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The Interaction of the β-Anomer of Doxorubicin with B and Z DNA

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SUMMARY

Equilibrium and kinetic studies on the interaction of daunorubicin, doxorubicin, and the β-anomer of doxorubicin with B and Z form DNA were made using spectroscopic and fluorometric methods. The β-anomer of doxorubicin binds more weakly to calf thymus DNA than do the parent compounds, with a binding constant over 2 orders of magnitude lower than that found for doxorubicin. The ionic strength dependence of the binding constant is identical for daunorubicin and the β-anomer of doxorubicin, indicating that the electrostatic contribution to the binding free energy is the same for the two compounds. Rate constants for steps along the dissociation pathway are larger for the β-anomer relative to the parent compounds, indicating a shorter lifetime for the β-anomer-DNA complex. Daunorubicin and doxorubicin were equally effective as inhibitors of the rate of the B to Z transition of polydeoxyguanylic-deoxycytidylic acid (poly(dGdC)) in 3.0 M NaCl. Both compounds bound cooperatively to poly (dGdC) under high salt conditions that initially favor the Z conformation. In contrast, the β-anomer of doxorubicin did not inhibit the rate of the B to Z transition under these conditions, and would not bind to poly(dGdC) in 3.0 M NaCl. The β-anomer did inhibit the rate of the transition of poly(dGm^{5}dC) to the Z form in 50 mM NaCl, 2.5 mM $MgCl_2$, although not as effectively as daunorubicin. Further, binding of the β-anomer to poly(dGm^{5}dC) under these conditions was cooperative, although the β-anomer was clearly a less efficient allosteric effector on the B to Z transition than was daunorubicin. These results emphasize the importance of the stereochemistry of the daunosamine residue in the specific and preferential binding of anthracycline antibiotics to B form DNA.

The anthracycline antibiotics doxorubicin (adriamycin) and daunorubicin (daunomycin) are among the most effective compounds in current use in cancer chemotherapy (1). Interaction of these drugs with DNA and the consequent inhibition of DNA function (2–4) is believed to be an important part of the molecular mechanism by which these antitumor agents inhibit cell growth (1). Consequently, the interaction of these antibiotics with DNA and chromatin has been widely studied by a variety of chemical and physical approaches (5–12). Several recent reviews summarize the current understanding of the anthracycline-DNA interaction and review the earlier literature (1, 13, 14).

Considerable effort has been made in the synthesis of novel anthracycline antibiotics in hopes of preparing new compounds with enhanced potency and efficacy or with reduced toxic side effects (1, 15). The β-anomer of doxorubicin (1′-epidoxorubicin) (Scheme I) is one compound to emerge from such efforts, and is identical with the parent compound, doxorubicin, except for the orientation of the daunosamine moiety (16). The β-anomer is, however, pharmacologically less effective than doxorubicin (17), binds more weakly to calf thymus DNA, but still alters the hydrodynamic properties of DNA in a manner consistent with an intercalative binding mechanism (18, 19). Aspects of the interaction of the β-anomer remain incompletely described, and are important for understanding the role of the duanosamine moiety in the specific interaction of anthracycline antibiotics with DNA. In particular, the ionic strength dependence of the β-anomer-DNA interaction and the dynamics of the β-anomer-DNA complex are poorly understood, and are explored in the present report. In addition, the interaction of the β-anomer with left-handed Z DNA was studied for reasons elaborated upon below.

Previous results from this laboratory showed that the anticancer drug daunorubicin binds preferentially to alternating purine-pyrimidine sequences (6). Since these are the sequences that may undergo the transition from the right-handed B form to the left-handed Z form (20), the effect of the drug on Z DNA was studied. Daunorubicin was found to inhibit the rate of the B to Z transition of poly(dGdC), and would convert Z DNA

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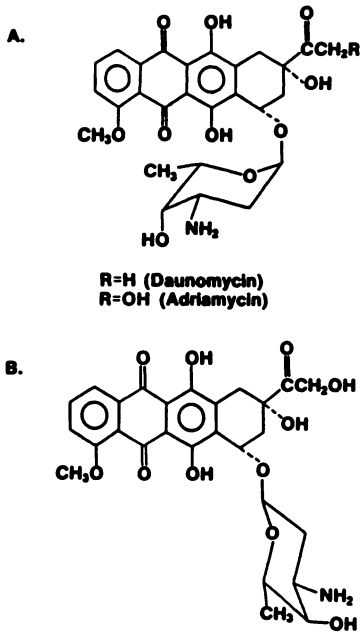
ABBREVIATIONS: poly(dGdC), polydeoxyguanylic-deoxycytidylic acid; poly(dGm^{5}dC), methylated polydeoxyguanylic-deoxycytidylic acid; bp, base pairs; AM, adriamycin, DM, daunomycin.



