Interactions of Acridine Antitumor Agents with DNA: Binding Energies and Groove Preferences†

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ABSTRACT: Absorbance spectroscopy is used to examine the thermodynamic properties associated with the interaction of the experimental antitumor agents *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide (AAC) and *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) with nucleic acids. Placement of the amino substituent at the C9 position on the acridine ring results in marked changes to the acridine chromophore's electronic properties, with the overall charge of AAC increasing to +2 in comparison to DACA's charge of +1 at neutral pH. In comparative DNA binding studies, we examine the influence that the electrostatic properties of these ligands have on the binding energies as well as their effects on enthalpy and entropy contributions. These studies show that placement of the amino moiety at C9 results in 6 times greater DNA binding affinity as compared the deamino analog (DACA). Comparisons of ionic strength dependence for these two analogs reveal a difference in the binding energies of the compounds which can be attributed to electrostatic effects. Further dissection of the enthalpy and entropy components of the binding energy reveals the enhanced electrostatic effects are related to an increased entropy contribution upon formation of the AAC−DNA complex. Groove selectivity of these acridine analogs was probed by examining the binding profiles to native and groove-modified DNAs which included glycosylated T4 DNA and the distamycin−DNA complex. These studies are indicative of minor groove interactions for both compounds with DNA.

Two acridine-based compounds, N-[2-(dimethylamino)-ethyl]-acridine-4-carboxamide, DACA,[1] (NSC 601316) and N-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide, AAC, have been widely studied as cancer chemotherapeutic agents, with DACA currently in phase I clinical trials. Though structurally very similar, AAC and DACA (shown in Figure 1) have been categorized as separate drug classes due to dissimilar antitumor specificities and structure−activity relationships (Atwell *et al.*, 1987). AAC has been demonstrated to be highly active against leukemia cell lines, exhibiting higher dose potencies than the clinically used *m*-AMSA (Denny *et al.*, 1987; Denny & Wakelin, 1986). In contrast, DACA warranted clinical trials on the basis of its unique selective activity against solid tumors (Atwell *et al.*, 1987). The structural difference between the two compounds resides in the presence of a C9 amino substituent which imparts significant changes to the electronic properties of the acridine chromophore as illustrated by the marked shift in the pK_a of the ring nitrogen from 8.3 (AAC) to 3.5 (DACA). These pK_a values ensure protonation of the acridine chromophore of AAC at physiological pH (Denny *et al.*, 1987; Atwell *et al.*, 1987). A positive charge resides on the C4 side-chain distal nitrogen (pK_a 8.6) of both compounds, resulting in AAC carrying a total charge of +2 and DACA a total charge of +1 at neutral pH.

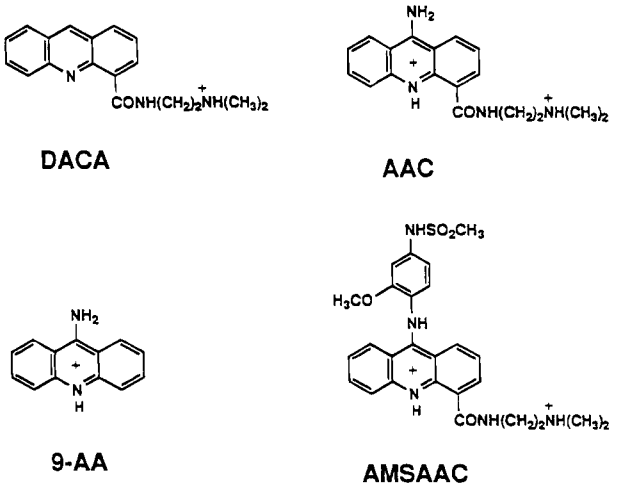

FIGURE 1: Chemical structure of acridine analogs *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) and the 9-amino analog, *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide (AAC). Also included is a structure of the parent compound 9-aminoacridine (9-AA) and the threading agent 9-[[2-methoxy-4-(methylsulfonylamino)phenyl]amino]-*N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (AMSAAC).

