be achieved when all of these factors are taken into consideration.

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Base- and Sequence-Dependent Binding of Aristololactam β-D-Glucoside to Deoxyribonucleic Acid

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ABSTRACT: The dependence on base-pair composition and sequence specificity of the (aristololactam β-D-glucoside)–DNA interaction was examined by spectrophotometric, spectrofluorometric, spectropolarimetric, thermal melting, thermodynamic, and viscometric studies. Binding of this alkaloid to various natural and synthetic DNAs was dependent upon the base composition and sequences of DNA. The binding parameters obtained from spectrophotometric analysis, according to an excluded-site model, indicated a relatively high affinity of the alkaloid binding to GC-rich DNA and alternating GC polymer. This affinity was further evidenced by the quenching of fluorescence intensity, decrease in quantum yield, and perturbations in circular dichroic spectrum. The alkaloid stabilized all DNAs against thermal denaturation. The temperature dependence of the binding constants was used to estimate the thermodynamic parameters involved in the complex formation of the alkaloid with various DNAs. The negative enthalpy and entropy change increased with increasing GC content of DNA and also compensated one another to produce a relatively small Gibbs free energy change. Viscometric studies showed that in the strong binding region the increase of contour length of DNA depended strongly on its base composition and sequence of bases, being larger for GC-rich DNA and alternating GC polymer. On the basis of these observations, it is concluded that the alkaloid binds to DNA by a mechanism of intercalation and exhibits considerable specificity toward alternating GC polymer.

During recent years there have been great advances in elucidating the factors that govern the affinity and specificity of binding of many naturally occurring and synthetic compounds to DNA. One important class of these compounds comprises those that bind to DNA by a mechanism of intercalation. Such compounds are important tools in molecular biology, and some are used for the treatment of cancer in man (Waring, 1981a,b; Wilson & Jones, 1982; Neidle & Waring, 1983; Neidle et al., 1987; Wilson, 1989).

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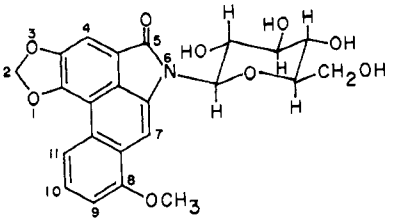

FIGURE 1: Chemical structure of aristololactam β-D-glucoside.

The *Aristolochia* group of alkaloids and their glucoside derivatives have attracted recent attention for their prospective clinical and pharmacological uses [for reviews, see Chen and Zhu (1987) and Cassady et al. (1990)]. Among this group of alkaloids, aristololactam β-D-glucoside (ADG), or 6-β-D-glucopyranosyl-8-methoxybenzo(*f*)-1,3-benzodioxolo[6,5,4-*cd*]indol-5(6*H*)-one (Figure 1), a phenanthrenic lactam derivative, is of particular interest to us for its attached glucoside ring. Recently it has been shown that (i) ADG has pH-dependent spectral changes from the standpoint of its absorption and fluorescence spectra (Chakraborty et al., 1989a), (ii) it binds to DNA by intercalation (Chakraborty et al., 1989b, 1990), (iii) it exists as a monovalent cationic ligand at neutral pH (Chakraborty et al., 1990), and (iv) the interaction has a large favorable nonelectrostatic part (Chakraborty et al., 1990), but the molecular nature of its interaction with DNA is obscure.

In order to gain some insight into the molecular nature of its specificity toward sequences of base pairs, we have carried out a series of physicochemical measurements on the interaction of ADG with several natural and synthetic DNAs of varying base composition and sequence.

Materials and Methods

Clostridium perfringens (CP) DNA (Type XII, 30 mol % GC,) calf thymus (CT) DNA (Type I, 42 mol % GC), *Escherichia coli* (EC) strain B DNA (Type VII, 50 mol % GC), *Micrococcus lysodeikticus* (ML) DNA (Type XI, 72 mol % GC), poly[d(G-C)]·poly[d(G-C)], poly[d(A-T)]·poly[d(A-T)], poly(dG)·poly(dC), and poly(dA)·poly(dT) were obtained from Sigma Chemical Co. (St. Louis, MO) and were tested for their nativeness and purity (Maiti, 1986; Maiti & Nandi, 1987a,b; Debnath et al., 1989). Each natural DNA exhibited a characteristic ultraviolet absorption spectrum with an A_{260}/A_{280} ratio between 1.88 and 1.93 and an A_{260}/A_{230} ratio between 2.12 and 2.22. The thermal melting temperatures (T_m) in 0.1× SSC (0.15 M NaCl + 0.015 M sodium citrate, pH 7.0) were 84, 74, 71, and 66.2 °C for ML, EC, CT, and CP DNA, respectively, with hyperchromicity varying from 30 to 37%. All polymers exhibited B-form structure as evidenced from circular dichroic (CD) spectra. Each polymer concentration was determined spectrophotometrically by using the molar extinction coefficients reported earlier (Nandi, 1986; Debnath et al., 1989).

