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Inhibition of the Thermally Driven B to Z Transition by Intercalating Drugs†

Jonathan B. Chaires
Department of Biochemistry, The University of Mississippi Medical Center, Jackson, Mississippi 39216-4505
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ABSTRACT: Poly(dG-m^{5}dC) in phosphate buffer containing 50 mM NaCl and Mg^{2+} will undergo a reversible thermally driven conversion from the B to the left-handed Z conformation. The temperature at the midpoint of the thermally driven B to Z transition (denoted T_z) is dependent upon the total Mg^{2+} concentration, with $[d(1/T_z)]/(d \ln [Mg]) = 0.0134\ K^{-1}$. The Mg^{2+} concentration at the midpoint of the equilibrium B to Z transition curve, denoted $[Mg]_{1/2}$, is dependent on temperature, with $(d \ln [Mg]_{1/2})/(d \ln T) = -1.02$. Binding of the anticancer drug daunomycin to the polymer results in a pronounced increase in T_z, dependent on the molar ratio of added drug. T_z is increased by 71.9 °C with nearly saturating amounts of drug bound. Transition profiles are biphasic at less than saturating amounts of bound drug. By experiments monitoring such biphasic curves at a visible wavelength sensitive to the binding of daunomycin, it may be demonstrated that no drug is released until the later phase of the transition. These results are analogous to the effects of intercalating drugs on the thermal denaturation of DNA and indicate that drug molecules preferentially interact with B-form DNA and are redistributed to regions in the B conformation over the course of the transition. Comparative studies show that some intercalators stabilize right-handed DNA more effectively than others. At similar initial binding ratios, the following order, from most to least effective, was experimentally observed: actinomycin > daunomycin > ethidium > proflavin.

The transition of DNA from the right-handed B to the left-handed Z conformation is of current interest, both as a striking example of DNA polymorphism and as a potential mechanism for gene regulation (Rich et al., 1984). Intercalators profoundly affect the stability of Z DNA and will, in general, inhibit the formation of Z DNA. Certain intercalators have been demonstrated to act as allosteric effectors on DNA conformation and will convert Z DNA to an intercalated right-handed form under solution conditions that would otherwise favor the Z form in the absence of the intercalator (Pohl & Jovin, 1972; Walker et al., 1985a,b; Chaires, 1985b, 1986). Mirau and Kearns (1983) noted striking quantitative differences in the ability of a variety of intercalators to inhibit the rate of the B to Z transition. The molecular details of the mechanism by which intercalators inhibit the B to Z transition and the origins of the observed quantitative differences among intercalators remain incompletely described.

The data presented here show that daunomycin and other intercalators inhibit the reversible thermally driven B to Z transition in poly(dG-m^{5}dC). Daunomycin increases the temperature at the midpoint of the transition in proportion to the amount of added drug. Below saturating drug binding ratios, transition profiles are biphasic and drug is not released until late in the transitions. These effects are entirely analogous to the effect of intercalators on the thermal denaturation of DNA, as explained by Crothers (1971) and McGhee (1976). Comparative studies show that intercalators vary in the effectiveness with which they stabilize the right-handed form of poly(dG-m^{5}dC). The method used thus provides additional insight into the mechanism by which intercalators inhibit the B to Z transition and provides a convenient means of assessing quantitative differences among intercalators.

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Materials and Methods

Polynucleotides. Poly(dG-m^{5}dC) was purchased from Pharmacia (Milwaukee, WI) and used without further purification. Samples were dialyzed against BP buffer, consisting of 2 mM NaH_2PO_4, 6 mM Na_2HPO_4, and 50 mM NaCl, pH 7.0. A molar extinction coefficient of 16 800 M^{-1} cm^{-1} was used to calculate polymer concentration.

