Selective Stabilization of Triplex DNA by Anthraquinone Sulfonamide Derivatives†

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ABSTRACT: A series of cationic anthraquinone derivatives was investigated for their ability to stabilize duplex and triplex DNA. Thermal denaturation experiments demonstrate that each of these compounds stabilizes the [poly(dT)·poly(dA)×poly(dT)] triplex without significantly affecting the [poly(dT)·poly(dA)] duplex. The amount of stabilization is determined by the number and placement of the cationic substituents on the anthraquinone skeleton. The stabilization arises primarily from higher affinity binding of the quinones to the triplex relative to the duplex structures. Phosphorescence quenching and viscometric titrations indicate that the quinones bind to the triplex by intercalation.

Certain base sequences of duplex DNA can form a triple helix (triplex) by binding a complementary third strand in the major groove through Hoogsteen hydrogen bonding. Triplex formation ordinarily requires a pyrimidine·purine× pyrimidine (the × indicates the Hoogsteen base pairing) base triplet composed of either T·A×T [poly(dT)·poly(dA)×poly(dT)] or C·G×C^+ [poly(dC)·poly(dG)×poly(dC^+)] (C^+ is protonated cytosine) (Felsenfeld *et al.*, 1957; Felsenfeld & Miles, 1957; Le Doan *et al.*, 1987; Moser & Dervan, 1987). Triplex formation provides an ability to recognize and associate small molecules specifically with selected sequences of duplex DNA (Scaria & Shafer, 1991; Wilson *et al.*, 1993; Mergny *et al.*, 1992). Important applications for triplex DNA are based on this property. For example, Dervan and Moser prepared a homopyrimidine oligonucleotide conjugated with iron−EDTA at the 5′-end that selectively cleaves DNA and is useful for chromosome mapping (Moser & Dervan, 1987). However, stabilization of these triplexes requires acidic pH or relatively high concentrations of divalent cations. Their instability under normal physiological conditions is a critical limitation that restricts the uses of triplex DNA *in vivo*. Consequently, various approaches are being explored to improve their stability.

Triplex DNA can be stabilized by modification of the base (Griffin *et al.*, 1992; Stilz & Dervan, 1993) or backbone (Wilson *et al.*, 1993; Zon, 1988; Nielsen, *et al.*, 1991), and by the addition of compounds that bind in the minor groove (Tung *et al.*, 1993) or by intercalation (Sun *et al.*, 1989). There are several recent reports of studies that examine association of intercalators and groove binders to triplex DNA, which often have significant effects on triplex stability. For example, polyamines, such as spermidine and spermine, stabilize the triplex presumably by reducing the electrostatic repulsion of the negatively charged phosphate groups (Hampel *et al.*, 1991). Acridines, when covalently linked to the 5′-end of the third strand, stabilize the triplex by intercalating at the duplex−triplex junction (Cassidy *et al.*, 1994). Wilson and Strekowski have undertaken a systematic search for intercalators that specifically stabilize triplex DNA (Wilson *et al.*, 1993). They reported that certain unfused aromatic quinolinium cations significantly increase the melting temperature ($\Delta T_{3\rightarrow2}$) of a T·A×T triplex while having a much smaller effect on the melting temperature of the T·A duplex ($\Delta T_{2\rightarrow1}$). Latimer and co-workers (1995) studied a series of protonated drugs related to the alkaloid sanguinarine chloride and also found a general stabilization for the T·A×T triplex compared with the T·A duplex but observed that these compounds did not bind well to triplexes having C·G×C^+ triplets. Hélène and co-workers described a series of benzopyridoindole derivatives that stabilize both T·A×T and C·G×C^+ containing triplexes more than they do the corresponding duplexes (Mergny *et al.*, 1992; Escudé *et al.*, 1995).

Fox and co-workers (1995) recently reported that certain 2,6-disubstituted cationic amidoanthraquinones stabilize the triplex formed by a T_5C_5 third strand but that the corresponding 1,4-disubstituted derivatives did not. Their findings are closely related to the work described in this report. Also of relevance to our work is the observation that some compounds intercalate into triplex DNA even when they bind by other means to duplex structures. In particular, Tuite and Nordén (1995) report that methylene blue binds to structures containing the T·A×T triplets by intercalation even though it binds the corresponding T·A duplex in its major groove.