ADG was extracted from *Aristolochia indica* and crystallized from ethanol. Its purity was checked by various physicochemical techniques (Chakraborty et al., 1989a,b). The concentrations of ADG were determined spectrophotometrically by using a molar extinction coefficient (ϵ) of 10930 M^{-1} cm^{-1} at 398 nm in dimethyl sulfoxide (DMSO). Except for T_m experiments, all DNA binding experiments were performed in a buffer, BPES-DMSO (20 mM Na^+), pH 6.9, containing 1.5 mM Na_2HPO_4, 0.5 mM NaH_2PO_4, 0.25 mM EDTA, 16 mM NaCl, and 240 mM DMSO. T_m measurements were conducted in BPE-DMSO (4 mM Na^+) buffer, pH 6.9, containing 1.5 mM Na_2HPO_4, 0.25 mM EDTA, and 240 mM DMSO. Glass-distilled deionized water and analytical-grade reagents were used throughout.

Single-stranded DNA was prepared by heating the duplex DNA samples at 98 °C for 10 min in the presence of formaldehyde (0.333 M) followed by rapid cooling as described (Maiti et al., 1984). Heat-denatured DNA was prepared in like fashion, omitting formaldehyde treatment. This heat-denatured DNA sample had approximately 15% residual double-stranded regions as evidenced from the measurements of absorbances at 260 nm.

Absorption and fluorescence spectra of ADG, either alone or mixed with various natural and synthetic DNAs, were obtained at 25 °C in a quartz cell of 1-cm path length by using a Shimadzu Model UV-260 automatic recording spectrophotometer and a Shimadzu spectrofluorometer RF-540 equipped with recorder DR-3 (Shimadzu Corporation, Japan), respectively, according to methods described earlier (Nandi et al., 1985, 1990). Uncorrected fluorescence spectra were reported. Quantum yields were calculated according to the equation of Parker and Rees (1960) as described in Maiti et al. (1983).

Spectrophotometric titration data were fitted to a theoretical curve, which was drawn according to the excluded-site model (Crothers, 1968) developed by McGhee and von Hippel (1974) as described earlier (Nandi & Maiti, 1985; Nandi et al., 1985):

$$r/C = K(1 - nr)[(1 - nr)/1 - (n - 1)r]^{n-1} \qquad (1)$$

where r is moles of alkaloid bound per mole of nucleotide, C is the molar concentration of free alkaloid, K is the binding constant to an isolated DNA binding site, and n is the number of nucleotides occluded after binding of a single alkaloid molecule. This program generated values of K and n for each data set. The analysis of a ln K vs $1/T$ plot obtained over the temperature range of the study enabled the calculation of ΔH, as the gradient was equal to $-\Delta H/R$. ΔG and ΔS were calculated in turn from the relationships $\Delta G = -RT \ln K$ and $\Delta G = \Delta H - T\Delta S$ as described in Chakraborty et al. (1990).

Melting profiles of DNA and ADG–DNA complexes were recorded on the Shimadzu UV-260 unit equipped with a temperature programmer (KPC-5) and a temperature controller (SPR-5) in a quartz cell of 1-cm path length; the change in absorbance of the DNA solution was monitored at the absorption maximum of natural and synthetic DNAs keeping a heating rate of 1 °C/min.

CD spectra were recorded at 25 °C on a Jasco J-20A spectropolarimeter equipped with a data processor attachment, Model J-DPY (Japan Spectroscopic Ltd., Japan), in a cylindrical quartz cell of 1-cm path length as described earlier (Maiti & Nandi, 1987a). The CD unit was routinely calibrated by using a solution of *d*-10-camphorsulfonic acid in water. The molar ellipticity [θ] was expressed in deg cm^2 $dmol^{-1}$. The CD spectra reported were averages of 2–4 scans.

