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Thermodynamics of the interactions of *m*-AMSA and *o*-AMSA with nucleic acids: influence of ionic strength and DNA base composition

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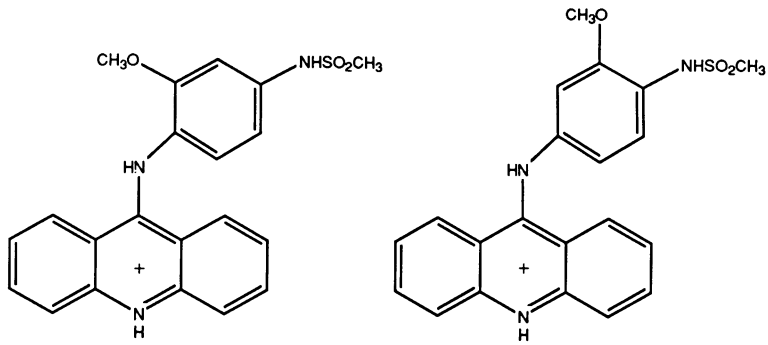
ABSTRACT

The equilibrium binding of the antitumor agent *m*-AMSA and its biologically inactive analog *o*-AMSA to native and synthetic DNAs are compared over a wide range of ionic strengths and temperatures. Although *o*-AMSA binds DNA with a higher affinity than *m*-AMSA it is not effective as an antitumor agent. Both m-AMSA and *o*-AMSA bind DNA in an intercalative manner. Indepth investigations into the thermodynamic parameters of these interactions reveal the interaction of *m*-AMSA with DNA to be an enthalpy driven process. In contrast, the structurally similar but biologically inactive *o*-AMSA binds DNA through an entropy driven process. The differences in thermodynamic mechanisms of binding between the two isomers reveal that the electronic and/or steric factors resulting from the position of the methoxy substituient group on the anilino ring directs the DNA binding properties of these compounds and ultimately the biological effectiveness as an antitumor agent.

INTRODUCTION

The acridine compound *m*-AMSA, (4'-(9-acridinylamino)methanesulfon-*m*-anisidide), has become an increasingly important drug as a treatment for a wide variety of tumors and leukemias (1-4). The mode of action of *m*-AMSA is thought to reside in the stabilization of the topoisomerase II-DNA complex (5-7); however, the precise mechanisms responsible for its cytotoxicity remain unknown. Recent studies by Schneider and coworkers have shown that stabilization of the ternary complex is not enough to promote maximal cytotoxicity but in addition RNA and protein synthesis must be maintained for effective toxicity thus suggesting that secondary events beyond ternary complex formation are involved in the antitumor activity of this compound (8). Interestingly, the structural isomer, 4'-(9-acridinylamino)methanesulfon-*o*-anisidide, or *o*-AMSA, demonstrates little or no antitumor activity at dosages of 75 to 100 times greater than that of *m*-AMSA (9). In addition, *o*-AMSA like the parent compound 9-aminoacridine does not induce topoisomerase II-mediated DNA strand breaks (7). The selective behavior of the two structurally isomeric compounds has prompted numerous investigations into the relative physical chemical properties associated with the interactions of these compounds with DNA. Thus far, a distinct relationship between their DNA binding properties and anti-tumor activities has not been established.

Both *m*-AMSA and *o*-AMSA whose structures are shown in Figure 1 have been shown to interact with DNA in an intercalative manner (10-12). The reversible interaction of *m*-AMSA



m-AMSA *o*-AMSA

Figure 1. Chemical structures of *m*-AMSA (left) and *o*-AMSA (right).

with DNA has been shown to be relatively weak with a binding affinity constant of approximately 10^4 M^{-1} in comparison to other antitumor antibiotics such as adriamycin, daunorubicin, and actinomycin D which show binding affinities in the range of 10^5 to 10^6 M^{-1}. In contrast, the *o*-AMSA isomer exhibits a binding affinity which is four times greater than that of *m*-AMSA, however this higher affinity for DNA is not reflected in antitumor activity. Earlier studies by Waring provided evidence of a binding selectivity for alternating purine-pyrimidine base pairs and a slight preference for guanine-cytosine base pairs for both the *m*-AMSA and *o*-AMSA isomers.

Although extensive information concerning the equilibrium binding of these compounds to DNA, relatively little attention has been given to the thermodynamics of the interactions of these compounds with DNA. This study examines the thermodynamic parameters associated with the interactions of both *m*-AMSA and *o*-AMSA with nucleic acids. Initial studies on the equilibrium binding of these compounds with DNA resulted in the observation of marked similarities in binding modes, relative binding strengths, and base sequence preferences. However, upon detailed examination of the thermodynamics associated with the interactions of these compounds with DNA, differences in thermodynamic parameters are observed which may reflect the differences observed in the antitumor activities of the *m*-AMSA but lacking with the *o*-AMSA. This report describes the influence of base composition and ionic strength on these thermodynamic properties associated with the interactions of *m*-AMSA and *o*-AMSA with DNA.

