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Thermodynamic studies on the interactions of di-substituted anthraquinones with DNA

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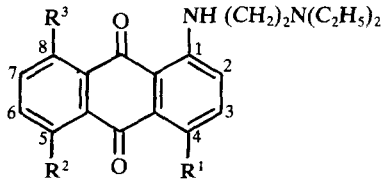
The DNA binding of the 1,4-, 1,5- and 1,8-bis[[(diethylamino)ethyl]amino] anthraquinones (**I–III**), which had been shown in an earlier study [1] to intercalate into DNA was investigated by spectrophotometric titration of the drug with DNA at temperatures between 25 and 50° enabling determination of the thermodynamic parameters for the intercalation process over this temperature range. At the ionic strength under which the determinations were carried out, external binding of the ligand to the sugar-phosphate backbone was precluded and so intercalation was the only binding mode.



I (1,4-bis-) R^1=NH$(CH_2)_2N(C_2H_5)_2$; R^2=R^3=H
II (1,5-bis-) R^1=R^3=H; R=NH$(CH_2)_2N(C_2H_5)_2$
III (1,8-bis-) R^1=R^2=H; R^3=NH$(CH_2)_2N(C_2H_5)_2$

The earlier study [1] involved computer graphics modelling of the intercalation of these same di-substituted anthraquinones into the self-complementary deoxynucleoside d(CpG) and revealed differences in binding properties. The thermodynamic parameters obtained in this study support the computer graphics modelling in that the manner of the binding of the 1,5-disubstituted compound by "straddling" is quite different from that of the 1,4- and 1,8-compounds, the results being consistent with initial binding to a non-base-paired region. The thermodynamic study does not support "straddling" as a binding mode for the 1,4- compound which the earlier work had proposed as a possibility, albeit after full minimization of the deoxynucleoside geometry had been performed. The manner of its binding would therefore appear to be similar to that of the 1,8-compound, that is insertion from the major groove of the helix with both substituents lying in that groove.

Methods

Solutions of ligand approximately 5×10^{-5} M were prepared in 0.3 M NaCl–0.008 Tris pH 7.0 buffer. Ligand solution (2.0 ml) was placed in each of three matched quartz cells and aliquots (1×15, 5×25, 4×65 and 4×100 μl) of approximately 2.5×10^{-3} M calf thymus DNA solution (calculated as DNA phosphate from the absorption at 260 nm where $\varepsilon_{260} = 6600$) in the same buffer were added sequentially to each cell using a Hamilton syringe which was also used to stir the mixture. The absorbance was determined at the λ_{max} of the unbound drug, after allowing time for equilibration, against a blank of buffer subjected to identical treatment. Titration was continued until there was no significant change in the absorbance values when dilution factors were taken into account, indicating that the drug was fully bound. The spectrophotometric studies were undertaken on a Beckman DU6B spectrophotometer in which the cuvette temperature could be maintained at temperatures up to 50° with temperature control of 0.25°.

The data from the spectrophotometric titrations were then subjected to non-linear regression analysis employing a NAG E04 FCF program [2].

This program generated values of K and n for each data set. Linear regression analysis on the ln K vs $1/T$ plots obtained over the temperature range of the study enabled the calculations of ΔH, since the gradient is equal to $-\Delta H/R$. ΔG and ΔS were calculated in turn from the relationships $\Delta G = -RT \ln K$ and $\Delta G = \Delta H - T\Delta S$.

Figure 1 indicates the relative positions and slopes of the van't Hoff plots for these di-substituted derivatives. At the high ionic strength prevailing (0.3 M NaCl), external binding of the drug was precluded and the results give an estimate of the intercalative binding alone.

Analysis of the binding of compounds **I–III** over the range 25–50° and calculation of ΔH and ΔS from van't Hoff plots showed marked differences between the 1,5-disubstituted compound and the other compounds (Table 1). To give a comparison of the relative binding affinities, the K values at 298 K are quoted in the table. The results of these DNA binding studies in solution can be correlated with the results from computer graphics modelling of the same compounds at an isolated DNA intercalation site of the self-complementary deoxynucleoside monophosphate d(CpG) [1].