For viscometric experiments, samples of linear duplex native DNAs were sonicated in a Labsonic 2000 sonicator (B. Braun Swiss) by using a needle probe of 4-mm diameter as described in Maiti et al. (1982, 1984). After sonication, the native DNA solution was extensively dialyzed under sterile conditions. The sonicated DNA samples had a molecular weight of the order of (2–3.5) × 10^5 (Nandi, 1986). Synthetic polynucleotides were used as such without sonication. Viscometric measurements were performed with a Cannon Manning Type 75 semimicroviscometer, mounted vertically in a constant-temperature bath (Cannon Instruments Co., State College, PA) maintained at 25 ± 0.05 °C. Flow times of DNA alone and DNA–alkaloid complexes were measured by an electronic stop watch (Fisher Scientific, Pittsburgh, PA) with an accuracy

Table I: Binding Parameters for the Interaction of ADG with Various Natural and Synthetic DNAs[a]

polymer	GC mol %	K ($\times 10^5$ M^{-1})	n[b]	α
CP DNA	30	1.7 ± 0.2	7.1 ± 0.1	
CT DNA	42	2.6 ± 0.2	6.1 ± 0.15	1.56[c]
EC DNA	50	2.8 ± 0.2	6.1 ± 0.10	1.65[c]
ML DNA	72	3.8 ± 0.2	5.9 ± 0.10	2.24[c]
heat-denatured CT DNA	42	0.55 ± 0.015	13.0 ± 0.2	
single-stranded CT DNA	42	0	0	
poly[d(G-C)]·poly[d(G-C)]	100	4.5 ± 0.25	4.2 ± 0.08	2.25[d]
poly[d(A-T)]·poly[d(A-T)]	0	2.0 ± 0.10	4.4 ± 0.05	
poly(dG)·poly(dC)	100	1.3 ± 0.1	6.0 ± 0.14	
poly(dA)·poly(dT)	0	0	0	

[a] Five determinations each. [b] n is the number of nucleotides occluded after binding of a single alkaloid molecule. [c] The ratio is based on the equation $\alpha = K_{DNA}/K_{CP\ DNA}$ calculated from the results as described in Nandi & Maiti (1985). [d] The ratio is based on the equation $\alpha = K_{DNA}/K_{alternating\ AT\ polymer}$.

of 0.01 s. The increase in helix length of sheared DNA and synthetic polynucleotides was calculated from the experimental data, which were transformed directly from flow times to values by using the expression

$$L/L_0 = \left(\frac{t_2 - t_0}{t_1 - t_0}\right)^{1/3} = 1 + \beta r \qquad (2)$$

where L is the contour length in presence of the alkaloid, L_0 is the contour length of free DNA, t_2 is the flow time for the complex, t_1 is the flow time for pure DNA, t_0 is the flow time for buffer at a given volume in the viscometer, and β is the slope when L/L_0 is plotted against r. The expression was derived directly from the theory of Cohen and Eisenberg (1969) with the added assumption that the intrinsic viscosity approximated the reduced viscosity for the complex as described earlier (Maiti et al., 1982).

Results

Absorption Spectra of the ADG–DNA Complex. The effect of progressively increasing concentrations of ML DNA and poly[d(G-C)]·poly[d(G-C)] on the absorption spectrum of ADG is illustrated in Figure S1 (supplementary material). The spectral changes essentially involved a gradual red shift and hypochromicity in the complex until saturation was reached. Similar and identical features in the spectral changes were observed with all other natural and synthetic DNAs except poly(dA)·poly(dT), where the quenching effect was only marginal.

The absorption spectra of ADG in presence of heat-denatured CT DNA and single-stranded CT DNA were done (data not shown). Single-stranded DNA did not produce any hypochromic effect on the absorption spectrum of ADG, while heat-denatured DNA produced a smaller hypochromic effect, suggesting the limited interaction between the alkaloid and the hairpin helical segments of heat-denatured DNA. It was observed that formaldehyde had no effect on either the alkaloid or native DNA or the ADG–DNA complex.