EXPERIMENTAL METHODS

MATERIALS

Drug Preparations. *m*-AMSA (NSC-249992) was obtained from the National Cancer Institute. Its purity was confirmed by TLC using KC18 (Whatman) plates and a methylene chloride-

methanol-water (100:20:2). *o*-AMSA was synthesized according to the method of Cain and coworkers (9) and used as the hydrochloride salt. The compounds were stored in the dark at -5°C until ready to be used. Due to their low aqueous solubility, dimethylsulfoxide (DMSO) was used to dissolve the compounds into concentrated stock solutions (~0.5 mg/ml). This DMSO-drug solution was then diluted 1/10 with 0.01 M sodium phosphate, 0.001 M disodium EDTA at pH 7.0. The sodium chloride concentration was adjusted as needed. This solution was then filtered through a 0.22 μ syringe filter (Millipore). The concentrations of the solutions were determined using visible spectroscopy with molar absorptivities reported by Wilson (12). The pKa's for both the *m*-AMSA and *o*-AMSA isomers were determined by visible spectroscopy. At pH 7.0, the *m*-AMSA was shown to be 89% protonated in contrast to the *o*-AMSA which was only 78% protonated.

DNA preparations. Calf thymus DNA (Type I) was purchased from Sigma (lot 115F-9500) and prepared as described previously (13). Concentrations were determined using the molar absorptivity ε_{260nm} = 13,200 M^{-1} cm^{-1}. The synthetic DNA's poly (dGdC)·(dGdC) (lot PD717910), poly (dAdT)·(dAdT) (lot AE7870102), and poly (dAdC)·poly(dGdT) (lot 005-27940) were purchased from Pharmacia and used without further purification after dissolving them into phosphate buffer. Concentrations of these nucleotides were determined using the ε_{260nm} of 14200 M^{-1} cm^{-1}, and ε_{260nm} of 13200 M^{-1} cm^{-1} for poly(dAdT)·poly(dAdT) (14) and ε_{260nm} 13000 M^{-1} cm^{-1} for poly(dGdT)·poly(dAdC) (15).

DNA Binding Studies. The optical titrations were performed using a Varian-Cary 2290 UV/visible spectrophotometer equipped with a Lauda RC-6 circulating water bath. Temperatures were monitored by immersion of the thermistor probe directly into the sample cell and displayed on the temperature-readout accessory. In an effort to maximize sensitivity, quartz cells of 10-cm pathlengths were used for these DNA binding studies. The drug's absorbance (ranging from 0.2 - 0.3 as measured in the 10 cm cells) was read directly from the digital display of the spectrophotometer operating in the statistical mode.

DNA binding isotherms for the two compounds were obtained by titrating measured quantities of a stock drug solution into a known concentration of calf thymus DNA and monitoring the change in the drugs absorbance (17). Values for the concentration of bound drug (C_b) is determined by:

$$\Delta A = \Delta \varepsilon C_b l \qquad [1]$$

where ΔA is the change in absorbance between free and bound ligand, $\Delta\varepsilon$ is the difference in molar absorptivity between the free and bound drug and l is the path length of the cell. The $\Delta\varepsilon$ values for *m*-AMSA and *o*-AMSA were equivalent to those reported by Wilson (12).

Data were plotted in the form of a Scatchard plot and analyzed using the cooperative ligand binding model of McGhee and von Hippel (18),

[2]

$$\frac{r}{C_f} = K(1-nr)\left(\frac{(2\omega-1)(1-nr) + r - R}{2(\omega-1)(1-nr)}\right)^{n-1} \left(\frac{1 - (n+1)r + R}{2(1-nr)}\right)^{2}$$

$$R = [(1-(n+1)r)^2 + 4\omega r(1-nr)]$$

where r is the number of moles of drug bound per mole of DNA base pair, C_f is the concentration of free drug in solution, K is the intrinsic binding constant, n is the binding size exclusion parameter, and ω is the cooperativity factor. Data were analyzed via a nonlinear least-squares fitting routine based on the Simplex algorithm (17). Binding constants determined in this manner are influenced by the degree of protonation equilibria of the drugs and can be illustrated by the equation:

[3]

$$K_{obs} = \frac{K_{binding}}{\left(\frac{K_a}{[H^+]}\right) + 1}$$

where K_{obs} is the intrinsic binding constant by analyzing the data by the McGhee and von Hippel equation, K_a is the acid dissociation constant for the drug, and $K_{binding}$ is the actual binding constant reflecting the drug-DNA interaction which has been corrected for the degree of protonation of drug species.

