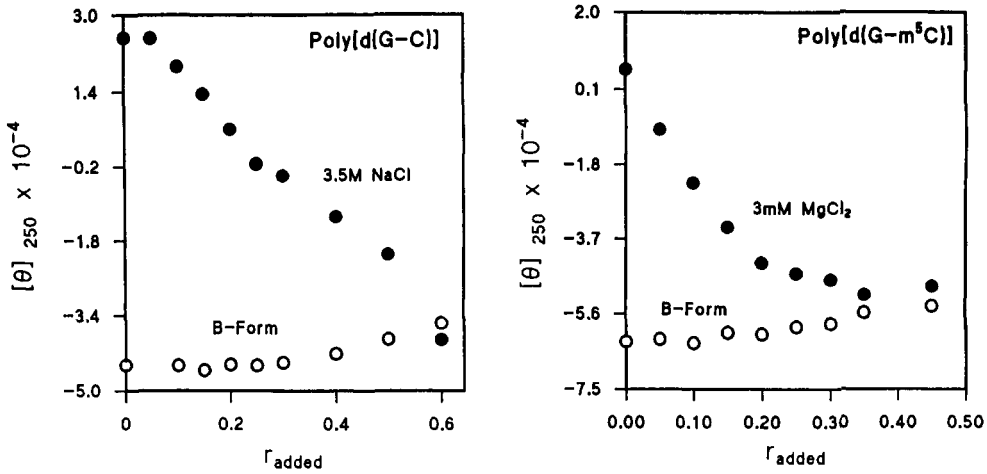

FIGURE 8: Plot of the molar ellipticity values at 250 nm as a function of different ratios of added elsamicin to poly[d(G-C)] (left panel) and poly[d(G-m^{5}C)] (right panel). The molar ellipticities were calculated from Figures 6 and 7.

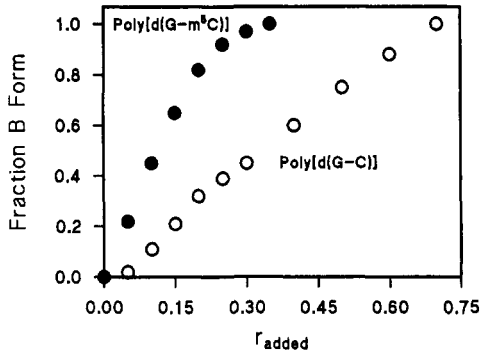

FIGURE 9: Plot of the fraction of poly[d(G-C)] and poly[d(G-m^{5}C)] in the B-form as a function of added elsamicin. The symbols refer to experimentally determined values obtained from Figure 8 as described in the main text.

at which to monitor B-to-Z transitions as elsamicin is added to the polynucleotides. Figure 8 displays plots of the molar ellipticity at 250 nm as a function of *r* added for the elsamicin titration of poly[d(G-C)] and poly[d(Gm^{5}C)] under B- and Z-conformation conditions. An elsamicin titration of Z-form poly[d(G-C)] in PE buffer containing 3.5 M NaCl produced an almost linear change at 250 nm as a function of added elsamicin. Addition of elsamicin to Z-form poly[d(G-C)] resulted (Figure 8) in a molar ellipticity versus *r* added plot which converged with the B-conformation data at an *r* added of approximately 0.6, indicating that the Z- and B-form conversion is completed at about 1 elsamicin added per 1.7 base pairs. On the other hand, addition of elsamicin to poly-[d(Gm^{5}C)] in the Z-form indicates (see Figure 8) that the conversion into a B-form is completed at about 1 antibiotic molecule added per 3.3 base pairs. The linearity of the data in the CD plots displayed in Figure 8 can be considered a good indication of a progressive conversion of Z-form of both poly-[d(G-C)] and poly[d(G-m^{5}C)] to a right-handed structure (Walker et al., 1985a). In fact, the linearity of the molar ellipticity data at 250 nm suggests that the polynucleotides consist of right-handed and left-handed regions without the presence of intermediates with alternate conformations, in clear agreement with the results on the B-to-Z transition described previously for other intercalating drugs (Chaires, 1986; Walker et al., 1985a,b). These plots can be used to calculate the fraction of right-handed helix as a function of added elsamicin using eq 3, and to infer the cooperativity of the B-to-Z transition (Chaires, 1986). Figure 9 displays the fraction of both polynucleotides in the B-form as a function of added elsamicin. If we consider that the DNA is in equilibrium between two conformations and that the antibiotic would bind to either conformational state with a characteristic affinity and cooperativity, the data agree with those found with other drugs (Chaires, 1986) to fit the allosteric model of Dattagupta et al. (1980), though we cannot use it to quantify parameters other than the fraction of polymer in B- or Z-forms because of the experimental drawbacks mentioned above, which thwarted the evaluation of the bound antibiotic to base pair ratio. The ionic strength dependence of the cooperative binding can be inferred from a comparison of the results displayed in Figure 8. An increase in ionic strength, which is needed for B–Z transition in poly[d(G-C)], results in a lower affinity of the drug for the B-form, as already observed if we compare the *r* added values where the data converged in the plots for poly[d(G-C)] and poly[d(G-m^{5}C)]. It is worth noting, by comparison of the left and right panels in Figure 8, the influence of the salt contribution to the number of bases converted per added elsamicin. Higher ionic strength is used to obtain a Z-form of poly[d(G-C)], resulting in a lower affinity of the drugs, and, of course, the B to Z equilibrium is also dependent upon salt concentration (Pohl & Jovin, 1984). From a qualitative point of view, the CD results presented here seem to agree with the "clustering model" of Krugh et al. (1987), in which left- and right-handed DNA exist together on the same polymer as two separate regions. There is a small difference in the amounts of the calculated B-form DNA after elsamicin titration of poly[d(G-C)], depending on whether we use the data from the kinetic studies in Figure 2 and Table I, or the CD experimental results depicted in Figures 6 and 9. However, they can be considered the same within the experimental error, and taking the slightly different salt concentration used in the two experimental approaches into consideration.