We have been examining the ability of certain cationic anthraquinone derivatives to cleave duplex DNA (Koch *et al.*, 1993; Armitage *et al.*, 1994; Breslin & Schuster, 1996; B. Armitage and G. B. Schuster, submitted; Ly *et al.*, 1996). In that connection we characterized the interactions of the anthraquinone sulfonamide derivatives, shown in Chart 1, with both the triplex T·A×T and the duplex T·A. This study reveals the effect of structural features, such as the position of the substituents on the anthraquinone and the charge of those substituents, on the binding of these anthraquinone derivatives to duplex and triplex DNA. In particular we find that 2,7-disubstituted anthraquinone tetracation (**6**) is a groove binder for T·A and does not stabilize it but that it is an intercalator for T·A×T and stabilizes it significantly.

MATERIALS AND METHODS

Nucleic Acids and Chemical Ligands. Synthetic DNA polymers were purchased from Pharmacia Biotech, Inc. (Piscataway, NJ) and used without further purification. Concentrations of all DNA polymers were determined

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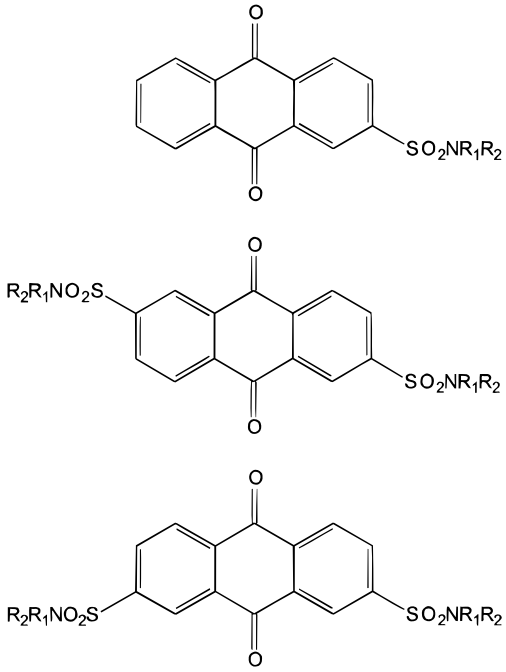
Chart 1



1 $R_1 = H$ $R_2 = (CH_2)_3NH_3Cl$

2 $R_1 = R_2 = (CH_2)_3NH_3Cl$

3 $R_1 = H$ $R_2 = (CH_2)_3NH_3Cl$

4 $R_1 = R_2 = (CH_2)_3NH_3Cl$

5 $R_1 = H$ $R_2 = (CH_2)_3NH_3Cl$

6 $R_1 = R_2 = (CH_2)_3NH_3Cl$

spectrophotometrically in PBS buffer (10 mM sodium phosphate, 100 mM NaCl, pH 7.0), using the following extinction coefficients in units of (mol of nucleotide/L)$^{-1}$ cm^{-1}: $\epsilon_{265} = 8700$ for poly(dT), $\epsilon_{260} = 6000$ for T·A. Solutions containing the T·A×T triplex were prepared by mixing T·A and poly(dT) in a 1:1 molar ratio, heating to 90 °C, and then cooling to room temperature.

The syntheses of cationic anthraquinone derivatives was reported previously (Armitage *et al.*, 1994). Concentrations were determined spectrophotometrically using the following extinction coefficients in units of (mol/L)$^{-1}$ cm^{-1} ($\lambda_{max} \cong$ 327 nm): $\epsilon_{PBS} = 5400$ for **1** and **2**, $\epsilon_{PBS} = 5900$ for **3** and **4**, $\epsilon_{PBS} = 6500$ for **5** and **6**.

UV Spectrophotometry. Absorbance versus temperature profiles were measured at 260 nm on a computer-interfaced Cary 1E spectrophotometer (Varian Techtron Pty. Ltd., Victoria, Australia) equipped with a thermoelectrically controlled cell holder. The heating and cooling rates in all experiments were 0.5 °C/min. The melting temperature was determined from first-derivative plots for each optically detected transition.

All thermal denaturation studies were conducted in PBS. [Triplexes formed spontaneously under these conditions without addition of polyvalent cations such as Mg^{2+} or spermine. Wilson and co-workers (1993) report similar procedures.] Polynucleotide solutions contained DNA at a concentration of 20 μM base pairs or base triplets. The substituted anthraquinone concentrations ranged from 0 to 20 μM.