Scheme I. Structures of anthracycline antibiotics. A, daunorubicin (daunomycin) and doxorubicin (adriamycin). B, the β-anomer of doxorubicin.

back to the B conformation under solution conditions that otherwise favor the Z form in the absence of drug (7). Under certain conditions, striking long range effects were observed, with a single drug molecule converting nearly two turns of the DNA helix from the Z to an intercalated B conformation (21). These results were rationalized by a model in which daunorubicin preferentially binds to B DNA, and acts as an allosteric effector on the B to Z transition (7). The daunosamine moiety was hypothesized to play a major role in the preferential interaction with B form DNA over the Z form since structural studies (22) indicate a favorable stereochemical fit of the sugar residue and the A ring of daunorubicin within the minor groove of B form DNA.

The studies reported here on the interaction of the β-anomer with Z DNA were designed, in part, to test this latter hypothesis. The β-anomer of doxorubicin is identical with the parent compound, doxorubicin, except for the orientation of the daunosamine moiety. The orientation of the daunosamine in the β-anomer is such that interaction with the minor groove of B DNA would probably not be possible. We find, in fact, that the β-anomer is considerably less efficient than daunorubicin or doxorubicin as an inhibitor of the B to Z transition. The β-anomer will not inhibit the high salt transition of poly(dGdC) at all, nor will it convert the Z form of the polymer back to the B form as will daunorubicin and doxorubicin. Under less extreme ionic conditions, the β-anomer will inhibit the transition of poly(dGm^{5}dC) to the Z conformation, but not as efficiently as daunorubicin. These effects arise from the lower affinity of the B anomer toward B DNA. These results emphasize the importance of the daunosamine stereochemistry on the preferential interaction of the anthracycline antibiotics with B DNA.

Materials and Methods

Anthracycline antibiotics. Daunorubicin and doxorubicin were obtained from Sigma Chemical Co. The β-anomer of doxorubicin and a sample of doxorubicin were supplied by Farmatalia-Carlo Erba, Milan, Italy. All compounds were used without further purification.

Polynucleotides. Poly(dGdC) (lot No. 782-73) and poly(dGm^{5}dC) (lot No. 782-26) were purchased from P-L Biochemicals Inc., and fractionated using Sepharose 4B as previously described (6). Concentrations were determined spectrophotometrically assuming a molar extinction coefficient for both polymers of 16,800 M^{-1} cm^{-1} at a wavelength of 254 nm. We use the nomenclature poly(dGdC) to refer to the double-stranded deoxypolynucleotide poly(dGdC)-poly(dGdC), and poly(dGm^{5}dC) to refer to its methylated counterpart.

Rate of the B to Z transition. The rate of the B to Z transition was monitored by absorbance at 295 nm using a Cary 219 spectrophotometer equipped with a Neslab circulating water bath and thermal programmer. For poly(dGdC), the reaction was initiated by a salt jump from 0.2 to 3.0 M NaCl. The transition of poly(dGm^{5}dC) was initiated by a $MgCl_2$ jump from 0 to 2.5 mM. Kinetic data were digitized, and fit to a single exponential using facilities available on the NIH PROPHET Computer Resource.

Binding studies. Binding of daunorubicin, doxorubicin, and the β-anomer of doxorubicin to DNA was determined by absorbance and fluorescence spectroscopy as previously described in detail (6–8, 10). The extinction coefficients of free and bound β-anomer were determined to be identical with those previously found for daunorubicin, as was the ratio of intrinsic fluorescence of the bound and free form of the drug. Where appropriate, binding data were fit to the neighbor exclusion model

$$r/C = K(1 - nr)\ ((1 - nr)/(1 - (n - 1)r))^{n-1} \qquad (1)$$

where K is the binding constant to an isolated site and n is the exclusion parameter in base pairs (23, 24). Data were fit using a nonlinear least squares fitting routine based on the Marquardt-Levenberg algorithm available on the NIH PROPHET Computer Resource.