Enthalpies of binding (ΔH°) were determined using linear least-square fits of the ln K versus 1/T°K plot, according to the van't Hoff relationship,

[4]

$$\frac{\delta \ln K}{\delta (1/T)} = -\frac{\Delta H^\circ}{R}$$

and the entropy of binding determined from the Gibbs free energy and enthalpy,

[5]

$$\Delta S^\circ = \frac{(\Delta G^\circ - \Delta H^\circ)}{T}$$

An alternate method used to obtain values of K as a function of temperature is described by Chaires (13) and Meehan (18). Briefly, data were obtained by mixing a known concentration of drug with a DNA solution and monitoring the resultant change in the drugs absorbance. From the absorbance change, values for bound and free concentrations of drug were determined as described earlier. The temperature was then adjusted as necessary, the drug-DNA complex allowed to reach equilibration, and the change in absorbance monitored as a function of temperature. Comparisons of the values obtained revealed that both methods are in excellent agreement. The enthalpy of binding of the drug is also influenced by the protonation equilibria and can be expressed by the equation:

$$\Delta H^{\circ}_{obs} = \Delta H^{\circ}_{binding} - \varphi \Delta H^{\circ}_{a} \quad [6]$$

where ΔH°_{obs} is the observed binding enthalpy, ΔH°_{a} is the enthalpy of proton dissociation from the drug, $\Delta H^{\circ}_{binding}$ is the corrected enthalpy of binding, and φ is the fraction of unprotonated drug molecule.

RESULTS

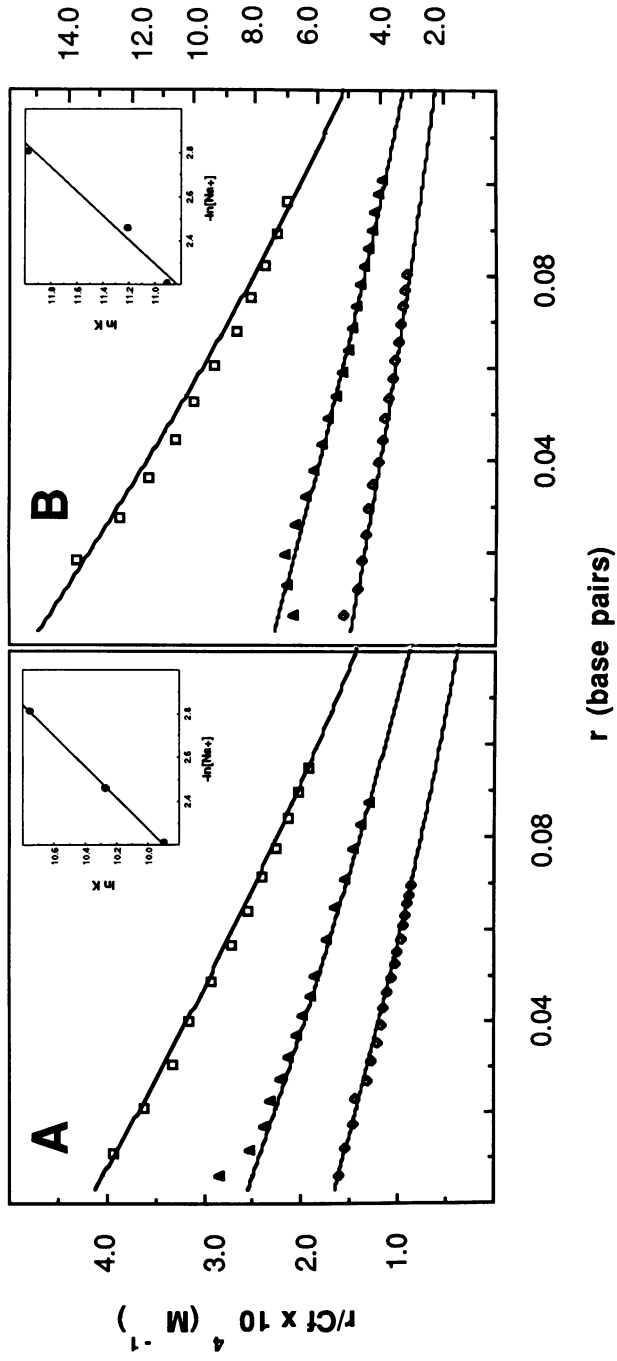
Equilibrium Binding of m-AMSA and o-AMSA to Nucleic Acids.