Fluorimetry. The equilibrium binding constants of the ethidium bromide (EtBr) with T·A×T triple helices and T·A duplexes were obtained by a fluorescence titration method in the absence of anthraquinones. Samples, typically 5 μM DNA in PBS, were titrated with 5 μL aliquots of 100 μM EtBr. Additions of 5 μL aliquots of a solution containing 10 μM DNA and the appropriate concentration of quinone were made at the same time in order to maintain the concentration of DNA as well as the ratio of quinone to DNA. Spectra were obtained with excitation at 510 nm, and the emission was monitored from 550 to 650 nm. Integrated spectra were used for data analysis. The data were analyzed according to the method of LePecq and Paoletti to obtain the bound (c_b) and free (c_f) concentrations of EtBr (LePecq & Paoletti, 1967). Plotting r/c_f vs r (where $r = c_b/[DNA]$) and fitting to the McGhee−von Hippel equation yielded a binding constant (K_{EB}) for EtBr. In the absence of quinone, this value is equal to the intrinsic equilibrium binding constant for EtBr. In the presence of quinone, K is an apparent binding constant and can be used to calculate the equilibrium binding constant for the quinone (K_{AQ}) using the following equation:

$$K = K_{EB}/[1 + K_{AQ}C_f^{AQ}]$$

where C_f^{AQ} is approximately equal to the total concentration of quinone. This method has been described in detail elsewhere (Armitage *et al.*, 1994).

Phosphorimetry. Phosphorescence measurements were carried out on a SPEX Fluorolog spectrofluorometer. The cell holder was maintained at a constant temperature of 77 K with liquid nitrogen. Experimental solutions in PBS buffer (30% v/v ethylene glycol) contained 10 μM of the quinone and 0 or 100 μM base pairs or base triplets of DNA. The phosphorescence emission spectra were measured from 400 to 650 nm, with an excitation wavelength of 340 nm. The phosphorescence intensities were obtained by integrating the emission spectra.

Viscometry. The viscosity measurements were carried out in a capillary viscometer submerged in a water bath maintained at 30.5 (±0.1) °C. Flow times were measured to ±0.1 s. Viscosity studies on T·A duplex and T·A×T triple helix were conducted in PBS buffer solutions. Aliquots (2.5−20 μL) of 1 mM quinone stock solutions were titrated directly into the solutions (1 mL) of 100 μM DNA (in units of base pair or base triplet), and flow times in the range 103−115 s were measured after each addition.

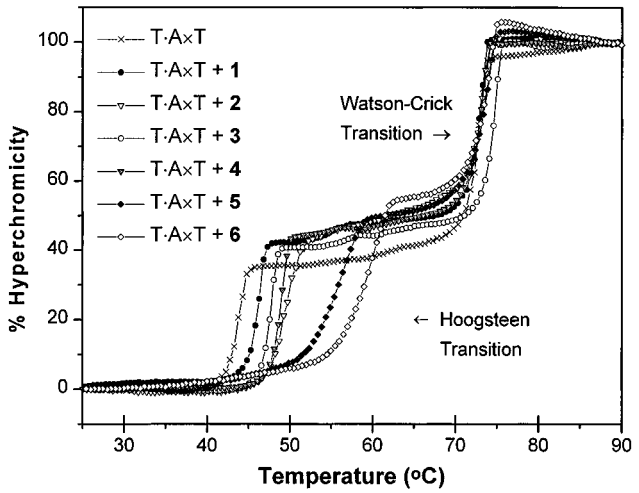


FIGURE 1: UV melting profiles at 260 nm for the T·A×T triplex and the complexes with anthraquinones **1**−**6** at ratio of triplets to AQ equal to 4. Base triplet concentrations were 20 μM in PBS buffer (10 mM sodium phosphate, pH 7.0, 100 mM NaCl). The Hoogsteen (HG) and Watson−Crick (WC) transitions are indicated.