Evaluation of Binding Parameters. The results of absorption titration were expressed in the form of Scatchard plots and were analyzed according to an excluded-site model (Crothers, 1968; McGhee & von Hippel, 1974). The binding isotherms of ADG to various natural DNAs and synthetic polynucleotides are illustrated in Figure 2. The quantitative data of the binding parameters presented in Table I indicated

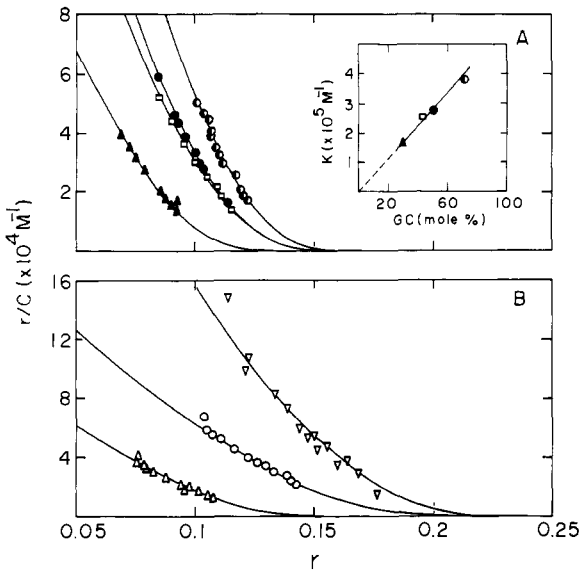

FIGURE 2: Representative Scatchard plots for ADG binding to various DNAs in BPES-DMSO buffer. (A) Binding isotherm for CP DNA (▲), CT DNA (□), EC DNA (●), and ML DNA (◐) with correlation coefficients of 0.997, 0.883, 0.995, and 0.915, respectively. (B) Binding isotherm for poly(dG)·poly(dC) (△), poly[d(A-T)]·poly[d(A-T)] (○), and poly[d(G-C)]·poly[d(G-C)] (▽) with correlation coefficients of 0.992, 0.988, and 0.974, respectively. Binding data are limited to ADG/DNA ratios corresponding to percentages of bound ADG ranging from 35 to 40% (lower limit) and 80 to 85% (upper limit). Inset represents a relation between K and GC content of natural DNA with correlation coefficient 0.987.

that the values for the GC-rich ML DNA differed from those of the AT-rich CP DNA, revealing that the higher GC content DNA bound with ADG more efficiently. This trend in the binding parameters was evident again in case of synthetic polynucleotides, where the binding efficiency was maximum with alternating GC polymer as compared to that of other polynucleotides. However, it was observed that ADG did not interact with poly(dA)·poly(dT).

The dependence of interaction of the alkaloid on base composition of DNA can be examined in terms of α-values obtained by excluded-site analysis described earlier (Nandi & Maiti, 1985). The α-values presented in Table I denoted GC specificity in binding (Müller & Crothers, 1975). Moreover, as shown in Figure 2A (inset), the binding constant increased linearly as a function of the GC content of the DNA.

Fluorescence Spectra of the ADG–DNA Complex. We observed a progressive quenching effect of the fluorescence spectrum of ADG with increasing concentrations of ML DNA (Figure S2, supplementary material). Similar features in the spectral changes were observed with the other polymers. Quantitative data on fluorescence quantum yield (Figure S3 and inset, supplementary material) of various ADG–DNA complexes were significantly dependent on base composition and sequence of DNA, being higher in GC-rich ML DNA and alternating GC polymer.

Thermal Stabilization of the ADG–DNA Complex. ADG enhanced T_m of all natural and synthetic DNAs studied except the homopolymer of AT. Further, the cooperativity of the melting transition of DNA was unaffected in presence of the alkaloid but a larger hyperchromicity of the ADG–DNA complexes as compared to DNA alone was observed, which indicated a contribution of the liberated ADG to the overall absorbance at 260 nm (data not shown). The value of ΔT_m (T_m of complex – T_m of native DNA) was dependent on the ADG/DNA ratio, but apparently there was no relation between stabilization and base composition of DNA. Table II

Table II: Observed Melting Temperature of Various DNAs in the Presence and Absence of ADG[a]

polymer	T_m (DNA) (°C)	T_m (complex) (°C)	ΔT_m (°C)
CP DNA	49.8	56.0	6.2
CT DNA	56.0	63.0	7.0
EC DNA	61.5	65.0	3.5
ML DNA	68.7	73.5	4.8
poly[d(G-C)]·poly[d(G-C)]	72.0	75.0	3.0
poly[d(A-T)]·poly[d(A-T)]	32.2	46.8	14.6
poly(dG)·poly(dC)	71.0	72.5	1.5
poly(dA)·poly(dT)	43.0	43.0	0

[a] Average from three experiments for each DNA.