Binding isotherms for the interactions of *o*-AMSA and *m*-AMSA to calf thymus DNA are shown in Figure 2. The solid lines drawn through the data represent a best fit using the McGhee and von Hippel equation. Parameters used to fit this equation to the experimental data are provided in Table I.

The slopes of these binding curves illustrate *o*-AMSA to bind more strongly to calf thymus DNA exhibiting a binding constant twice the magnitude to that of *m*-AMSA under identical binding conditions. The interaction of the two isomers with native DNA is shown to be influenced by the ionic strength of the buffer. Reduction of the sodium concentration from 0.11 to 0.02 results in an observed binding constant for *m*-AMSA of 2.6×10^5, an order of magnitude higher than the 0.11 ionic strength data. The value for n (binding site size) remains relatively constant over the salt concentrations studied, ranging only from 3.1 to 3.8 suggesting that within the salt concentrations considered, the mode of DNA binding is consistent. The binding of *o*-AMSA to DNA also shows marked ionic strength dependence, with the binding constant ranging from 1.4×10^5 to 4.2×10^4 over the ionic strength range 0.06 to 0.11. Values for n also remain relatively constant over this range, showing only slight deviations from 2.9 to 3.3.

The influences of base sequence composition on the interactions of *m*-AMSA and *o*-AMSA are shown in Figure 3. Scatchard analysis for the two isomers binding to the synthetic polynucleotides poly(dAdT)·poly(dAdT), poly(dGdC)·poly(dGdC) and poly (dAdC)·poly-(dGdT) reveal that both *o*-AMSA and *m*-AMSA show a distinct preference for binding to the poly(dAdT)·poly(dAdT) copolymer. A slight preference for binding to poly (dAdC)·poly (dGdT) over the poly(dGdC)·poly(dGdC) is observed for both compounds. Of the three alternating copolymers examined, both *o*-AMSA and *m-AMSA* demonstrate their lowest binding affinity to the poly(dGdC)·poly(dGdC) which is comparable to the binding constants observed for the interactions of these drugs with the calf thymus DNA.

The binding site exclusion parameter, n, for both isomers binding to poly (dAdT)·poly(dAdT) and poly (dAdC)·poly (dGdT) is slightly lower than values obtained for the binding to calf thymus DNA. Whether this implies that the drugs actually occlude fewer sites on the synthetic polymers or that negative cooperative effects are translated differently in the synthetic polymers is speculative, however, both compounds maintain the same values of n as obtained for the native DNA and poly (dGdC)·poly(dGdC).


A
B
r/Cf x 10 4 (M -1)
r (base pairs)
4.0
3.0
2.0
1.0
0.04
0.08
14.0
12.0
10.0
8.0
6.0
4.0
2.0

Thermodynamic Characteristics for the Binding of m-AMSA and o-AMSA to DNA.

The influence of temperature on the drug-DNA complex was used to determine the DNA binding enthalpies of both *m-AMSA* and *o*-AMSA and are presented in Table II. Effects of ionic strength on the binding enthalpies were examined and found to differ by less than 1 kcal/mol over the range of sodium chloride concentrations studied. The binding enthalpies were determined using classical van't Hoff analysis and by the constant ratio method described earlier. The van't Hoff data had a minimum correlation coefficient of 0.99 over the temperature range 25°C to 5°C, taken in five degree intervals and did not vary between experiments by more than ±0.3 kcal/mol. The $\Delta G°_{binding}$ data was calculated using the corrected binding constants provided in Table I. The $\Delta H°_{binding}$ was corrected for percent protonation from the observed $\Delta H°$ values as described in the experimental section. Typical heats of protonation for heterocyclic nitrogens such as acridine, 9-aminoacridine, 4-aminopyridine, and 4-aminoquinoline are on the order of +10 kcal/mol (19). This approximate value was used to correct for contributions to the observed $\Delta H°$ values from heats of protonation thus providing a more accurate description of the actual $\Delta H°_{binding}$. At pH 7, the *o*-AMSA was calculated to be 78% protonated compared to *m-AMSA*'s 89% protonation. Values of this magnitude could lead to significant errors in the data analysis if left unaccounted.

For example, the observed enthalpies of binding for *m-AMSA* to all DNA's (except poly (dAdC)·poly (dGdT)) are approximately 1 kcal/mol more negative than for the *o*-AMSA isomer. The contribution due to protonation of the *m-AMSA* enthalpy is -1.1 kcal/mol and is -2.2 kcal/mol for the *o*-AMSA . Correcting for different states of protonation result in enthalpy values which differ by approximately 2 kcal/mol, with the *m*-AMSA having the more negative value. Both isomers bind to poly(dAdC)·poly (dGdT) with a difference in the binding enthalpy of less than one kcal/mol.