RESULTS

Thermal Denaturation Study. The UV melting curves for triplex T·A×T in solutions containing the variously substituted anthraquinone sulfonamides are presented in Figure 1. The melting profiles are clearly biphasic, as is typically observed when T·A×T are formed (Wilson *et al.*, 1993). The low-temperature transition (Hoogsteen transition) corresponds to the conversion from the triplex to a duplex and a single-strand of poly(dT). The second transition (Watson−Crick transition) corresponds to the dissociation of the remaining duplex T·A to single strands, determined by comparison with authentic T·A duplex. Significantly, while none of the anthraquinone sulfonamide derivatives we examined stabilizes the Watson−Crick transition, each significantly stabilizes the triplex thereby increasing its observed melting temperature.

At the same loading density (the molar ratio of quinone to base triplet is 0.25), anthraquinone sulfonamides **1**−**6** display distinctly different capabilities for stabilizing the triple helix (Table 1). Of particular note, the Hoogsteen transition increases by 15.5 °C in the presence of **6**, however, the Watson−Crick transition is not affected within the limits of detection by this quinone.

For comparison, the effect of these quinones on the melting temperature of duplex T·A [i.e., in the absence of a single strand of poly(dT)] was also investigated. As shown in Table 1, there is no detectable stabilization of the duplex by any of these quinones, consistent with the observations for the T·A×T triplex. This demonstrates that the failure to stabilize the higher temperature transition in the T·A×T system is not due to binding of the quinones to the single strand of poly(dT) present in solution.

The data in Table 1 reveal two structural features of quinones that appear to control the stabilization of the triple helix. Except for **3**, a higher charge density on the quinone leads to greater triplex stabilization. However, the location of the positive charges on the quinone skeleton has a significant impact on the amount of stabilization. For example, the 2,7-disubstituted analogs **5** and **6** provide approximately twice as much stabilization as afforded by the corresponding 2,6-disubstituted isomers **3** and **4**. In fact, dication **3** is indistinguishable from monocation **1** in its stabilization of the T·A×T triplex. Similarly, tetracation **4** provides nearly the same stabilization as dication **2**, its monosubstituted analog. These data demonstrate that, at least for sulfonamide derivatives, 2,6-disubstitution affords no additional stabilization of T·A×T triplexes relative to 2-monosubstitution.

Table 1: Effect of Anthraquinones on the Thermal Stability of Triplex and Duplex DNA

		[triplets][a]	T·A×T		T·A
AQ	charge	[AQ]	$\Delta T_{3\rightarrow2}$ (°C)	$\Delta T_{2\rightarrow1}$ (°C)	$\Delta T_{2\rightarrow1}$ (°C)
1	+1	4	2.7[b]	0	0.5
		2	4.5	0	
		1	6.5	0.5	
2	+2	4	6.0	0.5	−0.5
		2	7.0	0.5	
		1	10.4	0.5	
3	+2	4	2.7	0.2	0.2
		2	4.5	0.5	
		1	7.3	0.7	
4	+4	4	7.2	0.5	0.1
		2	11.3	1.3	
		1	16.8	2.9	
5	+2	4	5.0	0.2	0.6
		2	7.5	0.4	
		1	11.0	1.0	
6	+4	4	15.5	0.5	−0.1
		2	19.5	2.0	
		1	23.4	2.9	

[a] Ratio of base triplets to quinone molecules in solution. The concentration of DNA is 20 μM in base triplets. [b] The experimental error in ΔT is ±0.5 °C determined by repetitive measurment.

Table 2: Binding Constants for Anthraquinones to Triplex and Duplex DNA

		binding constants ($\times10^{-5}$ M^{-1})	
AQ	charge	T·A×T	T·A
1	+1	2.13	0.51
2	+2	5.04	0.90
3	+2	1.91	0.86
4	+4	4.94	1.18
5	+2	5.28	1.56
6	+4	21.7	3.92
EtBr[a]	+1	2.87	0.44

[a] These values for ethidium bromide are about 10-fold greater than those reported by Scaria and Shafer (1991), where a UV−vis method was used to determine the binding constants. Their measurements were conducted in basic buffer (10 mM cacodylate buffer, pH 7.2, containing 0.5 mM EDTA) plus 100 mM NaCl for the T·A duplex, and the basic buffer plus 5 mM $MgCl_2$ for T·A×T.

