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Control of the DNA-Binding Specificity of 9,10-Anthraquinone by the Nature and Positions of Substituents

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Anthraquinone intercalator 1s carrying amino groups through a spacer at positions 1 and 8 of the ring stabilized calf thymus DNA more than poly [d(A-T)]$_2$ and polydApolydT. By contrast, N-acetylated derivative 2s stabilized the AT polymers more and showed preference for poly[d(A-T)]$_2$ to polydApolydT. This result is explained by postulating that the terminal acetylamino groups interact with adenines in the major groove of DNA and that the symmetric disposition of the substituents of 2s matches that of the alternating A-T sequence of poly[d(A-T)]$_2$.

Keywords: Anthraquinone intercalator, DNA, sequence specificity

INTRODUCTION

Intercalators exert their biological activities by the insertion between adjacent base pairs of DNA [1, 2]. Anthraquinone intercalators have a unique property of DNA binding mode and biological activity being dependent on the position of the substituents on the anthraquinone skeleton [3–6]. 1,5-Disubstituted anthraquinones bind to DNA as a threading intercalator, in which one of the substituents threads into between base pairs to protrude in the major or minor groove [3, 4]. 1,8-Disubstituted anthraquinones lay the same substituents in the major groove only, whereas substituents of all other known intercalators are positioned in the minor groove [3, 4]. In the meantime, it was demonstrated that the functional group contained in the substituent of an intercalator affects the preference of the intercalator for DNA bases. For example, the terminal amido moiety can interact with a guanine base on the surface of the DNA minor groove through hydrogen bonds [7].

If functional groups are introduced in the substituents of anthraquinone in positions 1 and 5, one of them is positioned in the major groove and the other in the minor groove. The former seems to interact most favorably with adenine and the latter with guanine. In 1,8-isomers the

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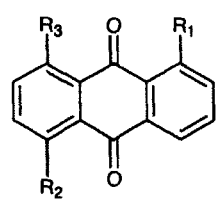
same groups are positioned in the major groove only and interact with adenines. If this argument is realized, anthraquinone intercalators should show preference for nucleotide sequences. Furthermore, the substituents of 1,5- and 1,8-disubstituted anthraquinones are disposed symmetrically about the C2 molecular axis. Since many DNA binding proteins are homodimers carrying a two-fold (C2) symmetry axis to interact with an invert-repeat DNA sequence which is also C2 symmetric, these compounds would be a better model of DNA-protein interactions [8].

In this paper, we report that the terminal amino (**1a**) and acetylamino (**2a**) forms of 1,5-disubstituted anthraquinone and their 1,8-counterparts (**1s** and **2s**) differ in the affinity and specificity of DNA binding (Fig. 1).

MATERIALS AND METHODS

Terminal amino derivatives **1a** and **1s** were synthesized by the methods described previously [9]. Terminal acetylamino derivatives **2a** and **2s** were synthesized by the following procedures.



	R_1	R_2	R_3
1a	X	X	H
2a	Y	Y	H
3a	Z	Z	H
1s	X	H	X
2s	Y	H	Y
3s	Z	H	Z

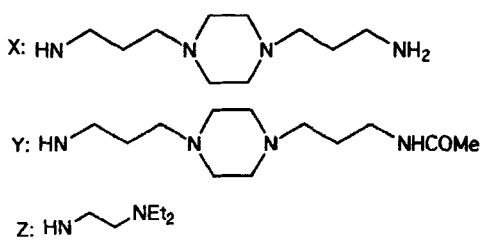


FIGURE 1 Structure of anthraquinone derivatives **1a**, **1s**, **2a** and **2s**.