Biophysical studies have provided insight into the sequence-specific interactions of these drugs with DNA. Characterization of the interactions of these compounds with synthetic DNAs revealed GC-rich segments to be preferred binding

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[1] Abbreviations: AAC, *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide; DACA, *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide; 9-AA, 9-aminoacridine; *m*-AMSA, 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide; AMSAAC, 9-[[2-methoxy-4-(methylsulfonylamino)phenyl]amino]-*N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide; CT, calf thymus; T4, coliphage T4 DNA.

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sites for both AAC and DACA (Denny *et al.*, 1987; Atwell *et al.*, 1987). From these studies, a structural binding motif was proposed in which the carboxamide side chain resides in the minor groove of B-DNA and is stabilized through specific hydrogen-bonding between the carboxamide and the carbonyl oxygen of an adjacent cytosine base (Wakelin *et al.*, 1987; Wakelin *et al.*, 1990; Moyer *et al.*, 1990). Recently, these studies were further corroborated by Bailly *et al.* (1992), who demonstrated strong GC preferences for AAC using DNase I footprinting methods. Interestingly, these studies demonstrate that the 4-carboxamide moiety to be the primary determinant in directing this observed GC specificity.

In the present work, visible spectroscopy is used to probe differences in DNA binding of these biologically active acridine analogs. The association constant of the dication AAC is found to be 6 times higher than that of monocation DACA. An aim of this study was to probe the influence of the formal charge on the molecule on binding energetics and thermodynamic properties. The studies presented here demonstrate that the enhanced binding of AAC is due to changes in the electronic properties of the acridine chromophore and can be attributed to enhanced electrostatic contributions to the free energy of binding. Examination of the enthalpy and entropy components describing the formation of the drug−DNA complex reveals the enhanced electrostatic free energy to be a result of more favorable binding entropy associated with the AAC analog.

In an effort to determine DNA groove specificity, the interactions of selected acridine analogs with DNAs having the major and/or minor grooves sterically blocked are examined. Included in this study are 9-aminoacridine, which has been shown by X-ray crystallography to intercalate from the minor groove of the DNA (Sakore *et al.*, 1979), and the threading agent, 9-[[2-methoxy-4-(methylsulfonylamino)-phenyl]amino]-*N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (AMSAAC), whose side chains are thought to occupy both DNA grooves when the chromophore is bound to DNA (Wakelin *et al.*, 1990). DNA-binding affinities for each of the drugs were determined using several DNAs having comparable GC content, including calf thymus DNA (grooves unmodified), coliphage T4 DNA (major groove blocked via glycosylation) (Revel & Luria, 1970), calf thymus DNA in the presence of distamycin (minor groove blocked), and coliphage T4 DNA plus distamycin (both major and minor grooves blocked). These studies reveal that blockage of the major groove (as in the case of coliphage T4 DNA) had no appreciable effects on binding of these compounds to DNA. In contrast, blockage of the minor groove by distamycin markedly decreased the binding affinities of these compounds with DNA, suggesting both AAC and DACA preferentially intercalate via the minor groove.

MATERIALS AND METHODS

Drug Preparation. The antitumor agents AAC, DACA, and AMSAAC were synthesized as described as the dichloride salts (Denny *et al.*, 1982, 1987; Atwell *et al.*, 1987). Distamycin A and 9-aminoacridine and were purchased from Sigma Chemicals, Inc., and used without further purification. Molar absorptivities for the free and bound drug (ϵ_f and ϵ_b) for 9-AA and AMSAAC were obtained from the literature (Wilson *et al.*, 1981; Wakelin & Denny, 1982). The values for ϵ_f were determined to be 9200 M^{-1} cm^{-1} at 409 nm for AAC and 10 300 M^{-1} cm^{-1} at 358 nm for DACA. Molar absorptivities for the bound drug species (ϵ_b) were determined by monitoring the absorbance change upon titration of aliquots of concentrated calf thymus DNA into a dilute drug solution and extrapolating to infinite DNA concentrations. Values for ϵ_b of 3500 and 5100 M^{-1} cm^{-1} for AAC and DACA, respectively, were obtained.