Stopped-flow dissociation kinetic studies. The rates of dissociation of anthracyclines from calf thymus DNA were determined using the sodium dodecyl sulfate dissociation method of Mueller and Crothers (25). A Dionex D-110 stopped-flow spectrometer was used for kinetic studies. Preformed drug-DNA complex was flowed against a buffered solution containing sodium dodecyl sulfate to produce a final sodium dodecyl sulfate concentration of 0.5%. The dissociation reaction was monitored by absorbance at 480 nm. Data were recorded photographically, digitized, and fit to multiple exponentials as previously described in detail (9).

Results

Binding of anthracycline antibiotics to B form DNA. Binding isotherms for the interaction of daunorubicin, doxorubicin, and the B-anomer of doxorubicin with calf thymus DNA are shown in Fig. 1. Data may be fit to the neighbor exclusion model (Equation 1) with the results shown in Table 1. The β-anomer binding constant is over an order of magnitude lower than the binding constants of daunorubicin and doxorubicin. Similarly, the binding of the β-anomer to poly(dGdC) in BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 0.185 M NaCl, pH 7.0), and to poly(dGm^{5}dC) in BPE (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA) + 50 mM NaCl is considerably weaker than the binding of doxorubicin and daunorubicin (Table 1).

Fig. 2 shows the effect of ionic strength on the binding of daunorubicin and the β-anomer to calf thymus DNA in the form of a Record plot (26) according to the equation

$$d \ln (K)/d \ln (M) = -Z\mu$$

where z is the charge on the ligand and μ the fraction of counterions associated with each DNA phosphate. The slope of the lines in Fig. 2 for daunorubicin and the β-anomer are,

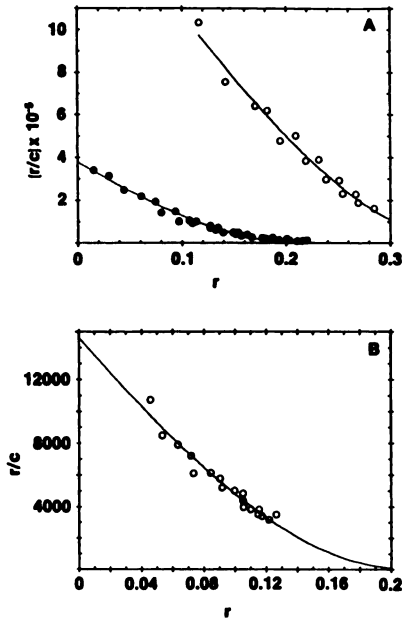


Fig. 1. Binding of anthracycline antibiotics to B form calf thymus DNA. A, Scatchard plot for the interaction of doxorubicin (O) and daunorubicin (●) to calf thymus DNA. B, Scatchard plot for the interaction of the β-anomer of doxorubicin with calf thymus DNA. Solution conditions were BPES buffer, 30°. The *solid lines* are the least squares fits to the neighbor exclusion model (Equation 1) with the results given in Table 1.

TABLE 1

Binding constants for the interactions of anthracycline antibiotics with DNA

All constants were determined at 30°. BPES buffer was used for calf thymus DNA and poly(dGdC) determinations, while BP buffer + 50 mM NaCl was used for studies with poly(dGm^{5}dC).

Polynucleotide and antibiotic	K	n
	M^{-1}	bp
A. Calf thymus DNA		
Daunorubicin	3.8×10^5	4.2
Doxorubicin	1.8×10^6	3.6
β-Anomer	1.5×10^4	4.3
B. Poly(dGdC)		
Daunorubicin	1.5×10^6	3.3
Doxorubicin	1.8×10^6	3.0
β-Anomer	3.2×10^4	3.1
C. Poly(dGm^{5}dC)		
Daunorubicin	2.1×10^6	3.0
Doxorubicin	2.2×10^6	2.5
β-Anomer	1.5×10^5	2.0

