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Binding of a homologous series of anthraquinones to DNA

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Abstract—The DNA binding characteristics of a series of homologous 2,6-disubstituted anthraquinone threading intercalators bearing one to four ethylene glycol units in their side chains have been studied. Binding constants were measured via surface plasmon resonance (SPR). These compounds bind to an AT-rich hairpin with slightly higher affinity than to a GC-rich hairpin. The binding constants decrease as the length of the side chain increases.

The factors that control small molecule intercalation in DNA continue to be the focus of study.[1–9] Overall, the hydrophobicity of the molecule and net charge are usually the most significant. Additional factors specific to the side chains include their steric bulk, isohelicity of the side chains with the minor groove, and phasing of the ligand subunits with the edges of the base pairs.[10–12] Loss of rotational and translational degrees of freedom upon binding have also been considered, though contributions from these have generally been difficult to assess.[2] One approach to analyzing the contributions of the factors controlling binding is to compare binding of a series of homologous compounds.

In the present study, we report the synthesis and DNA binding characteristics of a homologous series of 2,6-disubstituted anthraquinones with increasing side chain size (Fig. 1). These symmetrical molecules are threading intercalators, that is with one chain each in the major and minor groove.[1,3] The side chains were designed such that the cationic charge (protonated secondary amine) would have a constant position in the series. The side chains were based on the polyethylene glycol repeating unit. This allows a significant increase in the length of the side chain with a relatively small change in the hydrophobicity of the molecule.

The anthraquinones were synthesized by condensing an excess of the appropriate amine with 2,6-bis(3-bromopropionamido)anthracene-9,10-dione, synthesized in turn from 2,6-diaminoanthracenedione and bromopropionyl chloride.[13–15] The amines were either available from commercial sources (**I** and **II**) or synthesized from the glycol methyl ether and acrylonitrile, followed by reduction with hydrogen and a catalyst (**III** and **IV**).[16]

Surface plasmon resonance (SPR) based Biacore technology[17] was used to measure the binding of compounds **I**–**IV** to AT- and GC-rich DNA hairpins (Fig. 2).[18] The instrument response (RU) was plotted as a function of time to generate sensograms. Data from the sensograms were cast into binding plots of response as a function of the concentration of free drug (C_{free}) (Fig. 2). In each case, the observed response (RU_{obs}) was greater than the maximum predicted response ($RU_{max,pred}$)[18] indicating that there were multiple binding sites. Scatchard and Hill analyses revealed that each compound bound non-cooperatively to each DNA sequence. Therefore, the data were fit by non-linear least squares fitting analysis to a multiple equivalent site equilibrium model (eq 1):[19]

$$RU_{obs}/RU_{max,pred} = nKC_{free}/(1 + KC_{free}) \quad (1)$$

where n is the number of binding sites and K is the binding constant. Fits of the data to other binding models gave much larger errors.

Table 1 shows that the binding affinity for the AT-rich sequence was 2–3 times greater than for the GC-rich sequence for all four compounds in the series. Tanious et al. had previously studied the binding of several

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anthraquinone threading intercalators to poly(dA-dT) and poly(dG-dC) oligonucleotide sequences.[20] They found binding constants for the poly(dA-dT) sequence that were on average about 5 times greater than that for the poly(dG-dC) sequence, consistent with the current study.

The observed instrument response at high concentrations of added anthraquinone showed that each hairpin bound more than one molecule. Data fits indicated that the AT-rich sequence had approximately one more binding site ($n = 3.1 \pm 0.4$) than the GC-rich sequence ($n = 2.1 \pm 0.5$) for all four compounds. Neidle and co-workers, using optical titration methods and an extended McGhee–von Hippel model, found that related anthraquinones occluded 3–4 base pairs when binding to calf thymus DNA.[13] These two methods of measuring binding are consistent with one another, in that the stem portion of the hairpin (presumably the major binding site) has 8–9 base pairs.