DNA Preparation. Calf thymus DNA, obtained commercially (Sigma Chemicals, Inc., lot 71H9605), was sonicated, extracted with a 1:1 chloroform:phenol solution, and purified as previously described (Chaires, 1985). T4 coliphage DNA was purchased from Sigma (lot 74H6651) and was used without further purification. Concentrations were determined by UV absorbance at 260 nm using a molar absorptivity constant of 13 200 M^{-1} cm^{-1} (Graves *et al.*, 1981). All optical data were collected using a Cary 4 UV−visible spectrophotometer (Varian) equipped with a Lauda R61 circulating water bath.

DNA-Binding Studies. Absorbance titrations were performed in 10 cm cylindrical quartz cells. A constant temperature of 25 °C was maintained and monitored using a Markson 7001 microthermometer by immersion of the probe directly in the reference cell solution. Prior to use, optical cells and micropipett tips were poisoned with a dilute drug solution, extensively rinsed with Milli-Q ultrapure water, and air-dried to minimize the tendency of cationic drugs to adhere to the glass and plastic surfaces. In order to minimize aggregation effects of the drug, concentrations of free drug were maintained below 5 μM throughout all binding studies.

Binding isotherms were calculated from absorbance data collected over a wide range of drug/DNA ratios and salt concentrations. Aliquots of a stock drug solution were added to a known DNA concentration followed by thorough mixing and equilibration at 25 °C. Concentrations of bound drug were determined by monitoring the change in the absorbance of the drug resulting from complex formation. Data are presented as direct plots of r (bound drug per base pair) vs C_f (free drug concentration), and theoretical fits calculated

$$\frac{r}{C_f} = K_{obs}(1 - nr)\left[\frac{1 - nr}{1 - (n-1)r}\right]^{n-1}$$

using a nonlinear least-squares analysis (KaleidaGraph, Synergy Software, Reading, PA) of the noncooperative neighbor exclusion model of McGhee and von Hippel (1974) where r is the molar ratio of bound drug per base pair, K_{obs} is the binding constant for the interaction of the acridine analog with an isolated DNA site (M^{-1}), and n is the neighbor exclusion size expressed in base pairs.

DNA-Binding Groove Selectivity. The groove selectivity was evaluated by comparing binding profiles obtained for these compounds with various DNAs whose major and minor grooves were blocked either by covalent glycosylation or by the addition of distamycin. The presence of the bulky glucose residues in the case of coliphage T4 DNA effectively blocks drug binding in the major groove (Yen *et al.*, 1983). Distamycin A (2 μM) was used to block access to binding sites within the minor groove of the DNA (Kopka *et al.*, 1985).