Synthesis of 2a and 2s

To a solution of 10 mL of acetic anthydride and 0.5 g (0.8 mmol) of **1a** or **1s** were added two drop of pyridine. The resulting solution was heated at 55 °C for 3 h and then poured into 100 mL of ice-water. After adjusting the pH to 7 with sodium hydrogencarbonate, the solution was extracted with dichloromethane and the organic layer was dried over sodium sulfate. After evaporation, the solid residue was dissolved in a small amount of methanol and poured into ether. The solid obtained was dried under reduced pressure to yield 0.3 g (54%) of **2a** as a red solid, mp 175–177 °C; ^{1}H-NMR ($CDCl_3$) δ=1.65 (4H, m), 1.89–1.95 (4H, m), 1.98 (6H, s), 2.40–2.70 (24H, m), 3.30–3.39 (4H, m), 3.39–3.45 (4H, m), 7.00 (2H, m), 7.15 (2H, brs, D_2O exchangeable), 7.50 (4H, m), 9.70 (2H, m, D_2O exchangeable) ppm; Anal. Calcd. for $C_{28}H_{56}N_8O_4 \cdot 3H_2O$: C, 61.50; H, 8.26; N, 15.10. Found: C, 61.10; H, 8.17; N, 14.80. **2s** was also synthesised analogously and purified to homogeneity by HPLC (Hitachi L-6200 chromatograph with a dual plunger pump; Inertsil ODS-2, 7.6 mm × 250 mm, GL Science Inc.; flow rate 1.0 mL/min; solution A, 0.1% TFA (trifluoroacetic acid); solution B, 0.1% TFA and 70% acetonitrile; 0–100% B in 50 min). The retention time of **2s** was 33.6 min; violet solid, mp 110–115 °C; ^{1}H-NMR ($CDCl_3$) δ=1.93 (4H, m), 1.96 (6H, s), 2.10 (4H, m), 3.08 (4H, m), 3.14 (4H, m), 3.27 (8H, m), 3.40 (16H, m), 7.05 (2H, dd, J=7.6, 1.2 Hz), 7.36 (2H, dd, J=7.3, 1.2 Hz), 7.43 (2H, dd, J=7.6, 7.3 Hz) ppm: Anal. Calcd. for $C_{28}H_{56}N_8O_4 \cdot 4CF_3COOH$: C, 48.30; H, 5.24; N, 9.79. Found: C, 48.50; H, 5.37; N, 9.46.

DNA-Binding Studies

Extinction coefficients and equilibrium constants were determined as previously described [10, 11] with a Hitachi U-3210 UV-visible spectrophotometer equipped with a temperature controller Hitachi SPR-10. In the thermal denaturation

experiments heating rate was set at 0.33 °C/min. The ΔT_m is defined as the difference in T_m of DNA in the presence and absence of ligand. Calf thymus DNA, poly[d(A-T)]$_2$ and polydApolydT were purchased from Sigma Chemical Co. Calf thymus DNA was purified as described previously [12]. DNase I digested DNA had mean base pairs, 50–200, T_m = 29 °C in 0.1 M NaCl and 0.1 mM MES buffer at pH 7.0. The DNA concentrations were determined by optical measurement. The following molar extinction coefficients (nucleotide phosphate unit) were used: calf thymus DNA, 6,600 $M^{-1}cm^{-1}$ at 260 nm; poly[d(A-T)]$_2$, 6,600 $M^{-1}cm^{-1}$ at 262 nm; polydApolydT, 6,000 $M^{-1}cm^{-1}$ at 260 nm.

RESULTS AND DISCUSSION

All of the compounds **1a**, **1s**, **2a** and **2s** ($12-16 \times 10^{-6}$ M) exhibited hypochromic and bathochromic shifts for the anthraquinone chromophore at 540 nm in absorption spectra upon binding to sonicated calf thymus DNA (3.0×10^{-3} M), indicative of DNA intercalation. Quantitative study of the interaction of these ligands with the DNA was hampered, however, because of precipitate formation for this DNA and **1s** or **2s**. Hence, spectrophotometric titrations of the ligands with the DNA solution were carried out with shorter DNA fragments generated by DNase I digestion. The data were analyzed according to the Scatchard procedure. Comparison of the experimentally obtained Scatchard plots with the theoretical curves generated by the binding equation of McGhee and von Hippel [13, 14] allowed estimation of the binding constant (K) (Tab. I). All of the titrations was carried out at 20, 22, 24, 26 and 28 °C and thermodynamic parameters were evaluated from such data (Tab. II).