Equilibrium Mg^{2+} Titration Experiments. Ultraviolet absorbance spectra of poly(dG-m^{5}dC) in BP buffer were recorded in a Cary 219 spectrophotometer as a function of added Mg^{2+} concentration. Samples were thermostated, and the temperature was controlled by a Neslab circulating water bath. Following each addition of Mg^{2+}, absorbance at 300 nm was monitored continuously with time to establish that equilibrium was reached. The complete ultraviolet absorbance spectrum was then recorded. The fraction of Z form θ was calculated by

$$\theta = (A_{300} - A^0_{300})/(A^F_{300} - A^0_{300})$$

where A^0_{300} is the absorbance at 300 nm in the absence of Mg, A_{300} the absorbance in the presence of a given concentration of Mg, and A^F_{300} the maximal absorbance at 300 nm upon total

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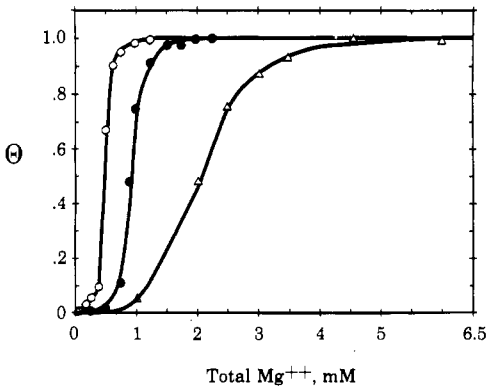

FIGURE 1: Equilibrium transition curves for the B to Z conversion of poly(dG-m^{5}dC). The fraction of the polymer in the Z form, determined from absorbance measurements as described in the text, is shown as a function of the total Mg concentration at 40 (△), 25 (●), and 10 °C (○). Solution conditions were 6.0 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 50 mM NaCl, pH 7.0.

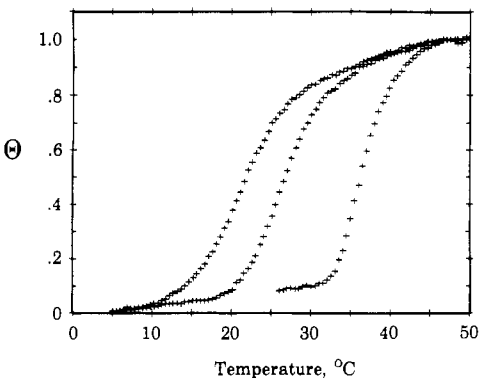

FIGURE 2: Thermal transition profiles for the B to Z conversion in poly(dG-m^{5}dC). Data from three selected total Mg concentrations are shown, corresponding to (from left to right) 2.0, 1.0, and 0.5 mM. A heating rate of 0.1 K min^{-1} was used for these transitions.

conversion of the polymer to the Z form.

Thermal Transition Experiments. The thermally driven B to Z transition was measured by absorbance with a Cary 219 spectrophotometer equipped with a temperature readout accessory and cell programmer, and thermostated by a Neslab circulating water bath controlled by an ETP-4RC temperature programmer. Typically, Mg^{2+} was added to poly(dG-m^{5}dC) solutions [40 μM [base pairs (bp)] in BP buffer] to reach the desired concentration. Samples were heated at 0.25 K min^{-1}, and the absorbance at 300 nm was continuously recorded, while the chart x axis was driven with the "chart" utility of the temperature readout accessory. Data were digitized, and the derivative dA/dT °C was calculated with facilities available through the NIH PROPHET Computer Resource as previously described (Chaires et al., 1983).

Drug Binding. The binding of daunomycin was measured by absorbance and fluorescence methods as previously described in detail (Chaires et al., 1982; Chaires, 1983).

Results and Discussion

Poly(dG-m^{5}dC) undergoes a thermally driven conversion from the B to the Z form in phosphate buffer containing 50 mM NaCl and Mg (Roy & Miles, 1982). Recent studies using differential scanning calorimetry (Chaires & Sturtevant, 1986) have further characterized this transition. The calorimetric enthalpy was found to be small, with $\Delta H_{cal} = 0.61$ kcal (mol of bp)$^{-1}$, but the large size of the cooperative unit, 110 bp, results in an appreciable van't Hoff enthalpy of 68 kcal mol^{-1} to drive the transition. The results presented here provide additional fundamental characterization of the transition and show that intercalators stabilize the right-handed form of poly(dG-m^{5}dC), thus increasing the temperature at the midpoint of the transition.

Figure 1 shows the effect of temperature on the equilibrium transition curves for the B to Z transition in poly(dG-m^{5}dC). Increasing temperature results in a decrease in the Mg^{2+} concentration at the midpoint of the transition. The dependence of the logarithm of $[Mg]_{1/2}$ on the logarithm of the temperature T is linear and is described by

$$\ln [Mg]_{1/2} = -3.85 - 1.02 \ln T$$

This relation may be used to predict at 5 °C a value of $[Mg]_{1/2} = 4.1$ mM, in excellent agreement with the value of 4.0 mM determined at the same temperature by Roy and Miles (1983).