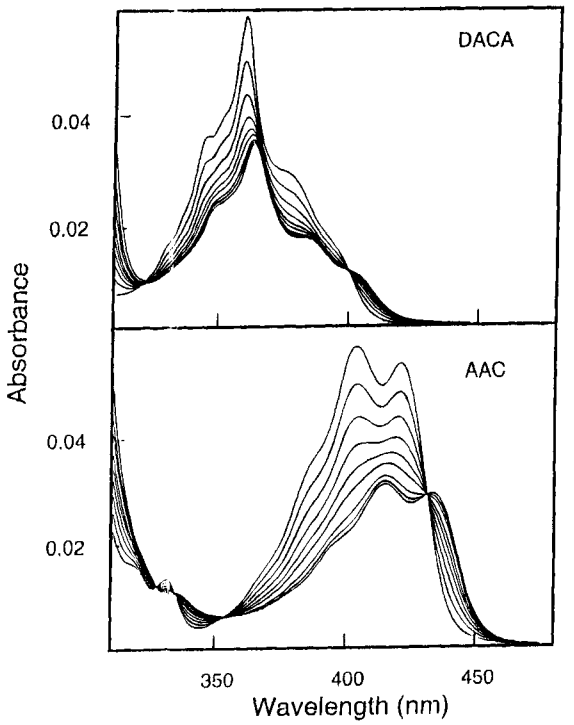

FIGURE 2: Influence of DNA binding on the visible absorption spectra of DACA (top panel) and AAC (bottom panel). Fixed concentrations of drug were titrated with increasing concentrations of calf thymus DNA. Drug concentrations were maintained below 5 μM throughout the experiment. Experiments were performed using a Cary 4 spectrophotometer equilibrated to 25 °C. Buffer and salt conditions are as described in Materials and Methods.

Thermodynamic Binding Profile. Thermodynamic data were obtained for the interactions of AAC and DACA to calf thymus DNA by monitoring the equilibrium binding of these antitumor agents to DNA as a function of temperature. van't Hoff binding enthalpies were determined by obtaining drug−DNA binding isotherms at several temperatures and using the "fixed ratio" technique as previously described by Chaires (1985) and Shimer *et al* (1988). With this method, a drug−DNA complex having a ratio of approximately 0.15 bound drug per base pair is prepared. The absorbance of the drug is recorded at 5 deg increments over the range 5−50 °C allowing 1.5 h equilibration at each temperature. Values for the intrinsic binding constants are determined for each temperature using the neighbor exclusion model and are presented in the form of van't Hoff plots. Binding enthalpies can then be obtained from the slopes of these plots and used to estimate the binding entropy. Although the van't Hoff method has limited utility due to restrictive temperature range that can be studied, this method is very useful in applying to drugs which exhibit limited aqueous solubility precluding the use of scanning or titration calorimetry. Results of Sauer et al. (1984) demonstrate the use of this method to provide results which are comparable to those obtained by calorimetric measurements.

RESULTS

The addition of the amino substituent at C9 results in marked changes to the electronic properties of acridine chromophore as evidenced by the change in the visible absorption spectra shown in Figure 2. The λ_{max} of AAC shifts to 409 nm which is 50 nm higher than the λ_{max} of 358 nm for DACA. The resonance-stabilizing influence of the amino substituent results in the distribution of an additional positive charge on acridine ring and considerably modifies the binding properties of this compound to DNA. Upon complex formation with DNA, characteristic changes to the visible absorption spectra of both drugs are evident. Both hypochromic and bathochromic shifts relative to their free spectra are observed with spectral shifts of 14 and 5 nm for AAC and DACA, respectively. Composite spectra exhibit well-defined isosbestic points for both drugs, indicative of two distinct absorbing species (free and bound drug).

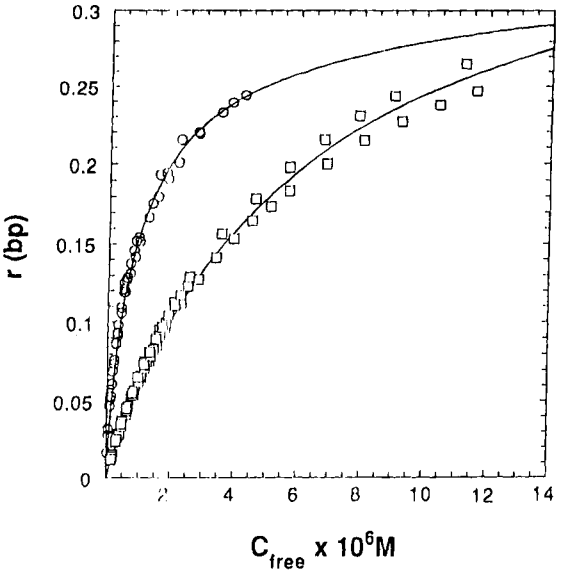

FIGURE 3: Binding isotherms in the form of direct plots obtained for the interactions of AAC and DACA with calf thymus DNA. Binding data were obtained at 25 °C in 10 mM sodium phosphate, pH 7.0, 1 mM Na_2EDTA, and 100 mM sodium chloride. Solid lines represent the best fits to the neighbor exclusion model. AAC is represented by the open circles and DACA the open squares.