Binding Constants. The equilibrium binding constants for the quinone sulfonamides with duplex and triplex DNA were obtained by competition with ethidium bromide. The results are summarized in Table 2. In each instance, the equilibrium constant for binding to the triplex is greater than it is for the duplex. For the case of the 2,7-tetracationic quinone **6**, the binding constant with the triplex is more than five times greater than it is for the duplex. The binding constants of these quinones with triplex DNA follow the same structural trends as does their ability to stabilize the triplex: larger K_{assoc} values correlate with larger $\Delta T_{3\rightarrow2}$.

Phosphorescence Quenching Studies. We have discovered that the ability of duplex DNA to quench the phosphores-

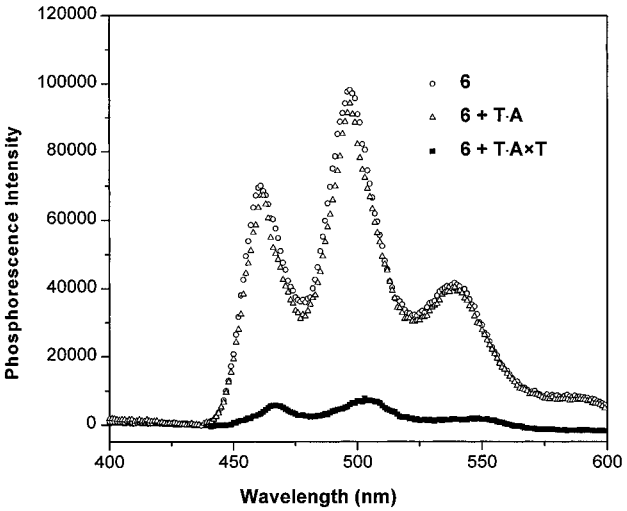


FIGURE 2: Phosphorescence emission spectra of AQ **6** when free in solution or bound to either the T·A duplex or the T·A×T triplex. Solutions contain 10 mM sodium phosphate (pH 7.0), 100 mM NaCl, and 10 μM AQ. For AQ−DNA complexes, the ratio of base pair or base triplet to AQ is 10. Excitation wavelength is 340 nm, and the temperature was controlled with liquid N_2.

Table 3: Effect of Anthraquinone Binding to Triplex or Duplex on Phosphorescence.

		phosphorescence quenched (%)	
AQ	charge	T·A×T	T·A
1	+1	75	14
2	+2	89	31
3	+2	67	23
4	+4	81	23
5	+2	98	38
6	+4	97	3

cence of these substituted quinones is controlled by the binding mode (Breslin & Schuster, 1996). The phosphorescence of intercalated quinones is nearly completely quenched, while groove binding has a much smaller effect on the emission. We applied this assay to the investigation of these quinones with triplex DNA.

The phosphorescence of quinone **6** was monitored in frozen ethylene glycol-containing buffer solutions at 77 K. The effect of adding duplex or triplex DNA to these solutions is shown in Figure 2. The phosphorescence of **6** is essentially unaffected by the T·A duplex, but it is nearly completely quenched by the T·A×T triplex. The other quinones we examined show different but consistent results (Table 3). In each instance the duplex and triplex substantially lowers the phosphorescence intensity of the quinone. However, in every case the quenching by the triplex is more extensive than it is by the duplex. Significantly, the relative effectiveness of the phosphorescence quenching by triplex DNA follows the same order as the ability of the quinone to increase the melting temperature of T·A×T.

Viscometric Measurements. The change in viscosity of DNA-containing solutions is a clear indicator of ligand binding mode. Intercalation between the base pairs of both natural and synthetic duplex polynucleotides results in an increase in solution viscosity due to ligand-induced lengthening of the helix (Bloomfield *et al.*, 1974). On the other hand, binding to the minor groove causes very little change to the viscosity (Wilson *et al.*, 1990). Similar, though more complex, observations have been reported for triplex DNA

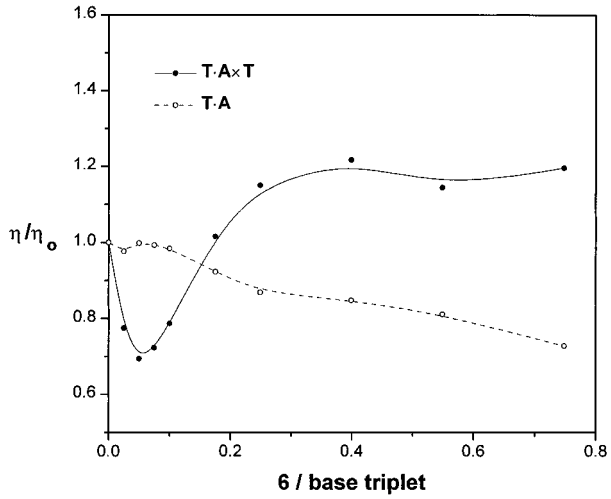


FIGURE 3: Viscometric titrations of the duplex T·A and triplex T·A×T with AQ **6** at 30.5 °C.