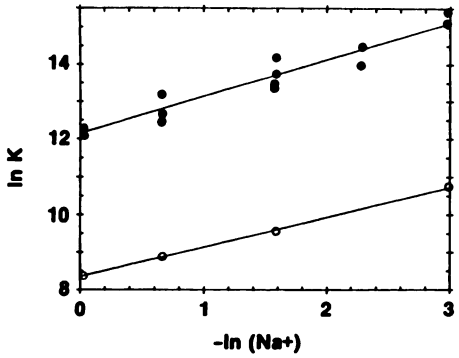


Fig. 2. The salt dependence of anthracycline antibiotic binding to B form calf thymus DNA. Data for daunorubicin (●) and the β-anomer of doxorubicin (O) are shown in a plot of ln K versus ln [NaCl] according to the theory of Record *et al.* (26). The slopes are −0.98 ± 0.08 and −0.79 ± 0.02 for daunorubicin and the β-anomer of doxorubicin, respectively.

within experimental error, identical, and are near the value of −0.88 expected for the interaction of a singly charged ligand with double-stranded DNA (26).

Dissociation rates from calf thymus DNA. The sodium dodecyl sulfate dissociation method of Mueller and Crothers (25) was used to measure dissociation rates of daunorubicin, doxorubicin, and the β-anomer of doxorubicin from calf thymus DNA. Sample time courses for the dissociation reaction for doxorubicin and its β-anomer are shown in Fig. 3. The dissociation reaction is complete for the β-anomer by 0.15 sec, but requires nearly 7 sec for doxorubicin. The lifetime of the doxorubicin-DNA complex is thus considerably longer than the lifetime of the corresponding β-anomer complex. Two exponentials were required to fit the dissociation data for both doxorubicin and the β-anomer, in accord with previous detailed kinetic studies on the daunorubicin-DNA interaction (9). Results of dissociation experiments are summarized in Table 2. The dissociation rates increase in the order AM<DM<B-AM.

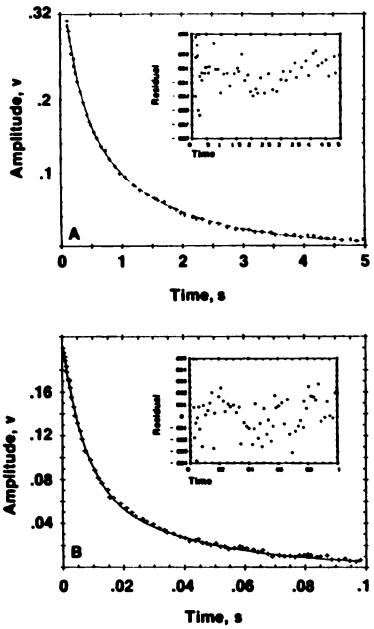


Fig. 3. Dissociation of anthracycline antibiotics from B form calf thymus DNA. The sodium dodecyl sulfate method of Mueller and Crothers (25) was used to determine the rate of dissociation of drug from calf thymus DNA. A, the dissociation of doxorubicin. The *solid line* is the fit of the data to two exponentials, with the residuals of the fit shown in the *inset*. B, the dissociation of the β-anomer. The *solid line* is the two exponential fit, with the residual plot shown in the *inset*. Table 2 summarizes the values determined for the dissociation rates for daunorubicin, doxorubicin, and the β-anomer.

TABLE 2

Dissociation rates of anthracycline antibiotics from calf thymus DNA

First order dissociation rate constants were determined by stopped-flow methods as described in Methods. Solution conditions were BPES buffer, 20°

Compound	r^o_b	k_{32}	k_{43}
		sec^{-1}	sec^{-1}
Daunorubicin	0.049	6.6 ± 1.5	1.3 ± 0.2
	0.11	8.3 ± 2.0	1.5 ± 0.3
	0.256	9.0 ± 2.0	1.6 ± 0.3
Doxorubicin	0.06	4.6 ± 2.5	0.5 ± 0.3
	0.11	3.3 ± 0.3	0.4 ± 0.05
β-Anomer	0.08	97 ± 13	24 ± 2.7
	0.232	100 ± 8.8	26 ± 4.0

The β-anomer of doxorubicin does not inhibit the rate of the B to Z transition of poly(dGdC) in 3.0 M NaCl. Fig. 4 summarizes comparative kinetic studies of the effects of anthracycline antibiotics on the rate of the B to Z transition in poly(dGdC) following a salt jump from 0.2 to 3.0 M NaCl. Daunorubicin and doxorubicin both are effective inhibitors of the rate of the transition. The β-anomer of doxorubicin, in contrast, is totally ineffective as an inhibitor, and in fact slightly accelerates the transition.