UV−visible titration data were used to construct binding isotherms for the two acridine analogs. Each binding isotherm contains data sets from multiple experiments and is presented in the form of a direct plot shown in Figure 3. Comparison of the binding isotherms obtained for AAC and DACA demonstrates the considerable influence the 9-amino substituent exerts on DNA-binding properties. Nonlinear least-squares analyses of the neighbor exclusion model are used to fit the data for determination of values of binding constant, K_{obs}, and the neighbor exclusion parameter, n. These studies reveal AAC to bind DNA with almost an order of magnitude higher affinity as compared with DACA. The interaction of AAC with calf thymus DNA is characterized by a K_{obs} value of 4.1 (±0.2) × 10^5 M^{-1} and binding site size of 3 (bp) as compared to value of 7.4 (±0.3) × 10^4 M^{-1} and binding site size of 2 (bp) observed for DACA. The absence of the amino substituent at C9 has an overall effect of reducing the binding constant by a factor of 6.

Thermodynamic properties associated with the interactions of these ligands with DNA are obtained by van't Hoff analyses (Figure 4) and results are presented in Table 1. AAC ($\Delta G°_{obs}$ −7.7 kcal mol^{-1}) exhibits an overall binding free energy that is 1 kcal mol^{-1} more favorable than that of DACA ($\Delta G°_{obs}$ −6.6 kcal mol^{-1}). The influence of the ionic strength on the binding of these compounds to DNA was investigated and used to quantitate both the effective charge of the ligands and the electrostatic contribution ($\Delta G°_{el}$) of

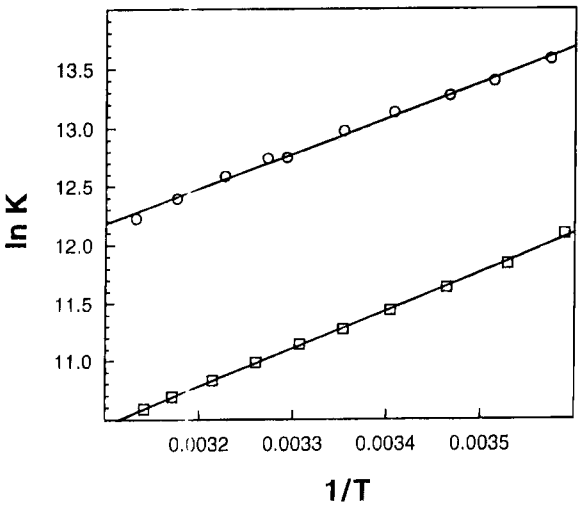

FIGURE 4: van't Hoff plots for the interaction of AAC (circles) and DACA (squares) to calf thymus DNA. Binding isotherms were obtained as described in the legend of Figure 3. Intrinsic binding constants were obtained using the neighbor exclusion model. Linear least-squares fits to the data are shown by the solid lines (R^2 of 0.995 and 0.999 for AAC and DACA, respectively). The slopes of these straight lines are used to quantitate binding enthalpies using the van't Hoff equation.

Table 1: Summary of Thermodynamic Binding Parameters for the Interactions of Acridinecarboxamides to DNA[a]

compd	$G°_{obs}$[b]	$G°$[c]	$G°_{el}$[d]	$H°$[e]	$S°$[f]
AAC	−7.7 ± 0.2	−4.8 ± 0.1	−2.8 ± 0.1	−6.1 ± 0.2	5.2 ± 0.2
DACA	−6.6 ± 0.1	−4.9 ± 0.1	−1.7 ± 0.2	−6.3 ± 0.2	1.1 ± 0.1