DISCUSSION

The results presented in this paper demonstrate that the antitumor antibiotic elsamicin A inhibits both the rate and extent of the B-to-Z transition of poly[d(G-C)] and poly-[d(G-m^{5}C)]. Elsamicin can convert Z-DNA back to an intercalated B-form, under all conditions which otherwise favor the Z-form. CD spectra similar to those presented in Figure 6 and 7 have been obtained with other DNA-binding ligands (Walker et al., 1985a; Krugh et al., 1987) and used to infer that the local environment of the polynucleotide at the binding site is a right-handed helix, which implies that the binding of the ligand would require the nucleation of two B (+drug)–Z

interfaces like for ethidium (Walker et al., 1985a). This interpretation agrees with the results reported in this paper, and it is substantiated by the DNase I experiments, which show that elsamicin A (Figure 4), like daunomycin (Chaires, 1985), binds to poly[d(G-C)] and poly[d(G-m^5C)], changing the size of the minor grooves through a B-to-Z conformational change.

Ethidium (Pohl et al., 1972; Krugh et al., 1987), actinomycin D (Walker et al., 1985b), and daunomycin (Chaires, 1986) have thus far shown cooperative binding effects which are similar to those presented in Figure 9 for elsamicin A, while proflavine binding does not show such a cooperative behavior (Pohl et al., 1972). At identical molar ratios of added drug per base pair the rate of the Z–B conversion is somewhat faster for elsamicin than for either ethidium or daunomycin (Table I and Figure 4). If, as has been recently shown, the size of the antibiotic is a crucial determinant of its efficiency as an inhibitor of the B-to-Z transition (Gilbert et al., 1991), these differences might be a direct consequence of the larger elsamicin site [about four base pairs (Párraga & Portugal, 1992)]. We have used both kinetic and CD experiments to determine the fraction of right-handed helix as a function of added elsamicin. Table I and Figure 9 show the fraction of B-form helix as a function of the added molar ratio of drug to DNA (base pairs). Although our data have to refer to added drug (and not to bound drug, for the reasons given above), it is worth mentioning that from a qualitative point of view there is a clear coincidence between our experimental data and those described elsewhere for different drugs (Chaires, 1986; Krugh et al., 1987), showing how an energetically unfavorable Z-to-B transition can be driven by coupling it to the energetically favorable interaction of elsamicin A with the B-form DNA. An allosteric model like the one described for ethidium and daunomycin (Chaires, 1986; Krugh et al., 1987) can also satisfactorily describe the elsamicin results. The model would allow us to describe, although only qualitatively, the sequential Z-to-B conformational change as the antibiotic binds. The location of the experimental points in the plot displayed in Figure 8, i.e., the shape of any curve that might be drawn on them, leads us to suggest that they would also fit the allosteric model of Dattgupta et al. (1980) [cf. how they nicely match with those reported for daunomycin (Chaires, 1986), though there is a difference since we represent r added in the x axes instead of r bound]. In any case, it is highly likely that the conversion of the polymer from a Z-form to a B-form is a sequential one, so elsamicin does not need to bind to Z-DNA, although a transient interaction cannot be ruled out. So far, this is the mot coherent interpretation of the linearity observed in the plots of the molar ellipticity versus r added displayed in Figure 8, from which we calculated the fraction of polymer in B-form after the addition of elsamicin.

Elsamicin A binds to alternating cytosine plus guanine sequences (Salas & Portugal, 1991; Párraga & Portugal, 1992), and it is an effective inhibitor of the B-to-Z transition and an effector of the Z-to-B conformational change. If, as is generally reckoned, Z-DNA plays a role in the control of the cell processes, our results suggest a potential mechanism by which the antibiotic might inhibit replicative events and gene transcription, probably leading to its usefulness as an antitumor antibiotic.

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