Table III: Viscometric Properties of the Binding of ADG with Various Natural and Synthetic DNAs[a]

polymer	β	Δ_L[b] (nm)	% helix length enhancement at r_{max}
CP DNA	1.40 ± 0.07	0.24 ± 0.012	8.5
CT DNA	1.95 ± 0.05	0.33 ± 0.009	13.6
EC DNA	1.97 ± 0.02	0.335 ± 0.003	12.0
ML DNA	2.30 ± 0.05	0.39 ± 0.009	21
poly[d(G-C)]·poly[d(G-C)]	2.40 ± 0.04	0.408 ± 0.007	24
poly[d(A-T)]·poly[d(A-T)]	1.46 ± 0.04	0.25 ± 0.007	14
poly(dG)·poly(dC)	0.90 ± 0.03	0.153 ± 0.005	5.2
poly(dA)·poly(dT)	0	0	0

[a] Five determinations each. [b] Length enhancement per intercalation site.

illustrated the values of ΔT_m at the saturation for each complex. It indicated that the alkaloid stabilized alternating AT polymer more as compared to other polymers.

CD of the ADG–DNA Complex. Further evidence for the interaction of the alkaloid with various DNAs was obtained from the CD spectral measurements. In the presence of ADG, the B-form CD spectrum of ML DNA (Figure S4, supplementary material), poly[d(G-C)]·poly[d(G-C)] (Figure S5A, supplementary material), and poly[d(A-T)]·poly[d(A-T)] (Figure S5B, supplementary material) was perturbed. In addition, a rather weak extrinsic CD was seen for the complexes, predominantly positive in character, centered at 315 nm. The perturbation at 235 nm was maximum for the complexes with GC-rich DNAs (Figure S4, inset, supplementary material). The CD spectrum of poly(dG)·poly(dC) showed very little effect with increasing concentrations of ADG and it did not produce any extrinsic CD. No significant perturbation of the CD spectrum was observed in ADG–poly(dA)·poly(dT) complexes.

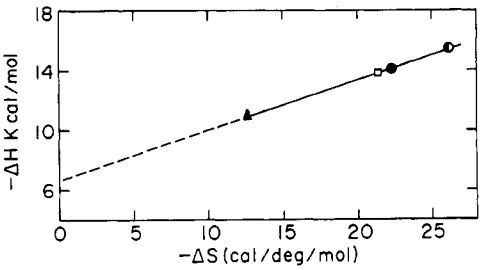

FIGURE 3: Plot of ΔH versus ΔS for binding of ADG to various DNAs represented by the symbols as in Figure 2. Data are fitted to a straight line with a slope of 338 K and a correlation coefficient of 0.999.

Hydrodynamic Properties of the ADG–DNA Complex. Viscosity measurements of DNAs of varying base composition and sequence in the presence of ADG were performed to analyze the intercalation process. It was observed that the relative length increase (L/L_0) against r for different DNAs was significantly dependent on the base composition of the DNA (data not shown). Table III illustrated that the changes of β and the enhancement of helix length (ΔL) depended on the base composition and sequence of DNA. The percent enhancement was maximum in the ADG–poly[d(G-C)]·poly[d(G-C)] complex.

Thermodynamics of ADG Binding. The spectrophotometric titration on the interaction of ADG with different DNAs at three temperatures, 15, 25, and 40 °C, were performed. Binding isotherms were fit to eq 1 and the results are presented in Table IV. It was seen from Table IV that the values of K decreased with increasing temperature, but the parameter n appeared to be invariant with the GC content of the DNA. Both the enthalpy and entropy change were strong functions of the base composition of the DNA. Figure 3 showed that the values for the enthalpy and entropy appeared to be linearly correlated and the changes in the enthalpy with increasing GC content of DNA were compensated by changes in the entropy to produce a relatively small change in free energy in all the cases.