The binding of daunorubicin and doxorubicin to poly(dGdC) in 3.0 M NaCl, conditions that initially favor the left-handed Z conformation, is strongly cooperative, as indicated by the convex Scatchard plots shown in Fig. 5. Cooperative binding isotherms such as those seen in Fig. 5 may arise from preferential drug binding to B form DNA, and concomitant conversion of Z DNA to an intercalated B form. A variety of physical methods were used to demonstrate this for the interaction of daunorubicin with poly(dGdC) (7, 21). The allosteric model proposed by Dattagupta *et al.* (33) to account for the cooperative binding of ligands to DNA may be used to analyze the binding isotherms shown in Fig. 5. Briefly, the allosteric model postulates that DNA is in equilibrium between two conformational states, which for the case at hand may be umambiguously assigned to the B and Z conformations. This equilibrium is described by an equilibrium constant, s, for the conversion of a base pair from the Z to the B conformation at a pre-existing B-Z interface, and by an equilibrium constant, $\sigma^2 s$, for the nucleation of a B form base pair within a stretch of helix in the Z conformation. Ligand may bind to either conformation with characteristic equilibrium constants (K_B and K_Z) and neighbor exclusion parameters (n_B and n_Z). Six parameters thus describe the model, and may be used to "fit" the data by trial and error adjustment (33). The data of Fig. 5 were analyzed as follows. Points greater than the r value at the maximum in r/c were fit by nonlinear least squares to the neighbor exclusion model (Equation 1) to provide estimates of K_B and n_B. The intercept on the r/c axis defines K_Z, and was estimated by linear extrapolation of low r data. The remaining parameters s, σ, and n_Z were then estimated by trial and error adjustment, using a computer program made available by Prof. Donald Crothers that generated binding isotherms using the six input parameters (33). The results of this analysis are shown in Table 3. The parameters s and σ were determined to be identical for doxorubicin and daunorubicin, as they should be, since these parameters refer to the conformational transition of poly(dGdC) from the Z to the B form, under the same ionic conditions. K_Z is nearly the same for the two drugs. The primary difference is in K_B, consistent with the results of Table 1, and consequently in the ratio K_B/K_Z, which is the primary driving force of the allosteric conversion of the polymer from the Z to the intercalated B form.

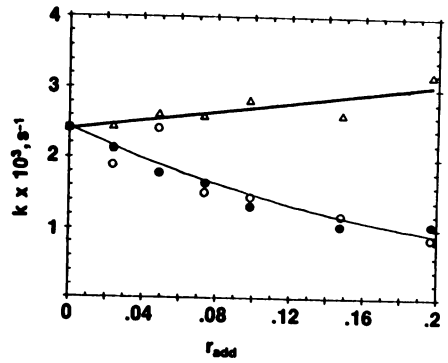


Fig. 4. Effect of anthracycline antibiotics on the rate of the B to Z transition. The rate of the transition of poly(dGdC) from the B to the Z form following a salt jump from 0.2 to 3.0 M NaCl was monitored by absorbance at 295 nm. The amount of added antibiotic, moles/mol of base pairs, is indicated for daunorubicin (●), doxorubicin (○), and the β-anomer of doxorubicin (△). Kinetic measurements were made at 30°.