[a] Data refer to solutions containing 10 mM sodium phosphate (pH 7.0), 1 mM disodium EDTA, and varied sodium chloride concentrations and temperatures. [b] Units are kcal mol^{-1}. $\Delta G°_{obs}$ is calculated from the binding affinity obtained at 25 °C and 0.1 M sodium chloride concentration. [c] $\Delta G°$ is the nonelectrostatic free energy component referring to a standard state of 1 M sodium chloride concentration. [d] The electrostatic free energy component, $\Delta G°_{el}$ refers to the polyelectrolyte contribution to the binding energy evaluated at 0.1 M sodium chloride concentration. [e] Enthalpy value evaluated from the van't Hoff equation, $\Delta H°/R = -\mathrm{d} \ln K_{obs}/\mathrm{d}(1/T)$ reported in kcal mol^{-1}. [f] Entropy (eu) estimated from the expression $\Delta S° = (\Delta H° - \Delta G°_{obs})/T$.

the overall binding free energy. The observed binding energy can be dissected into electrostatic and nonelectrostatic components from the expression $\Delta G°_{obs} = \Delta G°_{el} + \Delta G°$ (Chaires *et al.*, 1994). Data are presented in the form of a double-logarithmic plot of K_{obs} versus $-[Na^+]$ according to the polyelectrolyte theory of Record *et al.* (1978). As observed in Figure 5, increases in ionic strength result in decreased ligand-binding affinities. The slope determined from the linear least-squares fits of these plots of are used in calculating the electrostatic component ($\Delta G°_{el}$) of the overall binding free energy $\Delta G°_{obs}$

$$S = \frac{\delta \ln K_{obs}}{\delta \ln [Na^+]} \quad \text{and} \quad \Delta G°_{el} = SRT \ln [Na^+]$$

Using the equations shown above, electrostatic contributions ($\Delta G°_{el}$) for the overall binding free energies were found to be −2.8 kcal mol^{-1} for AAC and −1.7 kcal mol^{-1} for DACA and are presented in Table 1. The nonelectrostatic free energy components ($\Delta G°$) were determined to be −5 (±0.1) kcal mol^{-1} for both compounds, indicating that the intercalation geometries of DACA and AAC to be similar.

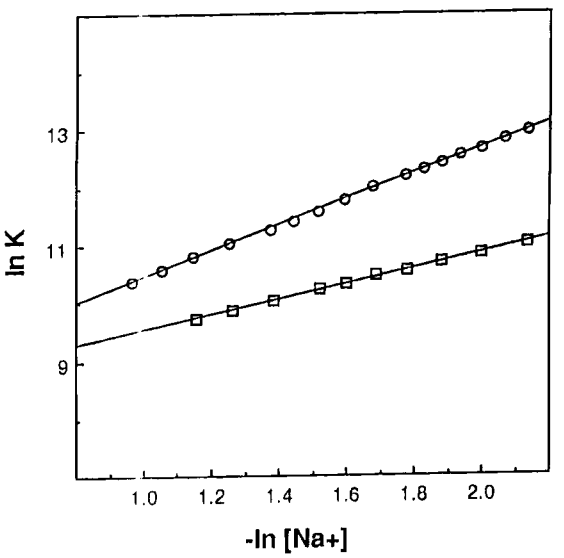

FIGURE 5: Dependence of the equilibrium binding constants on sodium chloride concentration for AAC (open circles) and DACA (open squares). Data are represented as a double-logarithmic plot according to the theory of Record *et al.* (1978). Linear least-squares fits to the data are shown by the solid lines and are used to quantitate values of S from the slopes.

Partitioning of the observed free energy into enthalpy and entropy components provides insight into the enhanced binding energy of AAC. Interestingly, binding enthalpies of both drugs were determined to be identical with both compounds exhibiting binding enthalpies of −6.2 (±0.2) kcal mol^{-1}. In contrast, a significant difference was noted when the binding entropy of AAC was compared with DACA (5.2 eu for AAC and 1.1 eu for DACA). This difference is indicative of the electrostatic component of the free energy being manifested through a more favorable binding entropy of the AAC analog.

