# Mechanism of DNA-binding of some aminoalkylamino-derivatives of anthraquinone and naphthacenequinone

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The synthesis of the aminoalkylamino-derivatives I-VII of anthraquinone and naphthacenequinone has been reported previously (Double & Brown 1975, 1976) and these compounds were shown to bind to DNA with affinity constants between $10^5$ and $5 \times 10^6$ M$^{-1}$. Although these compounds had been designed to intercalate into DNA, the previous studies did not fully identify the nature of the binding interaction. Whilst compounds I–V showed no in vivo activity vs L1210 leukaemia, a range of related aminoalkylaminoanthraquinones prepared independently by Zee-Cheng & Cheng (1978) and by Murdock et al (1978) showed activity vs P388 leukaemia. Highest activity was found where the side chains were 2-(hydroxyethyl)amino ethylamino-groups (increasing the length of the spacer groups led to decreased activity). Activity was enhanced further when the nucleus was a 5,8-dihydroxyanthraquinone. The most active of these derivatives was compound VIII. These anthraquinone and naphthacenequinone derivatives have been designed as simplified analogues of the important anthracycline antibiotic adriamycin (IX), and compound VIII has been shown, in a comparative study, to have activity equivalent to that of adriamycin in experimental tumour systems (Wallace et al 1979). Although adriamycin is active against a wide spectrum of tumours, it exhibits a cardiotoxicity which limits to 550 mg m$^{-2}$ the total cumulative dose which can be administered (Carter 1975). It has recently been shown (Cheng et al 1979) that the ratio of cardiotoxic dose to antitumour dose (P388 leukaemia) is higher for the aminoalkylaminoanthraquinones than for adriamycin. Also, compounds I, II, IV and V have been evaluated in the Ames test (Brown & Dietrich 1979) and no mutagenic activity was reported. The aminoalkylaminoanthraquinones therefore represent an important 'lead' in the development of improved analogues of adriamycin. Little is known of their action apart from the above-mentioned determination of binding constants from the spectral shifts shown by I–VII in the presence of DNA, and the finding that aminoalkylaminoanthraquinones show cross-resistance with adriamycin in the adriamycin-resistant P388 leukaemia sub-line (Cheng et al 1979). These data imply that these compounds have a similar action to that of adriamycin, a proven intercalating drug (Brown 1978) but cannot be taken as unequivocable proof of intercalating ability. In this study we have further examined the interactions of I–VI with DNA.

If these compounds bind to DNA by intercalation, as suspected, then this binding should result in increased stability of the helix to thermal denaturation. Consequently, for compounds II–VI, the absorbance of mixtures of drug and DNA ($1{\cdot}5 \times 10^{-5}$ and $1{\cdot}5 \times 10^{-4}$ M respectively—the latter calculated using the figure $\epsilon(P)_{260} = 6\,600$) in 0·018 M NaCl, 0·003 M Tris Cl buffer, pH 7·0, was monitored at 260 nm as the temperature was raised at a rate of 0·25 °C min$^{-1}$ and the temperature $T_m$ at which 50% of the absorbance change had occurred, was noted. The difference, $\Delta T_m$, between the values of $T_m$ for (DNA + drug) and DNA alone, was calculated, these determinations having been made simultaneously. The stabilization of the helix to melting due to the presence of compounds II–VI (Table 1) is less than that found, in the same system, for the anthracyclines adriamycin, daunomycin and nogalamycin ($\Delta T_m$ values of 16·25, 14·25 and 17·2 °C respectively) which were reported in previous studies (Plumbridge & Brown 1978, 1979a, 1979b). In these previous studies it was also shown that non-intercalating anthracyclines give values for $\Delta T_m$ of less than 3 °C. The values recorded here for compounds II–VI (Table 1) are therefore consistent with an intercalative mode of binding to DNA. In contrast to the results with DNA, none of the compounds I–VI showed significant stabilization of poly rI.rC (the $\Delta T_m$ values were all less than 2 °C). This parallels the finding that neither adriamycin nor its analogues can intercalate into this polyribonucleotide (Plumbridge & Brown 1979a). Whilst this work was in progress studies on the thermal denaturation of DNA in the presence of compound VIII and 13 of its analogues were published (Johnson et al 1979). All compounds showed significant stabilization of the helix, compound VIII giving a $\Delta T_m$ of 15·9 °C in a system where the $\Delta T_m$ for adriamycin is 13·6 °C.