The binding constants (K) of all of these compounds lie in the range of $2-10 \times 10^4$ M^{-1} and are comparable to those of simpler anthraquinones such as **3a** and **3s** carrying diethylaminoethylamino moieties ($4-5 \times 10^4$ M^{-1} in 0.2 M NaCl at 20 °C) [3, 4], demonstrating that the affinity of these intercalators is dictated for the most part by the anthraquinone ring. The binding constants of the amino derivatives **1a** and **1s** are larger than those of their acetylamino counterparts **2a** and **2s**. The amino derivatives, **1a** and **1s**, can be regarded as tetra-cationic intercalators whereas acetylated derivatives, **2a** and **2s**, as di-cationic ones under the experimental conditions. In general, the binding affinities of intercalators decrease with a decrease in the charge density of intercalators, because of a diminished electrostatic interaction with the polyanionic DNA polymer [1]. Our results are consistent with this rule.

The interaction of the amino intercalators with DNA is more favorable in terms of enthalpy but less so in terms of entropy than that of the acetylamino intercalators due possibly to desolvation of the ionic species. Whatever the mechanism, the data are consistent with the

TABLE I Binding affinity of anthraquinone intercalators for DNA[a]

Compd.	DNA	K × 10^{-4} (M^{-1})
1a	CT-DNA	8.0
1s	CT-DNA	9.8
2a	CT-DNA	2.1
2s	CT-DNA	2.2
2s	polydApolydT	3.0
2s	poly[d(A-T)]$_2$	21.0

[a] K values (0.1 M NaCl, 0.1 mM MES buffer, pH 7.0, 4% MeOH at 18 °C) were determined by a non-linear least-squares fit using the binding equation of McGhee and von Hippel. [13] CT-DNA stands for calf thymus DNA.

TABLE II Thermodynamic parameters for the intercalation of **1a**, **1s**, **2a** and **2s** into calf thymus DNA[a]

Compd.	ΔH (kcal mol^{-1})	ΔS (cal $mol^{-1}K^{-1}$)	ΔG_{298} (kcal mol^{-1})
1a	−12.4 ± 0.8	−20.0 ± 1.3	−6.4 ± 1.2
1s	−13.7 ± 0.4	−24.3 ± 0.8	−6.5 ± 0.7
2a	− 8.7 ± 0.2	−10.2 ± 0.2	−5.7 ± 0.2
2s	− 9.8 ± 0.1	−14.0 ± 0.1	−5.6 ± 0.1

[a] Experiments were conducted at 20, 22, 24, 26 and 28 °C in 0.1 M NaCl, 0.1 mM MES buffer, pH 7.0, and 4% MeOH.

notion that the amino and acetylamino substituents lie in the DNA groove to interact with the edges of the base pairs on the surface of the DNA groove, irrespective of the mode of interaction [15].

To evaluate the specificity of the intercalators for the GC content and the nucleotide sequence, thermal denaturation profiles for solutions of sonicated calf thymus DNA (GC content 42%), polydApolydT and poly[d(A-T)]2 (GC content 0%) were determined in the absence and presence of these compounds (ΔT_m defined as the difference in T_m, Tab. III). Since **1a** stabilized all DNA duplex by greater than 60 °C, the base-pair preference of **1a** could not be decided. On the other hand, the 1,5-derivative **1s** raised the T_m of calf thymus DNA more than that of poly[d(A-T)]2 or polydApolydT, suggesting that **1s** intercalates and stabilizes more the GC-rich DNA. By contrast, the acetylated derivatives **2a** and **2s** raised the T_m of calf thymus DNA less than that of poly[d(A-T)]2 or polydApolydT, indicating that **2a** and **2s** showed an AT-preference. It is also interesting to note that **2s** raised the T_m of poly[d(A-T)]2 more than that of polydApolydT by 10 °C in $\Delta\Delta T_m$ (difference in ΔT_m). This observation is consistent with the result of binding studies that **2s** can bind to poly[d(A-T)]2 7 times more strongly than to polydApolydT (Tab. II). Similarly, **2a** stabilized poly[d(A-T)]2 more than polydApolydT, albeit to a lesser extent of 4°C in $\Delta\Delta T_m$.