The interaction of *m-AMSA* with calf thymus DNA is characterized by a corrected binding enthalpy of -5.2 kcal/mol. The structurally similar *o*-AMSA binds calf thymus DNA with corrected binding enthalpy of -3.0 kcal/mol. This difference in binding enthalpies becomes more obvious when the binding entropies are considered. The entropy of binding for the *m-AMSA* -DNA interaction was found to be 2.0 cal/mol °K. In contrast, an entropy of binding of 11.3 cal/mol °K was observed for the *o*-AMSA -calf thymus DNA interaction, thus providing a distinct difference in the thermodynamic binding profile between the two isomers.

Figure 2. Scatchard analysis showing the effests of varying salt concentration on the binding properties of *m*-AMSA (A) and *o*-AMSA (B) to calf thymus DNA. Binding isotherms were measured at 20°C and the following sodium ion concentrations: 0.05 M (□-□), 0.085 M (Δ-Δ), and 0.11 M (◇-◇) . The solid lines are the least-square fits of the neighbor-exclusion model (Equation 2) to the experimental data usijng the Simplex algorithm. Fitting parameters are provided in Table I. Inset in both panels represents the dependence of the intrinsic binding constant K on the ionci strength expressed as $[Na^+]$.

Table I. Equilibrium binding of *m*-AMSA and *o*-AMSA to native and synthetic DNAs[a]

Drug	DNA	Ionic Strength	K_{obs} (M^{-1})	n	$K_{binding}$ (M^{-1})
m-AMSA					
	calf thymus	0.11	$1.7x10^4$	3.8	$2.0x10^4$
	calf thymus	0.085	$2.6x10^4$	3.1	$2.9x10^4$
	calf thymus	0.06	$4.2x10^4$	3.1	$4.7x10^4$
	poly (dGdC)·poly(dGdC)	0.11	$1.6x10^4$	3.1	$1.8x10^4$
	poly (dAdT)·poly (dAdT)	0.11	$3.4x10^4$	2.3	$3.8x10^4$
	poly (dGdT)·poly (dAdC)	0.11	$2.2x10^4$	1.9	$2.4x10^4$
o-AMSA					
	calf thymus	0.11	$4.2x10^4$	3.0	$5.4x10^4$
	calf thymus	0.085	$6.6x10^4$	2.9	$7.4x10^4$
	calf thymus	0.06	$1.4x10^5$	3.3	$1.6x10^5$
	poly (dGdC)·poly (dGdC)	0.11	$6.3x10^4$	2.9	$8.1x10^4$
	poly (dAdT)·poly (dAdT)	0.11	$1.4x10^5$	2.4	$1.8x10^5$
	poly (dGdT)·poly (dAdC)	0.11	$7.5x10^4$	2.6	$9.5x10^4$

[a] K refers to the intrinsic binding constant obtained from fitting the McGhee and von Hipple equation (equation 2) to the experimental data using the Simplex program with the ω term constrained to 0.45 and n is the exclusion parameter. $K_{binding}$ refers to the equilibrium binding constant which has been corrected for the degree of drug protonation. Data shown in this Table were obtained at 20°C using titration methods describe in the text.

Both isomers show a more positive enthalpy of binding to poly(dGdC)·poly(dGdC) than to native calf thymus DNA at 0.1 M NaCl. with the *m-AMSA* being 1.3 kcal/mol more positive. Furthermore, *m-AMSA* exhibits a three-fold increase in the entropy contribution upon binding poly(dGdC)·poly(dGdC) as compared to its binding to calf thymus DNA. The entropy contribution for the binding of *o*-AMSA to poly(dGdC)·poly(dGdC) also shows a marginally increased contribution of 2.3 cal/mol °K over that observed for the binding of calf thymus DNA.