On binding of a drug to DNA, not only are changes in the properties of the DNA indicative of the mechanism of binding, but so also are changes in the properties of the drug. As reported earlier (Double & Brown 1975, 1976) compounds I–VII show hypochromic and bathochromic shifts with an isosbestic point. Anthracyclines which do not intercalate into DNA only show slight shifts (if any) and no isosbestic point (Plumbridge and Brown 1979a,b) whereas all anthracyclines which intercalate show marked spectral shifts exhibiting an isosbestic point. On binding to

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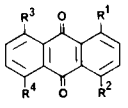
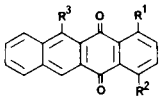
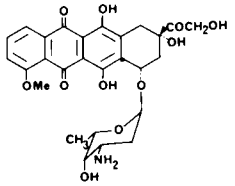


I $R^1 = X$ $R^2 = R^3 = R^4 = H$
II $R^1 = X$ $R^2 = OH$ $R^3 = R^4 = H$
III $R^1 = R^2 = X$ $R^3 = R^4 = H$
IV $R^1 = R^3 = X$ $R^2 = R^4 = H$
V $R^1 = R^4 = X$ $R^2 = R^3 = H$
VIII $R^1 = R^2 = NH(CH_2)_3NH(CH_2)_2OH$ $R^3 = R^4 = OH$



$X = NH\ CH(CH_3)(CH_2)_3N(C_2H_5)_2$



VI $R^1 = R^2 = X$ $R^3 = H$
VII $R^1 = R^2 = H$ $R^3 = X$

Table 1. Stabilization of DNA to melting in the presence of compounds II–VI (10:1, DNA/drug ratio).

| Compound | $\Delta T_m$ °C* |
|---|---|
| II | 3·5 |
| III | 3·4 |
| IV | 5·0 |
| V | 10·0 |
| VI | 3·9 |

* $\Delta T_m = T_m$ in presence of drug minus $T_m$ in absence of drug (0·018 M NaCl, 0·003 M Tris Cl buffer, pH 7·0).

DNA, there are often marked changes in the fluorescence properties of the drug and measurement of the degree of polarization of drug fluorescence when irradiated with polarized light indicates the degree of rigidity of binding of the drug to the helix (Plumbridge & Brown 1977). Unfortunately only compound IV had sufficient intensity of fluorescence for this test. Aliquots of DNA solution were added to a solution ($2·5 \times 10^{-6}$ M) of compound IV in 0·008 M Tris Cl, 0·05 M NaCl buffer, pH 7·0 and the degree of fluorescence polarization determined as previously described (Plumbridge & Brown 1977). At ratios of DNA/drug of 0, 5, 10 and 20, the values of fluorescence polarization were 0·07, 0·22, 0·30 and 0·33 respectively. If the drug were free to rotate in solution in the presence of DNA, the value would remain at about 0·07; the high values obtained with IV show that drug molecules are immobilized in the presence of DNA. This is consistent with intercalation as the mode of interaction.

If the anthraquinone derivatives intercalate into the helix as suggested by the above data, then they should be less available for polarographic reduction than free drug (Berg & Eckardt 1970). Solutions of compounds I–V ($5 \times 10^{-5}$ M) in 0·15 M phosphate buffer (pH 7·0) containing various ratios of DNA/drug (0–20) were analysed polarographically (dropping mercury electrode vs a saturated calomel electrode). The half-wave potentials were −0·55 V, −0·55 V, −0·65 V, −0·62 V and −0·64 V for I–V respectively. All compounds, like the adriamycin control, showed a reduction in wave height as the concentration of DNA was increased, adriamycin showing the largest percentage decrease in wave height (a 90% reduction at a ratio of DNA: drug of 20:1 compared with values of between 40 and 80% for I–V).