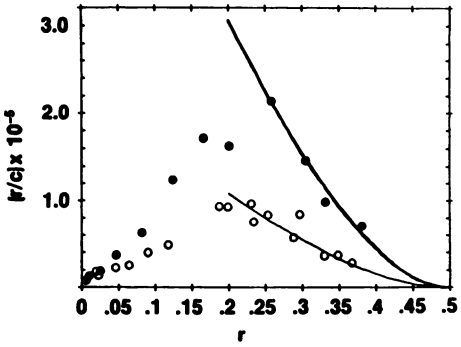


Fig. 5. Binding of anthracycline antibiotics to poly(dGdC). The binding of doxorubicin (●) and daunorubicin (○) to poly(dGdC) in BPE buffer + 3.0 M NaCl, 30°, was determined spectrophotometrically, and is presented here in the form of a Scatchard plot. The *solid lines* are least squares fits of the data for r 0.2 to the neighbor exclusion model (Equation 1). The fit for daunorubicin yields $K = 1.9 \times 10^5$ M^{-1}, $n = 2.0$ bp, while the fit for doxorubicin yields $K = 6.8 \times 10^5$ M^{-1}, $n = 2.0$.

In contrast to doxorubicin and daunorubicin, the β-anomer will not bind to poly(dGdC) under the ionic conditions described in Fig. 5, indicating that it is unable to convert Z DNA to the right-handed form. The reason for this is the low affinity of the β-anomer for B form DNA under these conditions. Extrapolation of the data in Fig. 2 predicts that the β-anomer would bind to B DNA with an affinity constant of 1.9×10^3 M^{-1} in 3.0 M NaCl. This is 2 orders of magnitude lower than the affinity of daunorubicin for B DNA under the same conditions, and is comparable to the affinity of daunorubicin for Z form DNA. Little of the added β-anomer would bind to either B or Z form DNA with a binding constant of that magnitude. Consequently, there is no driving force for the allosteric conversion of poly(dGdC) from the Z to a right-handed form provided by the β-anomer, and the polymer remains in the Z conformation.

The β-anomer will inhibit the rate of the B to Z transition of poly(dGm^{5}dC) in 50 mM NaCl, 2.5 mM $MgCl_2$. Poly(dGm^{5}dC) will undergo the B to Z transition under near physiological ionic strnegths. Fig. 6 shows that the β-anomer of doxorubicin will inhibit the rate of transition of this polymer to the Z form. Daunorubicin is, however, a more efficient inhibitor under these conditions than the β-anomer.

The binding of the β-anomer to poly(dGm^{5}dC) in 50 mM

TABLE 3

Parameters describing the allosteric binding of doxorubicin and daunorubicin to poly(dGdC) in 3.0 M NaCl

Parameters for the allosteric model of Dattagupta *et al.* (33) were estimated from the data of Fig. 5 as described in the text.

Compound	K_B	n_B	K_Z	n_Z	K_B/K_Z	s	σ
	$M^{-1} \times 10^{-5}$	bp	$M^{-1} \times 10^{-3}$	bp			
Doxorubicin	6.8	2.0	8.0	2.0	85	0.73	0.001
Daunorubicin	1.9	2.0	6.3	2.0	30	0.73	0.001

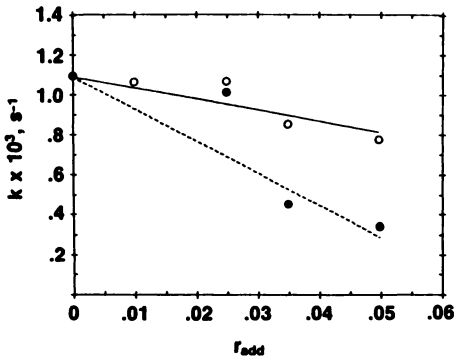


Fig. 6. The effect of anthracycline antibiotics on the rate of the B to Z transition of poly(dGdC). The rate of the transition of poly(dGm^{5}dC) in BP + 50 mM NaCl, 30°, following a magnesium jump from 0 to 2.5 mM was monitored by absorbance at 295 nm, in the presence of daunorubicin (●) or the β-anomer of doxorubicin (○).