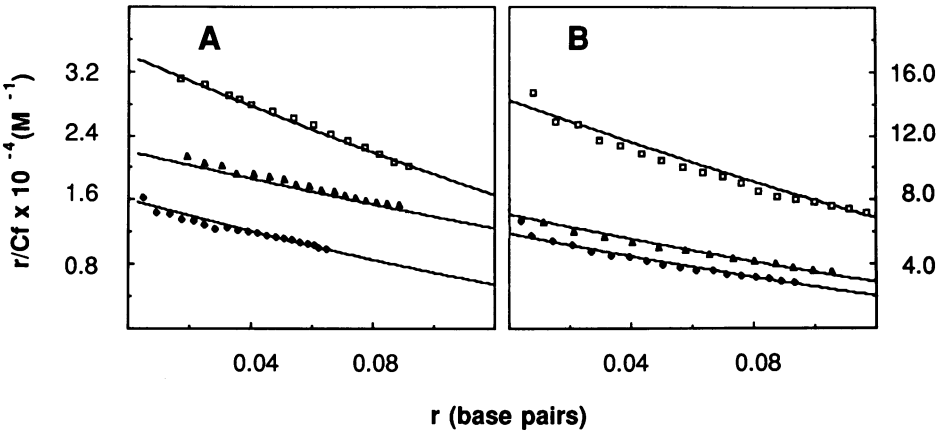


Figure 3. Effects of DNA base sequence on the interactions of *m*-AMSA (A) and *o*-AMSA (B) binding is shown by representative scatchard plots. The binding isotherms were obtained at 20°C and at a salt concentration of 0.1 M. The open squares (□-□) represent binding to poly(dAdT)·poly(dAdT), the open triangles (Δ - Δ) poly(dGdT)·poly(dAdC), and the diamonds (◇-◇) poly(dGdC)·poly(dGdC). The solid line drawn through the data is the least-square fit of the neighbor exclusion model (Equation 2) to the experimental data using the Simplex algorithm. Values used to fit this equation are provided in Table III.

Binding enthalpies for the two isomers to poly(dAdT)·poly(dAdT) show distinct variations also. The binding enthalpy of *o*-AMSA to poly(dAdT)·poly(dAdT) is equivalent to the values obtained for poly(dGdC)·poly(dGdC), both of which are only 0.6 kcal/mol more positive than the calf thymus DNA interaction. In contrast, *m-AMSA* demonstrates a distinct preference for binding to poly(dAdT)·poly(dAdT) copolymer over the alternating poly(dGdC)·poly(dGdC) copolymer by 1.4 kcal/mol. The enthalpy of binding of *m-AMSA* to poly(dAdT)·poly(dAdT) is identical to the value obtained upon binding native calf thymus DNA.

The entropic contributions are shown to increase for both the *o*-AMSA and *m-AMSA* in binding poly (dAdT)·poly(dAdT) . While the entropy of binding for *m-AMSA* is increased by only 0.7 cal/mol °K, the *o*-AMSA is characterized by much larger increase of 4.4 cal/mol °K over binding to calf thymus DNA under identical conditions.

The *o*-AMSA exhibits a preference for binding poly(dAdC)· poly(dGdT) over calf thymus DNA by 1 kcal/mol while the *m-AMSA* shows an identical affinity for binding poly(dAdC)·poly(dGdT) as to calf thymus DNA. The enthalpies of binding for *o*-AMSA and *m-AMSA* to poly(dAdC)·poly(dGdT) differ by less than 1 kcal/mol. The entropy of binding for *m-AMSA* is increased by 1.4 cal/mol °K upon binding the poly(dAdC)·poly (dGdT) when compared to calf thymus DNA binding. However, the binding entropy observed upon the interaction of *o*-AMSA with poly(dAdC)·poly(dGdT) is shown to decrease by 2.8 cal/mol K to a value of 8.5 cal/mol °K compared to binding to the calf thymus DNA.

Table II. Thermodynamic Characteristics for the interactions of *m*-AMSA and *o*-AMSA to native and synthetic DNAs

Drug DNA	Ionic Strength (M)	$\Delta G^{\circ}_{binding}$[a] (kcal mol^{-1})	ΔH°_{obs}[b] (kcal mol^{-1})	$\Delta H^{\circ}_{binding}$[c] (kcal mol^{-1})	$\Delta S^{\circ}_{binding}$[d] (cal mol^{-1} K^{-1})
m-AMSA					
calf thymus	0.11	-5.8	-6.3	-5.2	2.0
calf thymus	0.085	-6.0	-6.5	-5.4	2.0
calf thymus	0.06	-6.3	-7.3	-6.2	0.3
poly (dGdC)· poly (dGdC)	0.11	-5.7	-5.0	-3.9	6.1
poly (dAdT)· poly (dAdT)	0.11	-6.1	-6.4	-5.3	2.7
poly (dGdT)· poly (dAdC)	0.11	-5.8	-5.9	-4.8	.4
o-AMSA					
calf thymus	0.11	-6.3	-5.2	-3.0	11.3
calf thymus	0.085	-6.5	-5.2	-3.0	11.9
calf thymus	0.06	-6.9	-6.3	-4.1	9.6
poly (dGdC)· poly (dGdC)	0.11	-6.4	-4.6	-2.4	13.6
poly (dAdT)· poly (dAdT)	0.11	-7.0	-4.6	-2.4	15.7
poly (dGdT)· poly (dAdC)	0.11	-6.5	-6.2	-4.0	8.5