Discussion

The strong interaction of ADG with various DNAs is evident from the observation of the typical bathochromic and hypochromic shifts in the absorption spectrum, quenching of fluorescence intensity, decrease in quantum yield, hindrance to DNA strand separation, perturbation in the CD spectrum, increase in the contour length of sonicated rodlike DNA, and the sign and magnitude of the thermodynamic parameters. The bathochromic effect is similar to that observed with intercalated acridines, phenanthridines, or anthracyclines

Table IV: Binding and Thermodynamic Parameters for the Interaction of ADG with Various Natural DNAs[a]

polymer	temp (°C)	K ($\times 10^5$ M^{-1})	n	$-\Delta G$(25 °C) (kcal/mol)	$-\Delta H$ (kcal/mol)	$-\Delta S$(25 °C) (cal deg^{-1} mol^{-1})
CP DNA	15	3.2 ± 0.3	6.5 ± 0.1			
	25	1.7 ± 0.2	7.1 ± 0.1	7.17 ± 0.07	10.9 ± 0.4	12.5 ± 1.5
	40	0.72 ± 0.04	9.0 ± 0.2			
CT DNA	15	5.8 ± 0.3	5.2 ± 0.1			
	25	2.6 ± 0.2	6.1 ± 0.15	7.43 ± 0.0458	13.8 ± 0.25	21.5 ± 0.6
	40	0.8 ± 0.04	7.7 ± 0.2			
EC DNA	15	5.8 ± 0.025	5.0 ± 0.1			
	25	2.8 ± 0.2	6.1 ± 0.1	7.475 ± 0.0427	14.04 ± 0.6	22.19 ± 1.7
	40	0.82 ± 0.05	9.0 ± 0.2			
ML DNA	15	9.7 ± 0.4	5.0 ± 0.1			
	25	3.8 ± 0.2	5.9 ± 0.1	7.65 ± 0.0314	15.5 ± 0.5	26.24 ± 1.67
	40	1.2 ± 0.1	9.0 ± 0.2			

[a] Five determinations each.

(Waring, 1981a,b; Chaires et al., 1982; Maiti et al., 1982, 1984). Analysis of the absorbance quenching effects indicates that ADG binds to DNAs with binding constants ranging from 1.3×10^5 to 4.5×10^5 M^{-1}, which are of same order of magnitude as that reported for ideal intercalating compounds (Waring, 1981a; Maiti et al., 1982). No observable change in the absorption spectrum of ADG is produced with single-stranded DNA, which indicates that double-strandedness of DNA is an essential precondition for ADG binding. Similar results were also found for berberine–DNA (Maiti & Chaudhuri, 1981) and echinomycin–DNA (Waring & Wakelin, 1974) complexes.

The binding isotherms (Figure 2) fit reasonably well to the neighbor exclusion model used by others (Chaires, 1983, 1985; Chaires et al., 1982). Here the experimentally determined α-values (Table I) and quantum yields (Figure S3, supplementary material) clearly indicate that ADG exhibits GC specificity with a sequence preference for alternating GC polymer in binding. The majority of intercalators like sanguinarine (Nandi & Maiti, 1985), daunomycin (Chaires et al., 1982, 1987), echinomycin (Waring & Wakelin, 1974; Loretto Low et al., 1984), and others (Well & Larson, 1970; Müller & Crothers, 1975; Howe-Grant & Lippard, 1979) show GC specificity in DNA binding, and even those that do not, such as ethidium (Bresloff & Crothers, 1981), may exhibit a CG sequence preference in binding (Kastrup et al., 1978; Reinhardt & Krugh, 1978). Again, it is interesting to note that a linear dependence of the alkaloid binding constants (Figure 2A, inset) with increasing GC content of DNA is apparent. Thus, the presence of a single GC base pair is all that is required for preferred binding to an interaction site of natural DNA.

