A Zwitterionic Anthraquinone Derivative: First Zwitterionic DNA Binding Ligand

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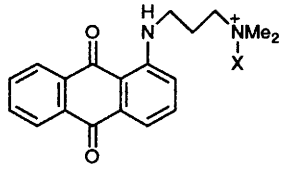
The anthraquinone derivative (**2**) is the first example of a zwitterionic DNA-binding ligand, interacting with DNA through outside binding rather than through intercalation which is characteristic of its cationic, parent compound (**1**).

A number of clinically useful anticancer drugs such as bleomycin, netropsin, actinomycin D, and m-AMSA bind to DNA by the outside binding mode or by the intercalation mode, showing more or less base-pair and base-sequence specifities.[1] Many studies have been devoted to understand the DNA-binding properties of these drugs and related compounds in efforts to develop chemicals of therapeutical value or to obtain a clue for understanding the molecular mechanism involved in DNA-binding proteins and restriction enzymes. However, the studies are mostly limited to cationic chemicals, emphasizing the electrostatic interaction between the polyanionic host DNA and the guest chemicals.

Only a few uncharged compounds have been studied in detail in their interaction with DNA. Uncharged polyaromatic compounds generally show weak to moderate affinity for DNA. The low water-solubility of this family of compounds also hampered such studies. A betaine-type dipolar molecule (which does not carry an overall net charge) was shown to lack DNA-binding ability; an ethidium-like structure-containing betaine did not interact with DNA,[2] though the parent ethidium ion itself is known to complex strongly with DNA. No zwitterionic type compounds (which again bear no net charge) have been studied for DNA interaction. Therefore, the structural features affecting the DNA-binding characteristics of various uncharged chemical species are not yet well understood. We have now prepared a zwitterionic anthraquinone derivative (**2**) and studied its DNA-binding properties as compared with its cationic parent compound (**1**).



(**1**) X = H
(**2**) X = $\sim\!\!\sim SO_3^-$

Compound (2) was prepared by the reaction of the anthraquinone (1) with propanesultone in dimethylformamide (DMF) at room temperature for 1 day. The reaction mixture was poured into excess of diethyl ether to give red crystals, which were filtered off and washed with diethyl ether (yield, 94%).

The binding of (2) to DNA was studied by absorption spectrometry, since (2), like (1), showed hypochromic and bathochromic shifts when it is bound to double-helical DNA (Table 1). The concentrations of the bound and the free (2) could be determined from the spectra assuming the formation of single association complex species. The data were analysed according to the Scatchard procedure. Comparison of the experimentally obtained Scatchard plot with the theoretical plot generated by the binding equations of McGhee and von Hippel[3] allowed estimation of the binding affinity (K) (Table 2). The binding affinity of (2) is of the same order as that of (1) which contains only a cationic site. This was rather unexpected since it means that the introduction of one negative charge into the monocationic ligand (1) to give the charge-neutral ligand (2) does not cause any substantial decrease in the binding affinity.

Figure 1 shows the logarithmic plots of the binding affinity K against the Na^+ concentration according to the theory developed by Wilson and Lopp,[4] which assumes two Na^+-releasing steps from the periphery of the polyanionic DNA chain, *i.e.*, DNA chain conformational change (chain elongation) and cationic ligand binding (cation exchange reaction). The slope (0.8) observed for (1) is in reasonable agreement with the predicted slope (1.0) for monocationic intercalators. In contrast, the observed slope for (2) is 0.0. This slope may be compared with the predicted slope of 0.2 for an uncharged intercalating ligand, which can cause Na^+ release only through a DNA chain-elongation effect (no cation-exchange reaction). Though no uncharged intercalators have so far been studied in detail to confirm the theory, the absence of Na^+ release (slope zero) in the binding of (2) with DNA suggests that the DNA chain does not undergo elongation on interaction with (2) and therefore (2) is not intercalating.

Further evidence on the nature of the (2)–DNA interaction came from viscosity measurements. The fractional increase in the length of DNA (L/L_0) was correlated with the intrinsic viscosities [η] and [η_0].[5] Figure 2 shows the experimental results obtained for both (1) and (2). The observed slope of the curve for (1) is lower than the predicted value, but is consistent with values obtained for similar intercalating molecules.[6] Considering the approximations involved in determining L/L_0, the agreement is reasonable. On the other

Table 1. Spectroscopic data of DNA-binding ligands[a] and effect on DNA-denaturation.

	Free ligand		Bound ligand			
Comp.	$\lambda_{max,f}$	ε_f	$\lambda_{max,b}$	ε_b	H[b]	ΔT_m /°C[a,c]
(1)	511	6000	525	4410	30	11
(2)	511	5140	516	4290	17	0

[a] Experiments were conducted at pH 7.4 in 20 mM Tris-HCl buffer containing 5% Me_2SO. [b] Spectra were measured for 40 μM (1) or (2) in the presence of 15 mM DNA (phosphate unit). H stands for percent hypochromicity [$\%H = (1 - \varepsilon_b/\varepsilon_f) \times 100$]. [c] ΔT_m stands for the difference in the transition temperature of DNA with and without (1) and (2) at a 1:10 ligand:DNA (phosphate unit) molar ratio.