DNA groove preference was evaluated by comparing the interactions of these compounds with DNAs having modified or blocked groove access. Examination of data in Table 2 shows that 9-AA, as well as AAC and DACA, binds calf thymus DNA (no groove modifications) or coliphage T4 DNA (major groove is modified by glycosylation) equally well. Thus, modification of the major groove of the DNA has little or no effect on the binding affinities or binding site sizes for any of the compounds examined. As an additional test, the interactions of AMSAAC (threading agent) to these DNAs were examined. DNA complex formation by this drug results in bulky substituents protruding into both the major and minor grooves of DNA. We observed a marked decrease in binding affinity to coliphage T4 DNA, indicating sensitivity to accessibility in the major groove.

Distamycin A was added to the DNA to block minor groove access by the acridine analogs. In the presence of distamycin A, the binding affinities of all compounds were significantly reduced which is consistent with the model proposed by Wakelin and Denny (1990) which suggests the formation of the acridine-DNA complex whereby the carboxamide side chains of both AAC and DACA reside in the minor groove of the DNA. In the case of the threading agent, modification of either of the DNA grooves results in decreased binding as was observed.

Table 2: Binding Parameters for the Interactions of Selected Acridine Analogs with "Groove-Modified" DNAs

	calf thymus DNA		coliphage T4 DNA		calf thymus DNA plus distamycin A		coliphage T4 DNA plus distamycin A	
compd	K_{obs}[a]	n[b]	K_{obs}[a]	n[b]	K_{obs}[a]	n[b]	K_{obs}[a]	n[b]
9-AA	7.2 (±0.4)	2.3	7.1 (±0.8)	2.3	2.2 (±0.5)	1.8	2.1 (±0.7)	1.8
AAC	41.1 (±1.2)	2.7	40.8 (±1.4)	2.7	13.2 (±0.5)	2.0	12.3 (±1.0)	2.7
DACA	7.4 (±0.3)	2.1	7.2 (±0.3)	2.1	3.4 (±0.8)	3.4	4.1 (±1.0)	3.3
AMSAAC	23.3 (±2.2)	3.2	13.7 (±2.5)	2.0	13.4 (±1.0)	9.2	8.5 (±1.2)	9.2

[a] Association constants (K_{obs}) are expressed (x 10^4 M^{-1}). [b] Binding site size (base pairs). All binding experiments are performed at 10 mM sodium phosphate, pH 7.0, 1 mM disodium EDTA, 100 mM sodium chloride and at 25 °C. In groove selectivity studies, an initial concentration of DNA of 5 μM (bp) and 2 μM distamycin (if present) were used. [a]Association constants are determined using the neighbor exclusion model (McGhee and von Hipple, 1974).

DISCUSSION

The present studies provide unique insight into the influence of substituent modification on the DNA binding properties of antitumor agents with DNA. From these studies, correlations between the biophysical properties associated with the interactions of these agents with DNA with the divergent antitumor activities of these structurally related but chemically different drug species are ascertained. A primary objective of these studies was to examine the influence of the amino substituent at C9 on the energetics, structural properties, and binding geometries associated with the interactions of these second-generation acridine antitumor agents with DNA. Properties such as binding affinities (K_{obs}) and binding site sizes (n) were obtained over a broad spectrum of temperatures and salt concentrations allowing us to characterize the electrostatic and nonelectrostatic components of the overall binding energies, and correlate these data with their influence on the enthalpy and entropy of this DNA binding process.

Comparison of binding isotherms obtained for DACA and AAC reveals that the presence of the amino substituent at the 9-position (AAC) results in markedly greater affinity for DNA. The binding constants, K_{obs}, are used to determine the overall binding energies of these compounds and subsequently when examined as a function of temperature can be dissected into enthalpy and entropy components. The interactions of both compounds with DNA are demonstrated to be enthalpy-favored processes, with both compounds exhibiting binding enthalpies of -6.2 kcal mol^{-1}. In contrast, a significant difference is observed in comparing the binding entropy of AAC with that of DACA. Both entropies are shown to contribute favorably to formation of the drug−DNA complex; however, AAC exhibits a binding entropy of 5.2 eu as compared to 1.1 eu for DACA. This increased binding entropy for AAC is likely due to the enhanced counterion displacement coupled with loss of water from the minor groove of the DNA resulting from the additional positive charge on AAC chromophore. Since both compounds have the same C4 side chain (i.e., N-[2-(dimethylamino)ethyl]acridine-4-carboxamide) available for interactions within the minor groove, the differences in binding entropies observed between AAC and DACA must reside with the additional +1 charge distributed on the acridine chromophore of AAC analog. The more favorable entropy is directly correlated with the observed electrostatic component of the binding energy ($\Delta G°_{el}$).