(Scaria & Shafer, 1991; Pilch *et al.*, 1993). We examined the change in solution viscosity on the addition of quinone **6** to T·A and T·A×T. The results are shown in Figure 3.

The total increase in viscosity and the shape of the curve seen for quinone **6** and T·A×T is similar to that reported for the binding of ethidium bromide or berenil to this polymer. In the latter case the initial decrease in viscosity seen for low concentrations is attributed to a ligand-induced conformational change (Pilch *et al.*, 1995). The addition of **6** to T·A results in only a slight decrease in viscosity that is typical of non-intercalative binding (D. T. Breslin and G. B. Schuster, submitted).

DISCUSSION

The experiments described above were designed to elucidate the mode of interaction of a family of anthraquinone sulfonamides with the T·A×T triplex. Their interactions with the T·A duplex were examined for comparison. The four sets of experiments we carried out lead to a consistent picture. Particularly in the case of quinone **6**, the results indicate binding by intercalation for the triplex and a nonintercalative binding for the duplex. This compound is the first we are aware of that exclusively stabilizes the triplex while having virtually no effect on the stability of the duplex.

The thermal denaturation and equilibrium binding studies show that these quinones bind more tightly to the triplex than to the duplex, and that they selectively stabilize the triple helix.[1] The magnitude of these effects depends on the specific quinone structure. We note that those quinones having greater positive charge density are the most tightly bound and have the greatest impact on the thermal stability of triple helix. Relief of electrostatic repulsions among the negatively charged phosphate backbones of the three DNA strands has been proposed as an important contribution to triplex stabilization (Cassidy *et al.*, 1994; Fox *et al.*, 1995). This may be one of the factors that contributes to stabilization of the triplex by quinone **6**. We also observed that the ability of the quinone to stabilize the triplex is dependent on the placement of the substituents: the 2,7-disubstitution pattern

[1] One explanation for the absence of stabilization of the poly[dA]-poly[dT] duplex by these quinones is that association of the quinone with the single strands is just as effective in modifying the free energy of the system.

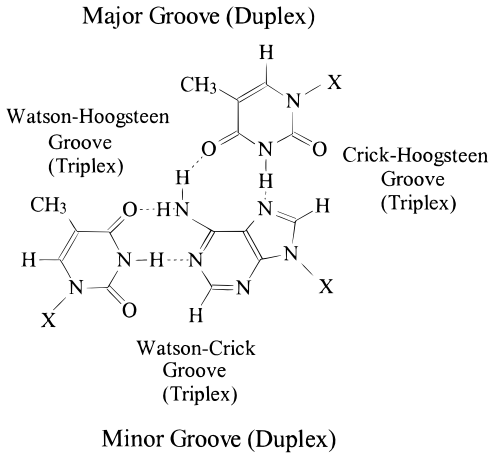


FIGURE 4: Model for triplex DNA.

is far more effective than is the 2,6-pattern. With respect to the work of Fox (1995), it is quite possible that 2,7-disubstituted amidoanthraquinones would give significantly greater effects than that reported for the 2,6-derivatives.

Our examination of **6** shows that it binds to triplex DNA by intercalation and to duplex DNA in the groove. The most compelling results come from the phosphorescence and viscosity experiments. The phosphorescence of **6** is essentially unquenched by duplex DNA and completely quenched by the triplex. The viscosity dependence of the triplex DNA with **6** follows the same pattern as ethidium bromide, which has been shown to be an intercalator in this polymer. In this regard, the behavior of **6** is similar to that of methylene blue which intercalates in the triplex and binds in the groove of the duplex but affords only minor triplex stabilization (Tuite & Nordén, 1995).