These results indicated that **2s** carrying acetylamino moieties can discriminate a subtle difference in the nucelotide sequence of A_n/T_n and $(AT)_n(TA)_n$. A previous paper implicated that the carboxamino group of an intercalator substituent can interact with a guanine base in the minor groove of DNA through hydrogen bonding [7]. When these groups are located in the major groove, they should interact with adenine bases, because adenines can serve both as a proton donor and a proton acceptor just as do guanines in the minor groove. An interaction of the carboxamino group with a thymine base is unlikely to occur, because the methyl moiety of thymine protrudes in the major groove to prevent it from hydrogen bonding.

As described in the Introduction, the acetylamino groups of **2s** are located in the major groove. They also are expected to interact with an adenine base because the acetylamino moiety has the same carboxamino functional group. The fact that **2s** showed a sequence preference for poly[d(A-T)]$_2$ and polydApolydT whereas **1s** having amino groups did not supports the contention that the acetylamino groups of **2s** actually interact with nucleic bases. Figure 2 illustrates putative structures of the complexes of **2s** with poly[d(A-T)]$_2$ and polydApolydT. As these structures suggest, the symmetry of the substituents of **2s** matches that of the alternating A-T sequence of poly[d(A-T)]$_2$, whereas the 1,5-geometry in **2a** hampers such an optimal interaction of acetylamino groups with adenine bases. This is exactly what we observed by experiments.

In summary, we showed that the nucleotide sequence specificity of intercalators can be altered readily by the nature of the substituents (amino vs. acetylamino) as well as by their positions on anthraquinone (1,5- vs. 1,8-). Synthetic intercalators which normally do not select nucleic bases may be made show high nucleotide sequence specificity by a more elaborate design of the ligand.

TABLE III Stabilization of DNA helices by **1a**, **1s**, **2a** and **2s**[a].

Compd.	ΔT_m(°C)[b]		
	CT-DNA	polydApolydT	poly[d(A-T)]$_2$
1a	>60	>60	60
1s	>60	34	34
2a	46	52	56
2s	41	43	53

[a] Experiments for synthetic DNAs were conducted in 0.1 M NaCl, 0.1 mM MES buffer, pH 7.0, 4% MeOH. In the case of calf thymus DNA, NaCl was omitted from the buffer. The concentrations of ligand and DNA were 10 μM and 50 μM, respectively. [b] ΔT_m is defined as the difference in helix-coil transition temperature of DNA in the presence and absence of the ligand. In this condition, T_m values were 38 °C for calf thymus DNA, 33 °C for polydApolydT and 23 °C for poly[d(A-T)]$_2$.

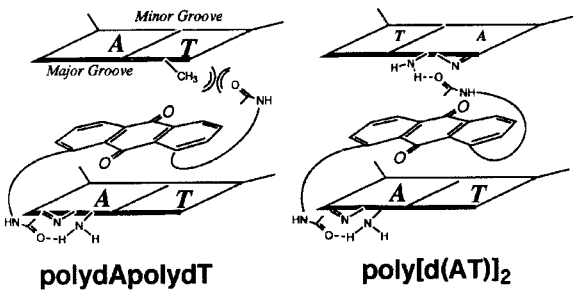


FIGURE 2 Model of the complex of **2s** with poly[d(A-T)]$_2$ and polydApolydT. This model assumes that the acetylamino groups interact with the nearest base pairs. If these substituents interact with the second nearest base pairs from the intercalated site, the basic structures are the same. This figure illustrates that the acetyamino moieties of **2s** serve as a proton acceptor and adenine as a proton donor in the major groove, but other modes of hydrogen bonding such as the one in which adenine serves as both a proton acceptor and proton donor are also possible [7].

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