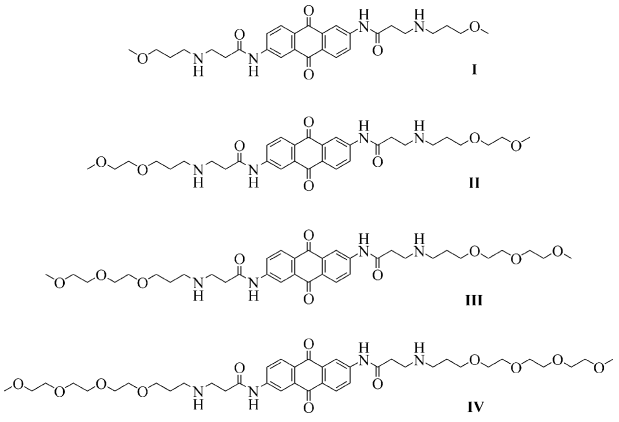
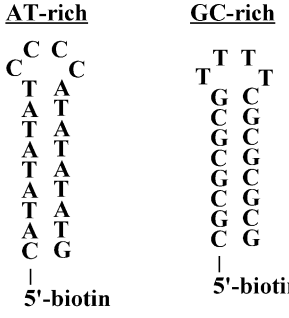


Figure 1. Homologous series of 2,6-disubstituted anthraquinones with polyethylene glycol side chains.



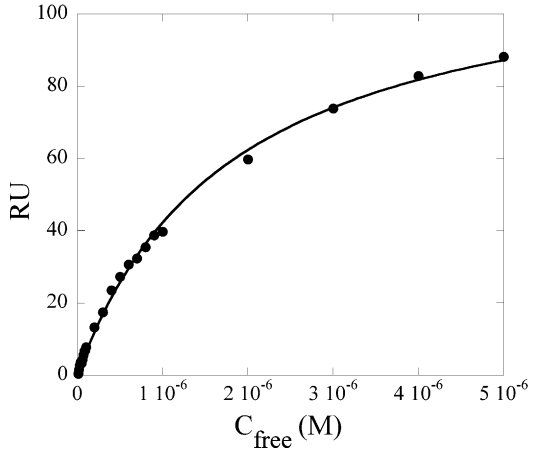


Figure 2. AT- and GC-rich DNA hairpins used for study (top). Binding plot for the binding of compound **II** to the AT-rich sequence (bottom).

The binding constant decreases with increasing length of the side chain for anthraquinones **I**–**IV** for both the AT- and GC-rich sequences. This result was consistent with DNA thermal melting studies,[21] which showed a decrease in ΔT_m from compound **I**–**IV** (Table 1). Figure 3 shows the binding constants in this study plotted as a function of the molecular volume of the molecule.[22] The data set can be expanded if we also look at the previous work of Neidle and co-workers on compounds with the same central core but different side chains.[13] The entire data set shows a consistent pattern: the binding constant decreases as the molecular volume increases.

It should also be noted that the hydrophobicity of the molecule decreases as the chain length increases for the intercalators studied herein. Specifically, the calculated octanol/water partition coefficient, ClogP, changes by −0.14 with the addition of each $–CH_2CH_2O–$ group.[23] Figure 3 plots the binding constants as a function of ClogP for both compounds **I**–**IV** of this study and the five related compounds from the study of Neidle and co-workers.[13] Overall, there is no dependence of the binding constant on hydrophobicity. Thus, molecular size is a better predictor of DNA binding than hydrophobicity for these anthraquinones, with molecules bearing larger side chains binding more poorly.