In our previous study (D. T. Breslin and G. B. Schuster, submitted), NMR spectroscopy, as well as other experimental results, indicates that quinone **6** is a groove binder in a DNA duplex structure. The propensity of quinone **6** to bind in the minor groove of duplex DNA may be related to its crescent shape. Since its phosphorescence is essentially unquenched in duplex A·T, the high charge density of its two substituents may immobilize it in the minor groove. The other quinones we examined behave as intercalators to mixed sequences of duplex DNA (unpublished results from our lab). The fact that their phosphorescence emission is only partially quenched may be due to partial intercalation in the well-known but unusual structure of T·A (Scaria & Shafer, 1991; Tuite & Nordén, 1995; Pilch *et al.*, 1993).

Another controlling structural feature of the interaction between these quinones and triplex DNA is the placement of the substituents. Formation of a triplex by binding a third strand in the major groove of the duplex splits it into two narrower grooves, see Figure 4. The minor groove of the duplex is comparatively unaffected by triplex formation. Fox and co-workers (1995) proposed that 2,6-amidoanthraquinone derivatives bind to the T·A×T triplex by "threading through". In this intercalative mode one substituent is in the minor groove (Watson−Crick groove), and the other is in the Watson−Hoogsteen groove (relatively wider than Crick−Hoogsteen groove, but narrower than Watson−Crick groove).

Within this model, the variable binding affinities and $\Delta T_{3\rightarrow 2}$ observed for different substituent placements may be explained in terms of either a base-stacking effect or by the control of electrostatic interactions. The intercalation geometry may depend on the substituent placement. A change of the orientation of the anthraquinone core relative to the base triplets in the intercalation site will affect stabilization through modulation of the π-electron overlap. If this is so, the 2,7-placement induces a more nearly optimal stacking interaction than the 2,6-placement of substituents.

A second explanation for the enhanced intercalation ability of the 2,7-disubstituted quinones is related to placement of the positively charged ammonium groups. For example, with the 2,7-placement one substituent can be in the Watson−Crick groove and the other in Crick−Hoogsteen groove. It has been suggested that the electrostatic potential of the minor groove of double helices is more negative than that of the major groove (Pullman & Pullman, 1981). We expect that the electrostatic potential of the three grooves in triple helices can be ordered as Crick−Hoogsteen > Watson−Hoogsteen > Watson−Crick. If so, and if one of the substituents in 2,7-placement can protrude into the Crick−Hoogsteen groove, a stronger electrostatic interaction between the quinone and triple helix DNA will result. Whatever the actual explanation for the enhanced stabilization caused by the 2,7-placement of substituents, this feature will be useful in the design of compounds that can specifically stabilize triplex DNA.

CONCLUSION

This study reveals that anthraquinones **1**−**6** bind to DNA triple helices primarily by intercalation, significantly increasing the triplex stability. All of the anthraquinones investigated display high affinity and selectivity for T·A×T triple helices *versus* T·A duplexes. While the current experiments have been restricted to T·A×T triplexes, the recent report by Neidle and co-workers demonstrating binding of AQ derivatives to mixed sequence triplexes (Fox et al., 1995) suggests that the AQ sulfonamides will also be able to selectively bind to such sequences. This prediction will be the target of future investigations.

We have found that the specific interaction of the quinone and DNA triplex is controlled by the number and placement of the positively charged sulfonamide side chains. Anthraquinones with 2,7-placement enhance stability of the triplex more than quinones with 2,6-placement do, i.e., **5** is better than **3** and **6** is better than **4**. This indicates that variation of the substituent placement may result in different intercalation geometries, which affect either the stacking forces between quinones and bases or the electronic interactions between the triplex grooves and substituents on the quinone.

The family of anthraquinones studied here initiate irreversible damage of duplex DNA at GG sites via a photoinduced electron transfer mechanism (Koch *et al.*, 1993; Armitage *et al.*, 1994; Breslin & Schuster, 1996; B. Armitage and G. B. Schuster, submitted; Ly *et al.*, 1996). The special ability of the quinones, tetracation **6** in particular, to intercalate in triplex DNA may provide a useful method for initiating electron transfer and base damage at GG sites within or near the triplex region. We are currently exploring this possibility.