Figure 2 shows sample temperature transition profiles for the conversion of poly(dG-m^{5}dC) from the B to the Z conformation. A single transition is observed for the sample used in these studies regardless of the wavelength chosen to monitor the reaction. The midpoint of the thermally driven transition, which is denoted T_z, is dependent upon the total Mg^{2+} concentration. T_z values were determined for 12 Mg^{2+} concentrations over the range 0.5–3.0 mM. The data are described by

$$1/T_z = 0.135 + 0.013 \ln [Mg^{2+}]$$

with a linear correlation coefficient of 0.999. This result indicates that the B to Z transition is coupled to Mg^{2+} binding and suggests that there is a preferential interaction of Mg^{2+} with the Z form. Increasing Mg^{2+} concentration thus decreases T_z.

The transition seen in Figure 2 is thermodynamically reversible by the following criteria. If poly(dG-m^{5}dC) samples are cooled following their transition to the Z form, absorbance and circular dichroic spectra slowly revert to show spectra characteristic of the right-handed B form of the polymer. If these samples are then rerun in an experiment such as that described in Figure 2, the observed transition profiles are superimposable upon the original experimental traces. This latter observation is the usual criterion normally applied to establish thermodynamic reversibility in differential scanning calorimetry experiments (Manly et al., 1985). Because the B to Z transition is slow, however, T_z is dependent on the heating rate used in an experiment. For example, a decrease in the heating rate from 1.0 to 0.1 K min^{-1} results in a 10.6 °C decrease in the apparent T_z. Transition profiles obtained by the stepwise increase in temperature, allowing 60 min for equilibration at each step, yield T_z values that are ca. 5 °C lower than those obtained by continuous heating at 0.1 K min^{-1}. Most of the experiments described here used a heating rate of 0.1 K min^{-1}.

Figure 3 shows the effect of daunomycin on the temperature-driven transition of poly(dG-m^{5}dC) from the B to the Z form. There are two noticeable effects. First, addition of the drug increases the midpoint of the transition, as shown in Figure 3A. Figure 4 documents the change in T_z (arbitrarily defined in the case of biphasic transition curves as the temperature at which the absorbance is half-maximal) with the molar ratio of added drug. Second, addition of daunomycin results in more complex, biphasic transition curves. Figure 3B shows data obtained by using two wavelengths to monitor the transition, one of them specific for daunomycin. Absorbance at 480 nm does not increase until late in the transition, indicating that daunomycin is not released until late in the transition. Both of these effects are entirely analogous to the effect of intercalators on the thermal denaturation of DNA, and it is therefore reasonable to assume that a similar mech-