Recently, Chaires *et al.* (1993) used the model of Friedman and Manning (1984) to correlate polyelectrolyte theory with binding energies associated with the interactions of monocationic and uncharged anthracycline antibiotics with DNA. We extend this work with our examination of a dicationic DNA binding ligand (AAC). Our data reveal the value obtained for S ($-\partial \ln K/\partial \ln [Na^+]$) for the interaction of AAC with DNA to be 2.2, in excellent agreement with the value of 2.24 as predicted by the model of Friedman and Manning (1984) for a ligand carrying a +2 charge. Similarly, the value of 1.3 obtained for the monocation DACA is also in excellent agreement with the 1.24 value predicted by the Friedman and Manning model for a monocationic ligand. Examination of $\Delta G°_{obs}$, $\Delta G°_{el}$, and $\Delta G°$ values listed in Table 1 reveals that the additional charge carried by AAC contributes an additional -1.1 kcal mol^{-1} to the electrostatic component of the free energy ($\Delta G°_{el}$) as compared with DACA. The 1 kcal mol^{-1} difference of $\Delta G°_{obs}$ values between the two compounds is manifested entirely by the polyelectrolyte contribution of the binding energy since the nonelectrostatic components ($\Delta G°$) for AAC and DACA are equivalent at -5 kcal mol^{-1}.

Groove selectivity of the binding of these agents to DNA was investigated through a comparison of binding properties for AAC and DACA with DNAs having blocked or limited groove access either by addition of the minor groove binder distamycin or covalent glycosylation of the major groove. The interaction of 9-AA with DNA is unaffected by blockage of the major groove of the DNA. Addition of distamycin, which binds exclusively in the minor groove, results in a marked decrease in the binding affinity of this drug to the DNA. Similarly, binding isotherms obtained for the interactions of AAC and DACA with groove-modified DNAs reveal the binding of both drugs to be unaffected by limited access to the major groove of coliphage T4 DNA. In contrast, both compounds exhibit markedly decreased DNA binding in the presence of distamycin, indicating that the interaction of AAC and DACA occurs via the minor groove of the DNA. The binding of the threading agent AMSAAC, which has substituent groups protruding into both the major and minor grooves, is decreased with modification of either groove (Wakelin et al., 1990; Wakelin & Denny, 1990). The studies presented here presume the difference in binding affinities to be directly influenced by the decreased access to the major or minor grooves of the DNA. Binding isotherms for these compounds reveal marked decreases in binding affinities to DNAs having distamycin blocked minor grooves. In contrast, binding to the coliphage T4 DNA was unaffected by the presence of glycosyl moieties within the DNA major groove. These results are indicative of binding of AAC and DACA via the minor groove. Similarly, our studies reveal the threading agent AMSAAC to bind calf thymus DNA with

almost twice the affinity as observed with coliphage T4 DNA whose major groove is blocked, consistent with the proposed complex geometry (Wakelin & Denny, 1990) which places a side chain of threading agents in both the major and minor groove of the DNA.

The antitumor activities of AAC and DACA appear to be contingent on their abilities to bind DNA, although the mechanism through which these effects are exerted are complex, depending on their influences on DNA-binding proteins such as topoisomerase II (Schneider *et al.* (1988). The present work characterizes energetic and structural features which describe the ligand−DNA interaction, thus providing insight into our general understanding of how these second-generation acridine antitumor agents function.

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