Our data may be compared with two other studies in which the DNA binding constant was measured as a function of the length of the side chain within a homologous series. Wakelin et al. studied five homologous 9-aminoacridinecarboxamides.[24] The series was synthesized by incorporating methylene units in the side chain, thus increasing the hydrophobicity of the molecule with increasing length of the side chain (ClogP increases by +0.53 with the addition of each methylene group). For this series, binding to poly(dA-dT) was independent of chain length; binding to poly(dG-dC) decreased slightly (by a factor of 2) over the series as the chain became longer. Maleev et al. studied the binding of four homologous disubstituted actinocin derivatives to calf thymus DNA.[25] They observed about an order of magnitude decrease in the binding constant for intercalation as the number of methylene groups in the side chains were

Table 1. Binding constants for compound **I**–**IV** binding to the AT- and GC-rich DNA sequence

Compd	Molecular volume (Å^3)	ΔT_m[a] (°C)	K (AT) (10^5 M^{-1})	K (GC) (10^5 M^{-1})
I	652	29.1	7.6±0.3	3.1±0.2
II	774	26.7	5.5±0.2	2.2±0.3
III	889	24.0	2.5±0.3	1.1±0.4
IV	1009	22.2	1.9±0.1	0.9±0.1

[a] ΔTm (Tm$_{complex} - T$m$_{DNA}$) were determined using calf thymus DNA.

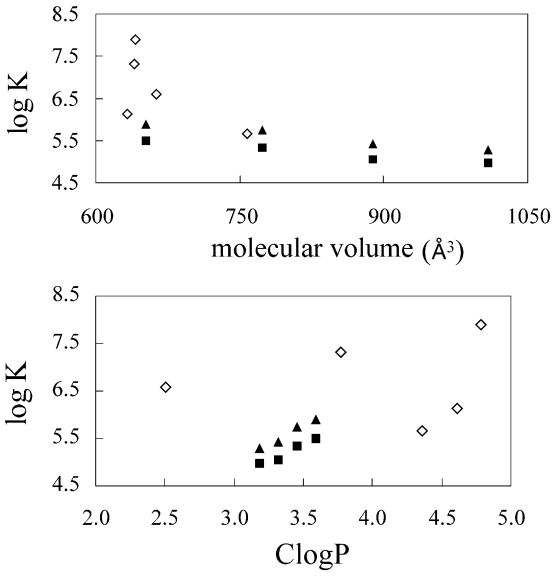


Figure 3. Plots of binding constant (log units) as a function of molecular volume (top) and ClogP (bottom). Data shown are for compound **I**–**IV** binding the AT-rich (closed triangles) and GC-rich (closed squares) sequences, and from work by Neidle and co-workers (open diamonds).

increased from two to five. The observation that the binding constants decrease as a function of the chain length for series where the hydrophobicity either decreases (anthraquinone series herein) or increases (9-aminoacridinecarboxamide and actinocin series) indicates the importance of chain length in determining the binding constant. Further analysis of structure-binding correlations among these series is complicated by differences in the distance between the intercalating moiety and the cationic charge in the side chains; the distance is constant in the anthraquinone series but increases as the side-chain length increases in the 9-aminoacridinecarboxamide and actinocin series.

The effect of side-chain size on DNA binding presumably results from loss of rotational degrees of freedom of the side chains on binding of the ligand to the DNA. The energetic cost resulting from this should increase from compound **I**–**IV**, resulting in the observed decrease in the binding constant. The observation that side-chain length is a better predictor of DNA binding than hydrophobicity for the anthraquinones, and the observation of decreased DNA binding with increasing side-chain length for series that both increase and decrease in hydrophobicity, indicate the importance of side-chain length as a determinant of the equilibrium constant for DNA binding.