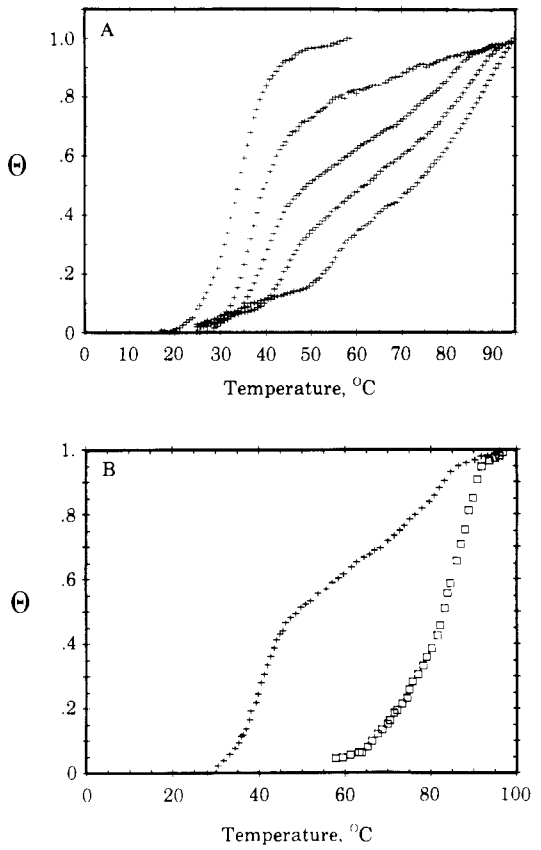

FIGURE 3: Thermal transition profiles for the B to Z conversion of poly(dG-m^{5}dC) in the presence of daunomycin. (A) Transition monitored at 300 nm in the presence of the following molar ratios of added daunomycin (DM) (left to right): 0, 0.04, 0.098, 0.137, and 0.196 mol of DM/mol of bp. (B) Transition profile in the presence of 0.098 mol of DM/mol of bp monitored at 300 nm (+) and at 480 nm (box). The latter wavelength is sensitive to drug binding, and the data indicate that no drug is released until the temperature is above 60 °C. The solution conditions for these experiments were 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 50 mM NaCl, and 1.0 mM *free* $MgCl_2$, pH 7.0. The heating rate was 0.25 K min^{-1}, and the total polymer concentration was 42.2 μM (bp).

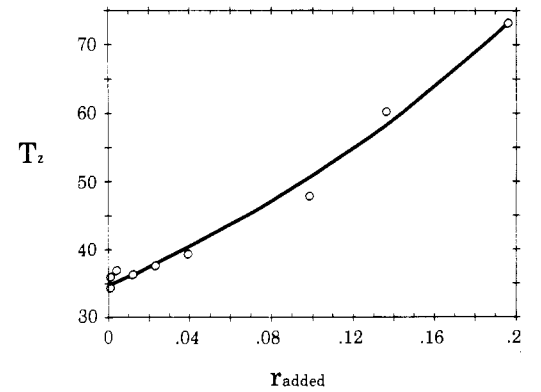

FIGURE 4: Temperature at the midpoint of the B to Z transition (T_z) as a function of the molar ratio of added daunomycin.

anism is operative. Crothers (1971) and McGhee (1976) have both proposed statistical mechanical models to account for ligand effects on DNA melting. Biphasic transitions are predicted to occur from these models and arise from the redistribution of ligand into regions of the polymer in the conformation with higher affinity for the ligand. That intercalators partition into regions in the right-handed conformation was previously suggested from the analysis of cooperative binding isotherms resulting from the interaction of drugs with Z DNA (Chaires, 1986; Walker et al., 1985a,b). The data of Figure 3B are entirely consistent with that suggestion.

The following equation describes the effect of reversible ligand binding on the DNA helix to coil transition:

$$\delta T_m^{-1} = 1/T_m^0 - 1/T_m = (R/\Delta H)[\ln (1 + KL)^{1/n}] \quad (1)$$

where T_m and T_m^0 are the transition midpoints in the presence and absence of ligand, respectively, R is the gas constant, ΔH is the enthalpy change for the transition of a base pair from the helix to the coil, K is the intrinsic ligand association constant at T_m, n is the exclusion parameter for the ligand–DNA interaction, and L is the free ligand concentration at T_m (Crothers, 1971; McGhee, 1976; Lazurkin et al., 1970). The theory assumes that the ligand–DNA interaction is rapid relative to the DNA transition so that the binding reaction is at equilibrium over the course of the transition. As written, the equation assumes negligible interaction of the ligand with the coil form. Equation 1 ought to describe the analogous effect of ligand binding on the temperature-dependent B to Z isomerization with appropriate redefinition of the parameters. This was experimentally tested as follows. Poly(dG-m^{5}dC) has T_z = 31.8 °C in the absence of daunomycin and T_z = 103.7 °C in the presence of the drug at a binding ratio of 0.353, with a heating rate of 0.1 K min^{-1} used in both cases. In the latter case, the total drug concentration was 25.8 μM, distributed initially at 20 °C as 2.3 μM free drug and 23.5 μM bound drug. Under these solution conditions, the interaction of daunomycin with the polymer is characterized at 20 °C by $K = 8.6 \times 10^6$ M^{-1} and n = 2.6 bp, as determined by absorbance and fluorescence titration experiments and analysis according to eq 10 of McGhee and von Hippel (1974). Assuming an enthalpy for the drug–polymer interaction of −19 kcal mol^{-1} (Chaires, 1985a), the equilibrium constant at T_z = 103.7 °C is estimated to be 2.5×10^4. The quantity KL may then be calculated and substituted into eq 1 by further assuming that L = 25.8 μM near the T_z. The enthalpy for the transition of a base pair from the B to the Z conformation has been determined calorimetrically to be 0.6 kcal mol^{-1} under the same solution conditions used here (Chaires & Sturtevant, 1986), and n is known from the titration experiments at 20 °C. With these values, eq 1 may be used to calculate a predicted value of $\delta T_m^{-1} = 0.6 \times 10^{-3}$, a value in good agreement with the experimentally observed value of $\delta T_m^{-1} = 0.61 \times 10^{-3}$. The agreement is good considering the assumptions made in the estimation of KL and the uncertainty in the enthalpy value for the drug–polymer interaction under these solution conditions. The calculation serves to reinforce the analogy between the effect of intercalation on DNA melting and on the B to Z transition and suggests that a similar underlying mechanism is operative in both cases.