[a] ΔG° was calculated from the relationship $\Delta G = -RT\ln K$, where K is the $K_{binding}$, corrected for fractionation of protonated drug at pH 7. [b,c] The ΔH°_{obs} and $\Delta H^{\circ}_{binding}$, corresponding to the uncorrected and corrected enthalpies, respectively, were obtained from the slope of the van't Hoff plots. [d] The entropies were determined from the relationship $\Delta S^{\circ}_{binding} = (\Delta G^{\circ}_{binding} - \Delta H^{\circ}_{binding})/T(^{\circ}K)$.

Ionic Strength Effects on the Binding of m-AMSA and o-AMSA to DNA. The influence of the ionic strength on the equilibrium binding of both *m-AMSA* and *o*-AMSA to native DNA were examined the results presented in Table III. These effects can be analyzed using the method of Record and Manning (20, 21) where:

$$\frac{\delta \ln K}{\delta \ln [Na^+]} = -Z\psi \qquad [7]$$

Table III. Ionic Strength Dependence of Binding Constants at 20°C for Amsacrine Analogs.

Compound	$[Na^+]$ (M^{-1})	K_{obs}	Z^a	Z^{*b}
m-AMSA				
	0.11	1.7×10^4	1.8	1.5
	0.085	2.6×10^4		
	0.06	4.2×10^4		
	0.02	2.6×10^5		
o-AMSA				
	0.13	4.0×10^4	1.9	1.6
	0.11	4.2×10^4		
	0.085	6.6×10^4		
	0.06	1.4×10^5		

[a] Z is the effective charge on the drug calculated by displacement of counterions on duplex DNA using equation 6. [b] Z* is the effective charge on an intercalating drug derived from equation 7.

In which K is the observed equilibrium, $[Na^+]$ is the sodium ion concentration, Z is the effective electric charge on the drug and ψ is the fraction of counter ions associated with each DNA phosphate. For double stranded DNA $\psi = 0.88$.

Alternatively, Wilson and Lopp (22) have derived a similar expression to account for changes in the DNA structure upon intercalation by a ligand. This expression:

$$\frac{\delta \ln K}{\delta \ln [Na^+]} = -2n (\psi - \psi^*) - Z^*\psi \qquad [8]$$

where K and ψ are as before and ψ^* is the fraction of counter ion per phosphate in the intercalated drug-DNA complex. Z* is the effective electric charge on the drug in this model and n is the number of neighboring sites excluded upon ligand binding. An average value of n = 3.0 and $\psi^* = 0.82$ has been used to calculate the Z* values in Table III.

These models provide consistent results demonstrating that both *o*-AMSA and *m-AMSA* bind to calf thymus DNA with similar effective charges. Both *m-AMSA* and *o*-AMSA are shown to carry a charge of between 1.5 to 2, based on the Z* and Z values observed in Table III. The DNA interactions of similar amsacrine derivatives which lack the methanesulfonamido functional group result in the determination of values of Z* close to 1.0, indicating that the presence of the methanesulfonamido group results in a partial positive charge on the molecule.

DISCUSSION

Binding of o-AMSA and m-AMSA to Calf Thymus DNA.

The present studies provide a unique insight into the mechanism(s) of the interactions of *o*-AMSA and *m-AMSA* with nucleic acids. Although these drugs are structural isomers, and both exhibit intercalative binding to DNA, the *m-AMSA* demonstrates effective antitumor activity while the *o*-AMSA is biologically inactive. An interesting paradox is observed in the correlation the DNA binding affinities of these compounds with the antitumor activities. The biological activity of the *m-AMSA* (and several other antitumor agents) seems to be manifested through its intercalative interaction with DNA, however, the biologically inactive *o*-AMSA demonstrates a higher affinity for binding DNA than does *m*-AMSA. Theoretical calculations and molecular modeling studies suggest that the differences in the antitumor activities of the two isomers may arise from differences in the mechanism by which these compounds inhibit topoisomerase II. The mechanism of this inhibition may be mediated through select DNA structural perturbations leading to changes in the enzyme recognition site or through ternary complexes formed between the enzyme and the drug-DNA complex. However, a more in-depth analysis of the equilibrium binding and thermodynamic properties associated with the interactions of these structural isomers with DNA reveals key evidence towards unraveling some of the basic differences in the mechanisms of complex formation between the *m-AMSA* and *o*-AMSA with DNA.