Acknowledgements

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15. As an example, for the synthesis of **III**, a 10-fold excess of 3-[2-(2-methoxyethoxy)ethoxy]propylamine (a mixture with the nitrile and starting alcohol) was allowed to react directly with 0.20 g (0.39 mmol) of bis(3-bromopropionamido)anthracene-9,10-dione by refluxing in ethanol for 36 h. The product was filtered and the filtrate evaporated under reduced pressure. Recrystallization was carried out from $CHCl_3$–EtOH (3:2, v/v) to give a yellow powder (0.19 g, 70%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.82 (m, 4H, $CH_2CH_2CH_2$), 2.82 (t, J = 6.6 Hz, 4H), 2.93 (t, J = 6.9 Hz, 4H), 3.23 (s, OCH_3), 8.13 (dd, J = 2.4, 8.4 Hz, 2H, Ar*H*), 8.19 (d, J = 8.4 Hz, 2H, Ar*H*), 8.50 (d, J = 2.4 Hz, 2H, Ar*H*). The other peaks are presumably underneath DMSO-d_6 and between 3.2 and 3.6 ppm. FAB-MS (thioglycerol) m/z 701.3753 ($M+H^+$ calcd 701.3762 for $C_{36}H_{53}N_4O_{10}$). For **III** and **IV**, the final products for DNA studies were purified by HPLC on a Beckman Ultrasphere ODS C-18 column with methanol as the mobile phase. The other anthraquinones were prepared in a similar fashion.
16. 3-[2-(2-Methoxyethoxy)ethoxy]propionitrile was synthesized by condensing di(ethylene glycol) methyl ether with an excess of acrylonitrile.[26] This was hydrogenated over Raney nickel at 55 psi[26] to give 3-[2-(2-methoxyethoxy) ethoxy]propylamine as a mixture with the starting materials. 3-2-(2-(2-Methoxyethoxy)ethoxy)ethoxypropylamine was synthesized similarly, except that 5% rhodium on alumina was used as the hydrogenation catalyst.[27]
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18. Binding interactions were measured with a BIACORE 3000 optical biosensor instrument (Biacore, Uppsala, Sweden). All interactions were measured on a streptavidin coated sensor chip (Biacore) at 25 °C. 5′-Biotin-labeled DNA hairpins were obtained as a gift from Dr. David Wilson. They were originally obtained as anionic exchange and HPLC purified products from Midland Certified Reagents (Midland, TX, USA) and were used without further purification. A GC base pair was placed at the end of the AT-rich hairpin in order to minimize the risk of fraying. DNA hairpins (25 nM) were dissolved in HBS-EP buffer and immobilized via biotin capture. A

flow rate of 2 μL/min was used in order to control the amount of DNA immobilized. In each case, the amount of DNA immobilized was equivalent to approximately 350 RU. For the binding analysis, several concentrations (5 nM–5 μM) of compound in MES 10 buffer [1×10^{-2} M MES (2-(*N*-morpholino)ethanesulfonic acid) containing 1×10^{-3} M EDTA and 0.1 M NaCl with the pH adjusted to 6.25 with NaOH] were injected at a flow rate of 20 μL/min. After each injection the compound was dissociated and the surface regenerated using running buffer and regeneration buffer (10 mM Gly, pH 2). The response from the reference channel (channel 1) was subtracted from the responses from the channels with DNA (channels 2 and 3). At steady-state, the instrument response is proportional to the amount of bound compound.[19] Average fitting of the response at steady-state was performed with the BIAevaluation 3.1 program and the binding constants determined using KaleidaGraph (version 3.5, Synergy Software, Reading, PA, USA). The maximum predicted response per bound compound ($RU_{max,pred}$) is given by: $RU_{max,pred}=(RU_{DNA}\times MW_{compound}\times R)/MW_{DNA}$, where RU_{DNA} is the response of the immobilized DNA, $MW_{compound}$ and MW_{DNA} are the molecular weights for the compound and DNA, respectively, and R is the ratio of the refractive index increment of the compound to DNA. For this series of compounds, R was taken to be 1.2.[28]

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22. Molecular volumes were calculated using PCModel (version 8.5, Serena Software, Bloomington, IL, USA). Data shown are for the Monte Carlo calculation. Data using the Connolly analytical formulation[29] were the same within 1%.
23. ClogP values were calculated using ChemDraw (version 7.0, CambridgeSoft, Cambridge, MA, USA). In view of the atom limit of the algorithm, the ClogP values are reported for the anthraquinone and one side chain only.
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