The effect of a variety of intercalators on the thermally driven B to Z transition is summarized in Table I. Different intercalators stabilize the right-handed form to differing extents, following the order (from most to least effective) actinomycin > daunomycin > ethidium > proflavin. Differences between compounds are striking. At the same ratio of bound drug, for example, actinomycin increases T_z nearly twice as much as ethidium. Mirau and Kearns found the same order of effectiveness (with the exception of daunomycin, which was not studied) with respect to the ability of intercalators to inhibit the initial rate of the B to Z transition in poly(dG-dC) (Mirau & Kearns, 1983). They emphasized that intercalators with the slowest dissociation rates were the most effective inhibitors of the B to Z transition and proposed that the kinetics of the drug–polymer interaction, rather than the affinity of the drug for DNA, was the prime determinant of inhibition. While a similar correlation could be made by using the data of Table

Table I: Comparison of the Effect of Various Intercalators on the Thermally Driven B to Z Transition in Poly(dG-m^{5}dC)

compound	ΔT_z[a] (°C)	K (10^6 M^{-1})	n (bp)
actinomycin[b]	80	14.0	3.0
daunomycin[c]	53.0	8.6	3.0
ethidium bromide[d]	41.7	1.6	2.0
proflavin[c]	36.7	2.0	2.0

[a] ΔT_z is the difference between the observed T_z in the presence of 0.2 mol (mol of bp)$^{-1}$ *bound* intercalator and T_z^0 = 31.8 °C, the midpoint of the transition in the absence of intercalator. Solution conditions were 2 mM NaH_2PO_4, 6 mM Na_2HPO_4, 50 mM NaCl, and 1 mM free $MgCl_2$, pH 7.0. A heating rate of 0.1 K min^{-1} was used. The value for actinomycin is the lower limit, since the optical methods used could not be used above 110 °C and the transition was incomplete at that temperature. [b] Values for K and n for the interaction of actinomycin with B-form poly(dG-dC) in 0.1 M Na^+ as reported by Winkle and Krugh (1981). [c] K and n determined for the interaction of drug with B-form poly(dG-m^{5}dC) in 50 mM NaCl by fluorescence titration in this laboratory. [d] K and n estimated for the interaction of ethidium with B-form poly(dG-m^{5}dC) in 50 mM NaCl by using the data of Walker et al. (1985a).

I, other factors clearly are of importance. In particular, the association constant and the exclusion parameters appear in eq 1 and directly influence the magnitude of the T_z shift. The data of Table I show that the compounds with the highest binding constants, actinomycin and daunomycin, are more effective in stabilizing B DNA than are the simple intercalators ethidium and proflavin. For the limited data in Table I, there is a fair linear correlation (correlation coefficient = 0.979) between the magnitude of ΔT_z and the binding constant K.

Conclusions

Intercalating drugs inhibit the thermally driven B to Z transition in poly(dG-m^{5}dC). The effect of intercalators on the B to Z transition seems analogous to their effect on the helix–coil transition in DNA, and it is therefore reasonable to assume that both phenomena are governed by similar underlying mechanisms. In both cases, intercalators increase the temperature at the midpoint of the transition and transition profiles are often biphasic at less than saturating binding ratios. In the experiments presented here, biphasic profiles must arise from preferential intercalator binding to right-handed DNA and from the redistribution of ligand to regions in the right-handed conformation over the course of the B to Z transition. Different intercalators stabilize right-handed DNA to differing extents, primarily on the basis of their affinity toward right-handed DNA. In the present study, the order (from most to least effective) actinomycin > daunomycin > ethidium > proflavin was experimentally established.

Registry No. Mg, 7439-95-4; poly(dG-m^{5}dC), 51853-63-5; actinomycin, 1402-38-6; daunomycin, 20830-81-3; ethidium bromide, 1239-45-8; proflavin, 92-62-6.

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