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REFERENCES

Armitage, B., Yu, C., Devadoss, C., & Schuster, G. B. (1994) *J. Am. Chem. Soc. 118*, 9847−9859.

Bloomfield, V. A., Crothers, D. M., & Tinoco, I. (1974) *Physical Chemistry of Nucleic Acids*, 442−443, Harper and Row, New York.

Breslin, D. T., & Schuster, G. B. (1996) *J. Am. Chem. Soc. 118*, 2311−2319.

Cassidy, S. A., Strekowski, L., Wilson, W. D., & Fox, K. R. (1994) *Biochemistry 33*, 15338−15347.

Escudé, C., Nguyen, C. H., Mergny, J. L., Sun, J., Bisagni, E., Garestier, T., & Hélène, C. (1995) *J. Am. Chem. Soc. 117*, 10212−10219.

Fox, K. R., Polucci, P., Jenkins, T., & Neidle, S. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 7887−7891.

Felsenfeld, G., Davies, D. R., & Rich, A. (1957) *J. Am. Chem. Soc. 79*, 2023−2024.

Felsenfeld, G., & Miles, H. T. (1967) *Annu. Rev. Biochem. 36*, 407−448.

Griffin, L. C., Kiessling, L. L., Beal, P. A., Gillespie, P., & Dervan, P. B. (1992) *J. Am. Chem. Soc. 114*, 7976−7982.

Hampel, K. J., Crosson, P., & Lee, J. S. (1991) *Biochemistry*, *30*, 4455−4459.

Koch T., Ropp, J. D., Sligar, S. G., & Schuster, G. B. (1993) *Photochem. Photobiol. 58*, 554−558.

Latimer, L. J. P., Payton, N., Forsyth, G., & Lee, J. S. (1995) *Biochem. Cell. Biol. 73*, 11−18.

Le Doan, T., Perrouault, L., Praseuth, D., Habhoub, N., Decout, J.-L., Thuong, N. T., Lhomme, J., & Hélène, C. (1987) *Nucleic Acids Res. 15*, 7749−7760.

Lepecq, J. B., & Paoletti, C. J. (1967) *J. Mol. Biol. 27*, 87−106.

Ly, D., Kan, Y., Armitage, B., & Schuster, G. B. (1996) *J. Am. Chem. Soc. 118*, 8747−8748.

Mergny, J. L., Duval-Valentin, G., Nguyen, C. H., Perrouault, L., Faucon, B., Rougée, M., Montenay-Garestier, T., Bisagni, E., & Hélène, C. (1992) *Science 256*, 1681−1684.

Moser, H. E., & Dervan, P. B. (1987) *Science 238*, 645−650.

Nielsen, P. E., Egholm, M., Berg, R. H., & Buchardt, O. (1991) *Science 254*, 1497−1500.

Pilch, D. S., Waring, M. J., Sun, J., Rougée, M., Nguyen, C. H., Bisagni, E., Garestier, T., & Hélène, C. (1993) *J. Mol. Biol. 232*, 926−946.

Pilch, D. S., Kirolos, M. A., Breslauer, K. J. (1995) *Biochemistry 34*, 16107−16124.

Pullman, A., & Pullman, B. Q. (1981) *Rev. Biophys. 14*, 289−380.

Scaria, P. V., & Shafer, R. H. (1991) *J. Biol. Chem. 266*, 5417−5423.

Stilz, H. U., & Dervan, P. B. (1993) *Biochemistry 32*, 2177−2185.

Sun, J., Francois, J. C., Montenay-Garestier, T., Saison-Behmoaras, T., Roig, V., Chassignol, M., Thuong, N. T., & Hélène, C. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 9198−9202.

Tuite, E., & Nordén, B. (1995) *J. Chem. Soc., Chem. Commun.* 53−54.

Tung, C. H., Breslauer, K. J., & Stein, S. (1993) *Nucleic Acids Res. 21*, 5489−5494.

Wilson, W. D., Barton, H. J., Tanious, F. A., Kong, S., & Strekowski, L. (1990) *Biophys. Chem. 35*, 227−243.

Wilson, W. D., Tanious, F. A., Mizan, S., Yao, S., Kiselyov, A. S., Zon, G., & Strekowski, L. (1993) *Biochemistry 32*, 10614−10621.

Zon, G. (1988) *Pharm. Res. 5*, 539−548.

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