The results of thermal stabilization of ADG-DNA polymers (Table II) do not show any relationship between base composition and enhancement of T_m. There are various factors that contribute to the stabilizing ability of the ligands on the DNA helix. These are (i) molecular shape of the complex, (ii) van der Waals interactions between ligand and DNA base pairs, and (iii) the formation of hydrogen bonding by the ligand with base pairs or to the groove of the DNA double helix. The present data show that ADG stabilizes less as compared to the other intercalators, like ethidium (Maiti et al., 1982), daunomycin (Chaires et al., 1982), and sanguinarine (Maiti et al., 1984), where not only van der Waals forces but also H-bonds play an important role for stabilization against thermal denaturation. In the case of the ADG–DNA complex, H-bond formation is not apparent (Chakraborty et al., 1990). The higher degree of stabilization in poly[d(A-T)]·poly[d(A-T)]–ADG complexes relative to the poly[d(G-C)]·poly[d(G-C)]–ADG complexes probably reflects differences in the molecular orientation at the interaction site and also the temperature at which the complex is melted. Again, the lesser value of quantum yield in poly[d(G-C)]·poly[d(G-C)] compared to ML DNA may be due to the different orientation of the ADG molecule at the intercalation site in the two polymers, as the glucoside ring in ADG being noncoplanar with the rest of the molecule produces significant effect in the fluorescence intensity (Chakraborty et al., 1989a).

Viscometric technique is well established as a method for investigating the extension of a DNA helix associated with intercalation (Wilson & Jones, 1982; Waring, 1981a,b; Fox et al., 1981). The slope β (Table III) is a parameter related to the functional increase in the contour length of rodlike DNA molecule induced by monofunctional intercalative ligands. The calculated value for helix length extension per bound alkaloid (Table III) in each complex lies well within the range of values reported for other intercalating agents (Waring, 1981a,b; Maiti et al., 1982). The main result is that the increase in contour length of DNA on binding to ADG is higher for GC-rich DNA than AT-rich DNA. These findings are also comparable with the results previously reported for proflavin–DNA (Ramstein et al., 1972) and sanguinarine–DNA complexes (Nandi & Maiti, 1985), where the increase in contour length of duplex DNA depends strongly on its base composition.

The temperature dependence of intercalation has been used to derive the enthalpy of ADG binding to the four natural DNAs studied. It has been observed that the binding of the alkaloid is an exothermic process and the binding free energy arises primarily from a large negative enthalpy due to intermolecular interactions at the intercalation site. With all four DNAs, intercalation is favored by a negative enthalpic contribution and is opposed by the decrease in entropy. It is interesting to note from Figure 3 that the negative enthalpy and entropy change increased with increasing GC content of DNA and also compensated one another to produce a relatively small Gibbs free energy change. Figure 3 clearly shows that the interaction reactions are enthalpy driven. The observed "compensation temperature" (the slope of the line in Figure 3; 338 K) is significantly higher than the mean harmonic temperature (299 K), indicating that the data are not artifactual, as proposed by Krug et al. (1976). A linear relation between Gibbs free energy change and the enthalpy change (data not shown) is also in favor of this proposal. The thermodynamic parameters obtained presently are comparable to those obtained for daunomycin–CT DNA complexes (Chaires, 1985). The values of negative enthalpy and entropy changes increase with increasing GC content of the DNA, which leads to a greater van der Waals stacking interaction between the phenanthrene moiety and the adjacent base pairs in GC-rich ML DNA. These results are in confirmity with the results obtained from spectroscopic and viscometric studies. Thus, it is suggested that enthalpy and entropy compensation may be a general feature of the intercalation reaction, which also depends on the GC content of the DNA, but the physical basis of this phenomenon remains unknown.

In conclusion, the results presented here indicate that the binding affinity of ADG is higher for GC-rich DNA than for AT-rich DNA, with considerable specificity toward alternating GC polymer. ADG binds to DNA by a mechanism of intercalation. The process of binding is enthalpy driven. Intercalation of ADG to four natural DNAs is characterized by a favorable negative enthalpy and opposed by a negative entropy change contribution to the binding site.

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Supplementary Material Available

Five figures showing absorption spectra of ADG–ML DNA and ADG–poly[d(G-C)]·poly[d(G-C)], fluorescence spectra of ADG–ML DNA, fluorescence quantum yield data, and CD spectra of ADG–ML DNA, ADG–poly[d(G-C)]·poly[d(G-C)], and ADG–poly[d(A-T)]·poly[d(A-T)] complexes (7 pages). Ordering information is given on any current masthead page.

Registry No. Poly[d(G-C)]·poly[d(G-C)], 36786-90-0; poly[d(A-T)]·poly[d(A-T)], 26966-61-0; poly(dG)·poly(dC), 25512-84-9;

poly(dA)·poly(dT), 24939-09-1; aristololactam β-D-glucoside, 132491-66-8; guanine, 73-40-5; cytosine, 71-30-7.

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