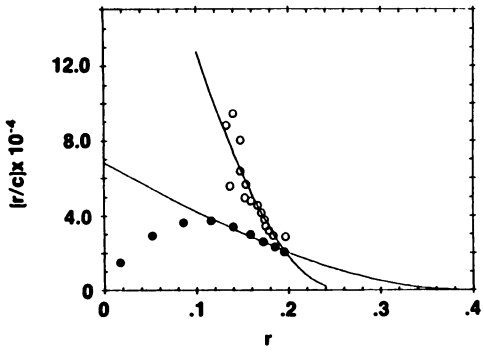


Fig. 7. Binding of antrhacycline antibiotics to poly(dGm^{5}dC). The binding of daunorubicin (○) or the β-anomer of doxorubicin (●) to poly(dGm^{5}dC) in BP buffer, 50 mM NaCl, 2.5 mM $MgCl_2$, is presented in the form of a Scatchard plot. *Solid lines* indicate the fits of the data to the neighbor exclusion model yielding for daunorubicin $K = 2.9 \times 10^5$ M^{-1}, $n = 3.6$ bp, and for the β-anomer $K = 6.8 \times 10^4$ M^{-1}, $n = 2.6$ bp.

NaCl, 2.5 mM $MgCl_2$ is shown in Fig. 7. The binding is cooperative, indicating that under these conditions, the β-anomer can convert the polymer from the Z to the B conformation. The conversion is complete at $r = 0.1$, indicating that one bound drug molecule per 10 base pairs will completely convert the polymer to the B conformation. Under these conditions, however, only one daunorubicin per 20–25 base pairs is required for the complete conversion of the polymer to the B form (21). The β-anomer of doxorubicin is, then, less effective as an allosteric effector of the B to Z equilibrium than is daunorubicin.

Discussion

The results presented here emphasize, above all, the profound importance of the orientation of the daunosamine moiety in the specific interaction of anthracycline antibiotics with B form DNA. Alteration of the orientation of the daunosamine, as in the β-anomer of doxorubicin, leads to a lessened affinity of the drug toward B form DNA, and a shorter lifetime of the drug-DNA complex relative to the parent compounds. Both of these observations correlate with the ineffectiveness of the β-anomer as a chemotherapeutic agent (1, 17), and as an inhibitor of the B to Z transition in DNA.

The results presented in Fig. 1–3 provide new fundamental information on the interaction of the β-anomer of doxorubicin with B DNA that extends our previous understanding of the binding reaction. The binding data of Fig. 1 show that the binding constant for the interaction of the β-anomer with calf thymus DNA is over 2 orders of magnitude lower than that found for the interaction of doxorubicin. This corresponds to a difference in the binding free energy between the two compounds of nearly 3 kcal/mol. The salt dependence of the binding constants (Fig. 2) for daunorubicin and the β-anomer of doxorubicin is essentially identical, with $Z\mu$ near the expected value of −0.88 for the binding of a ligand carrying a single charge to double-stranded DNA (26). This indicates that the electrostatic contribution to the binding free energy is identical for both daunorubicin and the β-anomer of doxorubicin. Specifically, knowledge of the value of $Z\mu$ enables us to assess quantitatively the thermodynamic equilibrium constant for the experimentally observed results. From the results of Record *et al* (26)

$$\ln K_{obsd} = \ln K_T^{\circ} + Z\xi^{-1} \ln (\gamma \pm \delta) - Z\mu \ln [M^+] \quad (2)$$

where K_{obsd} is the observed equilibrium constant and K_T is the thermodynamic equilibrium constant (corrected for the free energy of ion release by using a standard state in which all reactants including ions have unit concentration). $Z\mu$ is defined previously, $\gamma \pm$ is the mean activity coefficient of the Na^+ ion, and the remaining terms are constants for double-stranded DNA: $\xi = 4.2$ and $\delta = 0.56$. Since $Z\mu$ is identical for daunorubicin and the β-anomer, the last two terms of Equation 2 will be identical for the two compounds under the same ionic conditions, and the difference in observed binding constants thus arises from a difference in the thermodynamic equilibrium constant, K_T°. Previously, it was proposed that the charged amino residue of the daunosamine may participate in a direct interaction with the negatively charged DNA phosphates (13, 19, 27). Such an interaction was not observed in the daunorubicin-oligonucleotide crystal structure (22), nor is such an interaction necessary to account for the observed salt dependence of the daunorubicin-DNA interaction (10). The behavior of daunorubicin and the β-anomer of doxorubicin seen in Fig. 2 emphasize that the salt dependence of binding is a general electrostatic effect in which the charge on the drug molecule replaces the charge neutralization function of the condensed counterion, and need not participate in a specific interaction with DNA phosphate as previously proposed. The altered geometry of the sugar residue in the β-anomer would render such a specific interaction improbable. The 3 kcal difference between the binding free energy of doxorubicin and the β-anomer probably arises (primarily) from the loss of favorable van der Waals interaction of the sugar with the minor groove of the DNA, although the attribution of binding free energy contributions to specific molecular constituents must surely be made with caution (28).