As a final test of the nature of the binding of these anthraquinone derivatives to DNA, their effect on the binding to DNA of the archetypical intercalating drug ethidium was evaluated. The fluorescence intensity of ethidium was monitored ($\lambda_{max}$ excitation, 476 nm: $\lambda_{max}$ emission, 596 nm) as aliquots of a solution of ethidium ($2 \times 10^{-5}$ M) in 0·008 M Tris Cl 0·05 M NaCl buffer, pH 7·0, were added to solutions (in the same buffer) of DNA ($2 \times 10^{-5}$ M), DNA plus the drug under test ($2 \times 10^{-5}$ and $2·5 \times 10^{-6}$ M respectively), drug ($2·5 \times 10^{-6}$ M) and buffer; the latter two acting as controls. Considering firstly compound II which had no effect on the fluorescence properties of unbound ethidium (since both controls gave identical fluorescence intensity readings), the fluorescence enhancement of ethidium due to binding to DNA was reduced in the presence of drug (Fig. 1). This is almost certainly due to competition between ethidium and compound II for binding sites on DNA, confirming the ability of com-

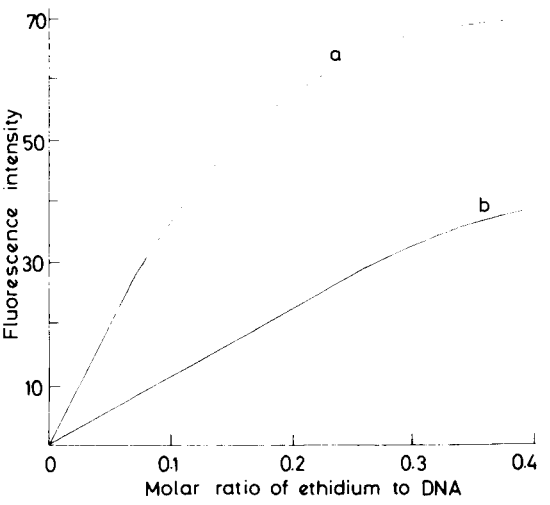



FIG. 1. Fluorescence of ethidium on addition of aliquots of a solution of ethidium to DNA (curve a) and to an 8:1 (molar) mixture of DNA and compound II (curve b).

pound II to intercalate into DNA. A less likely explanation for the lower enhancement of fluorescence in the solution of DNA with compound II compared to DNA alone, is that energy transfer occurs between intercalated ethidium molecules and drug. If this were the case, the drug molecules would need to bind between base pairs in close proximity to the ethidium, again implicating an intercalative binding. The other compounds evaluated by this method (IV and V) also caused a marked fluorescence reduction in solutions of DNA plus ethidium. These compounds themselves, however, do show some fluorescence under the conditions used and mixtures of drug and ethidium show a quenching of fluorescence; it is possible that the fluorescence reduction in DNA + ethidium is due to these effects rather than to intercalation of drug. However, if the compounds did not intercalate into DNA, ethidium would have the same affinity for DNA whether drug were present or absent and at high ratios of DNA to ethidium, the ethidium would be essentially totally bound and the fluorescence would be similar whether drug were present or absent. This was not the case, for even at ratios (molar) of DNA:ethidium 100:1, the fluorescence of ethidium was markedly lower in presence of drug than in absence of drug (DNA to drug ratio of 8:1).

None of the above tests alone is sufficient to establish the nature of the interaction of aminoalkylaminoanthraquinones and related compounds with DNA. Taken collectively, however, the data show that these compounds bind to DNA by an intercalative mechanism, Johnson et al (1979) in the recent study of antitumour activity of VIII and 19 analogues against several murine tumours found no correlation between in vivo activity and $\Delta T_m$. They concluded that antitumour activity might be due to some mechanism other than DNA-binding. Several facts however show that differences in distribution may be important. For example, it was found that compound VIII was much more potent than the corresponding compound with no phenolic groups, the latter only being effective when administered at the site of tumour implantation (Johnson et al 1979). A study of the distribution properties of these compounds might resolve this apparent anomaly.

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