The similarity in ΔH and ΔS values for the 1,4- and 1,8-compounds would suggest a similarity in the nature of the intercalative process for these two compounds in contrast to that of the 1,5-compound which has markedly different thermodynamic parameters. This similarity of the results for the 1,4- and 1,8-compounds is consistent with results from computer graphics modelling [1] and with a study of the dissociation kinetics of the DNA–drug complexes of these compounds [3]. By contrast, the 1,5-compound was found to have the slowest rate of dissociation from DNA. This was attributed to the findings from the computer graphics modelling studies [1] which showed that DNA-

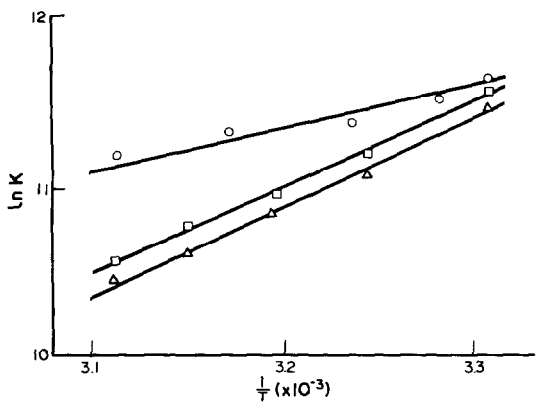


Fig. 1. van't Hoff plots for the di-substituted derivatives of 1-[[(diethylamino)ethyl]amino]-anthracene-9,10-dione; △, 1,4 derivative; ○, 1,5 derivative; and □, 1,8 derivative.

Table 1. Binding and thermodynamic parameters of the di-substituted derivatives of 1-[[(diethylamino) ethyl]amino]-anthracene-9,10-dione

Compound	$K(M^{-1})$ at 298K $\times 10^5$	$\Delta G°_{298}$ (kJmol^{-1})	$\Delta H°_{298}$ (kJmol^{-1})	ΔS(Jmol^{-1}K^{-1})	Correlation coefficient for van't Hoff plot
I 1,4-bis-	1.23	−29.1	−43.0	−46.8	0.991
II 1,5-bis-	1.41	−29.4	−25.3	+13.8	0.941
III 1,8-bis-	1.12	−28.9	−41.8	−43.2	0.973

breathing (transient base-pair unstacking) had to occur to allow docking of the 1,5-compound with (and so also release from) the receptor site.

The lower ΔH value for the intercalation of the 1,5-compound is evidence for a two stage process; an initial interaction with non-base-paired DNA residues provides the interaction energy which drives formation of the double helical structure (but with an unwinding) in order to accommodate the 1,5-compound. Computer graphics indicated that the 1,5-compound alone could not be simply inserted into the intercalation site without some base-pair unstacking whereas both the 1,4- and 1,8-compounds could readily intercalate from the major groove. In consequence the ΔH value for the intercalative process in these two cases was substantially higher.

It is interesting to note that the computer graphics indicates the 1,5-compound intercalates by "straddling" across the helix with consequent high stabilization; it has the highest affinity for DNA of the three compounds. The 1,8-compound can only intercalate from the major groove side due to steric hindrance in the minor groove with the chromophore parallel to that of the base pairs whereas the 1,4-compound bound with the chromophore in a perpendicular orientation with the side chains in the major groove.

An alternative model for intercalation of the 1,4-compound involving "straddling" analogous to that of the 1,5-compound could be obtained using full geometric minimization of the dinucleoside geometry with optimization of the bond lengths and the angles. This derivative would also require base–pair separation before interaction could take place but the thermodynamic parameters clearly show that this mode of binding does not occur. This is supported by consideration of the differences in the ΔS values. It would be expected that a side-chain projecting into the minor groove could disrupt the DNA spine of hydration and/or be desolvated by the binding process [4]. This would release water to the bulk medium with a consequent increase in ΔS. A contribution to the different ΔS value for the 1,5-compound could also result from the transient base–pair unstacking which is necessary to accommodate the molecule in the DNA molecule which leads to an altered conformation of the DNA–drug complex.

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