As shown in Table I, *o*-AMSA is shown to exhibit a higher affinity for calf thymus DNA when compared to *m-AMSA*. However, the thermodynamic contributions governing the formation of the drug-DNA complex are different for the two molecules. The interaction of *o*-AMSA with DNA is characterized by an enthalpy of -3.0 kcal/mol which is approximately one-half the value of the free energy of binding. However, the entropy value for this interaction was found to be 11.3 cal/mol°K, revealing the binding of *o*-AMSA to DNA to be an entropy driven process. Positive entropic values of this magnitude suggest that the hydrophobicity of the compound plays an important role in the transfer of the drug molecule from the solvent to the intercalation site (23).

In contrast, the interaction of *m-AMSA* with DNA exhibits a binding enthalpy of -5.2 kcal/mol. This value comprises virtually all of the free energy of binding (-5.8 kcal/mol), indicative of an enthalpy driven process for the binding of the biologically active *m-AMSA* to DNA. The similarity of structure of the two molecules would seem to preclude these distinct differences in binding modes as being a result of different states of solvation. The observation that both compounds release equivalent amounts of counterions as shown in Table III suggests that both compounds interact with nucleic acids through a common mode via intercalation of the acridine ring between adjacent base pairs of the DNA. However, these studies demonstrate that the methanesulfonanidide ring and the placement of the methoxy groups at either the 2' or

3' position plays a crucial role determining whether the DNA binding process is entropy or enthalpy driven.

The data presented here suggests that the both *o*-AMSA and *m-AMSA* may be hindered from full insertion of their acridine rings into the DNA as evidenced by the low enthalpy of binding as compared to other intercalators such as ethidium bromide and 9-aminoacridine, however, both the *o*-AMSA and *m-AMSA* exhibit identical DNA unwinding angles of 20.5°(10-11). A plausible explanation for the difference in the binding enthalpies of the two isomers would be that the methanesulfonamido group of *m-AMSA* is more able to make a particular contact with the minor groove backbone of the DNA than the same group in *o*-AMSA. This could be due in part to steric or electronic interference by the methoxy group in the 2' position of the *o*-AMSA .

Hopkins (24) and Breslauer (25) have shown that the electrostatic interactions between ethidium, DAPI, and netropsin contribute little toward the overall binding enthalpies of these drug-DNA complexes. Thus, it is plausible to speculate that the methanesulfonamido group of *m-AMSA* might form an additional hydrogen bond in the minor groove of the DNA. Such a minor groove interaction would explain the observation of the loss in the entropic driving force of the DNA binding mechanism since the drug-DNA complex would be energetically constrained within a limited span of conformations.

Binding of o-AMSA and m-AMSA to Synthetic DNA's.

The base sequence composition is shown to greatly influence the thermodynamic binding properties of *o*-AMSA and *m-AMSA*. While *m-AMSA* binding to calf thymus DNA is predominantly enthalpy driven, its interaction with poly(dGdC)·poly(dGdC) appears to be a more entropic process as illustrated by the magnitude of the 3-fold increase in the ΔS° value from 2 to 6.1 cal $mol^{-1}{}^\circ K^{-1}$. A positive increase in the enthalpy of binding to the poly(dGdC)·poly-(dGdC) would be predicted since the energy needed for intercalation into the double helix of this polynucleotide (i.e., unstacking enthalpy) is greater than for randomly sequenced DNAs or the other alternating synthetic copolymers (26).

The binding of *o*-AMSA to poly(dGdC)·poly(dGdC) exhibits the same thermodynamic profile as observed with its interaction to calf thymus DNA. Complex formation with the poly(dGdC)·poly(dGdC) copolymer results a loss in the binding enthalpy of 0.6 kcal/mol for the *o*-AMSA . In addition, an increase in the binding entropy value is observed, and thus may explain the preferential binding of *o*-AMSA to poly(dGdC)·poly(dGdC) over calf thymus DNA.

Both *o*-AMSA and *m-AMSA* show higher affinities for the poly(dAdT)·poly(dAdT) and to poly(dAdC)·poly(dGdT) than to calf thymus DNA. The thermodynamic profile is similar to those for calf thymus DNA, suggesting that *m*-AMSA may preferentially bind to areas of high AT or AC·GT content in the native DNA. The preference of *o*-AMSA for the

poly(dAdT)·poly(dAdT) results mainly from the increase in entropy as compared to calf thymus DNA while the enthalpic component show a slight decrease. Conversely, the preference observed for poly(dAdC)·poly(dGdT) probably arises from the more negative enthalpic component and a decrease in entropy. The stacking energy of poly(dAdC)·poly(dGdT) is the lower than that for poly(dAdT)·poly(dAdT) and could result in the decreased binding enthalpy (26).

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