The kinetics of the daunomycin-DNA interaction have recently been described in detail (9), and provide the framework for understanding the dissociation kinetics shown in Fig. 3. The minimal mechanism for the daunomycin-DNA interaction was proposed to be

$$DM + DNA \rightleftharpoons (DM\text{-}DNA) \rightleftharpoons (DM\text{-}DNA)^* \rightleftharpoons (DM\text{-}DNA)^{**}$$

in which daunorubicin interacts with DNA in a bimolecular association step, and subsequently undergoes two isomerization events. The dissociation steps observed in Fig. 3 correspond to the back reactions in the slow isomerization steps. Notably, the

rates of these steps for the β-anomer are over an order of magnitude faster than for the comparable steps for doxorubicin. Consequently, the lifetime of the β-anomer-DNA complex will be considerably shorter than the doxorubicin-DNA complex. Pharmacological activity is often more readily correlated with kinetic rather than equilibrium properties of drug-DNA complexes (25), and this finding is in accord with the ineffectiveness of the β-anomer in chemotherapeutic trials (17). Indeed, the order of the dissociation rates found here, AM<DM<β-anomer, correlates with the known biological inhibitory action of the drugs, with the compounds with the longer complex lifetimes being more clinically effective. A recent kinetic study reported a similar pattern for doxorubicin, daunorubicin, and an additional anthracycline antibiotic, iremycin (29).

The chemistry and biology of Z DNA are of intense current interest (20).An increasing amount of evidence suggests a role for Z DNA in the control of gene expression (20). Regions of Z DNA in the genome may therefore be unique and import targets for pharmacologically active compounds. Intercalators are, in particular, inhibitors of the B to Z transition (7, 20, 30). In some cases intercalators have been shown to act as allosteric effectors on the B to Z transition, and will convert Z DNA to an intercalated B form under solution conditions that would otherwise favor the Z form in the absence of drug (7, 21, 31, 32). The mechanism by which intercalators exert these effects has not been described in detail. The results presented here for the effect of the β-anomer on the B to Z transition provide additional information toward understanding how the anthracycline antibiotics affect the B to Z transition and the stability of Z DNA.

Analysis of the binding data of Fig. 5 by the allosteric model (33) indicates that daunorubicin and doxorubicin bind preferentially to B form DNA, with ratios for the binding constants to B and Z form DNA, K_B/K_Z of 30 and 88, respectively. In contrast, the β-anomer will not convert Z DNA to the B form under these conditions due to its low affinity for B form DNA. Under low salt conditions (Fig. 7) the affinity of the β-anomer for B DNA is greater, and the compound will convert poly(dGm^{5}dC) from the Z to the B conformation, but still less effectively than daunorubicin or doxorubicin, which bind over an order of magnitude more strongly to B form DNA under these conditions, and consequently will have a considerably larger K_B/K_Z ratio.

The strong preference of daunorubicin for the B conformation as a binding site was previously proposed to arise from a favorable interaction of the daunosamine moiety of the drug with the minor groove of the B DNA (7), as visualized to atomic resolution in the structure of a daunorubicin-oligonucleotide complex (22). The studies reported here for the β-anomer support this notion. Alteration of the orientation of the sugar results in a compound that is considerably less efficient as an inhibitor of the B to Z transition. Intercalation of the anthraquinone ring system is sufficient to cause some inhibition of the B to Z transition and weak allosteric effects, as seen in Figs. 6 and 7, but the proper orientation of the sugar is clearly required for optimal effects. Analogous results were recently reported for actinomycin and actinomone (which lacks the cyclic peptide side chain found in actinomycin). Both compounds convert Z DNA to the B form, but actinomycin is considerably more efficient than actinomone (31). This suggests a crucial role of the cyclic peptide moiety of actinomycin in the specific recognition of B DNA, perhaps analogous to the role of the daunosamine moiety of the anthracyclines suggested from the results described here.

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