Table 2. Binding affinity to calf thymus DNA (K_C) and *E. coli* DNA (K_E).[a]

		Calf thymus DNA		*E. coli* DNA		
Comp.	$[Na^+]$/M	$K_C \times 10^{-4}$	n_C	$K_E \times 10^{-4}$	n_E	K_E/K_C[b]
(1)	0.05	3.50	6			
	0.10	1.20	5	1.40	6	1.2
	0.25	0.77	7			
	0.5	0.49	5			
(2)	0.05	0.72	7			
	0.10	0.88	10	1.30	14	1.5
	0.25	0.80	12			
	0.50	0.77	11			

[a] K and n values (5% Me_2SO, 20 mM Tris-HCl, pH 7.4) were determined by a nonlinear least-squares fit using the binding equation of McGhee and von Hippel. [b] The ratio of binding affinities for calf thymus DNA (GC content: 39%) and *E. coli* DNA (GC: 50%).

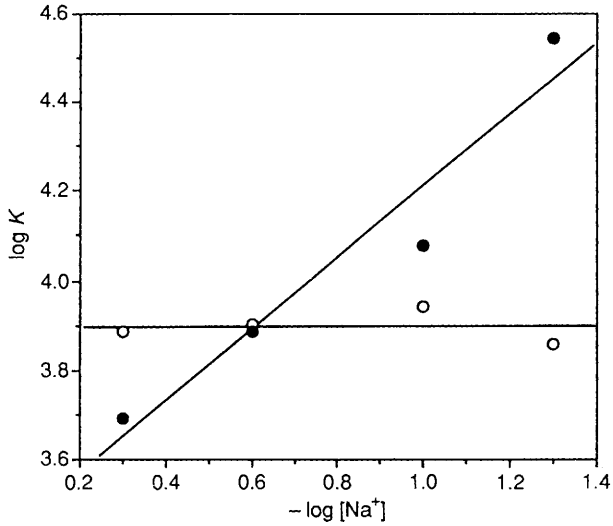


Figure 1. Log K values (Table 1) plotted as a function of $-\log[Na^+]$; ●, (1); ○, (2).

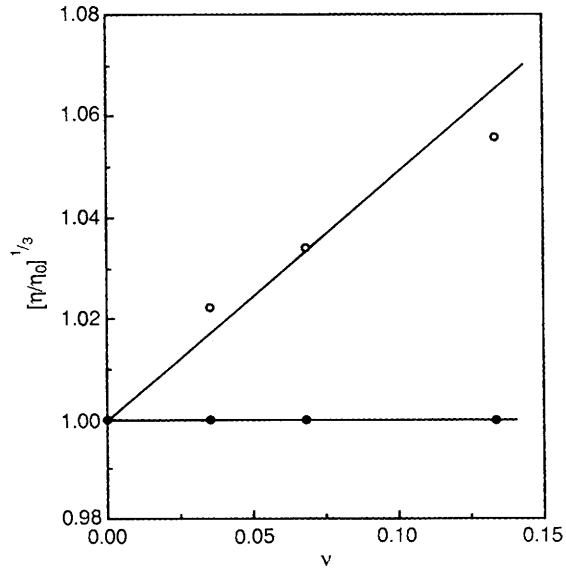


Figure 2. Viscometric titrations plotted as $[\eta/\eta_0]^{1/3}$ (which approximates L/L_0) as a function of v (mol ligand bound/mol DNA base pairs) for ○, (1); ●, (2), with sonicated calf thymus DNA. The slope of (2) is 0 while the curve for (1) has a slope of 0.5 ($L/L_0 = 1 + 0.5v$) compared to the ideal slope of 1.0.

hand, the presence of (**2**), in spite of the fact that its DNA binding affinity is comparable to that of (**1**), did not cause any change in viscosity of the DNA solution. These findings support (**2**) forming an outside type complex with DNA, where the interaction prevails between the hydrophobic surface of DNA (groove) and hydrophobic aromatic moiety of (**2**).

The effect of (**1**) and (**2**) on the thermal denaturation of DNA was assessed by the usual classical technique.[7] Thermal-denaturation profiles of calf thymus DNA were analysed in the absence or in the presence of (**1**) and (**2**) (ΔT_m) (Table 1). The transition temperature remained unchanged in the presence of (**2**) (ΔT_m 0 °C), while the transition temperature in the presence of (**1**) indicated a strong stabilization of the double helix of (**1**) through intercalation (ΔT_m 11 °C). This strengthens the idea that the binding mode of (**2**) is quite different from that of (**1**).

Table 2 includes the binding site sizes of the interacting ligands, which show a meaningful difference between (**1**) and (**2**), the binding site sizes of (**2**) being larger than those of (**1**). This is in accordance with the difference in the nature of binding mode suggested above. Table 2 also includes the K_E/K_C ratios of (**1**) and (**2**). The GC content of *E. coli* DNA is 50% and that of calf thymus DNA is 39%, and the K_E/K_C ratio should reflect the nucleic base-pair preference of the interacting ligands. The observed K_E/K_C ratios do not differ much between the two, and this indicates that, in spite of the difference in the nature of the interaction, the base-pair preferences of (**1**) and (**2**) are more or less similar.

A DNase I footprinting study was undertaken for possible differentiation between the two modes of DNA binding by (**1**) and (**2**). However, both the footprinting patterns of (**1**) and (**2**) are featureless, not showing any particular base-pair or base-sequence preference. The differentiation of the binding modes by 400 MHz ^{1}H NMR spectroscopy was not successful since neither (**1**) nor (**2**) gave meaningful chemical shift changes on interaction with DNA.

Further study is needed to decide if the changes observed are restricted to compound (**2**), or have some general meaning. Nevertheless, the present study strengthens the recent understanding that the two modes of interaction (intercalation and outside binding) may be influenced by a subtle balance in the energy involved in the interaction.[8]

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