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Parsing the Free Energy of Anthracycline Antibiotic Binding to DNA†

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ABSTRACT: The DNA binding free energy of eight anthracycline antibiotics was determined as a function of NaCl concentration. Compounds were chosen for study that differed from the parent compounds, doxorubicin or daunorubicin, at a single chemical substituent. Determination of the salt concentration dependence of the binding constant allowed us to dissect the DNA binding free energy of each compound into its component nonelectrostatic and polyelectrolyte contributions. Comparison of the nonelectrostatic free energy contribution allowed us to evaluate the net energetic contribution of specific functional groups to DNA binding. These quantitative data revealed a surprisingly large and favorable energetic contribution (2 kcal mol^{-1}) of the groove-binding daunosamine moiety and a substantial energetic penalty for alteration of its stereochemistry. The energetic cost of removal of hydroxyl groups at the C-9 and C-14 positions (which structural studies indicate may participate in hydrogen-bonding interactions with the DNA) was approximately 1 kcal mol^{-1}. Replacement of the 3′-amino group with a hydroxyl group led to a loss of 0.7 kcal mol^{-1} in binding free energy, above and beyond the energetic penalty resulting from the removal of its positive charge from the antibiotic. The results and analysis presented here provide a rigorous and detailed description of structure−DNA affinity relationships among anthracycline antibiotics. The results are of general interest in understanding how total ligand binding free energies are partitioned among substituents and will be useful in the formulation of rules for the rational design of novel DNA binding agents.

The rational design of new DNA binding agents requires a detailed understanding of the DNA binding properties of existing compounds with proven clinical utility. In addition to high-resolution structural data of drug−DNA complexes, an understanding of the energetics of complex formation is essential for a thorough understanding of the rules that govern the binding of small molecules to DNA. The anthracycline antibiotics are of proven clinical utility, with the parent compounds daunorubicin (daunomycin) and doxorubicin (Adriamycin) among the most important agents in use in cancer chemotherapy (Weiss, 1992; Priebe, 1995). The anthracyclines are, arguably, the best characterized DNA intercalators. Numerous high-resolution crystallographic structures have been reported for daunomycin and other anthracyclines bound to different hexanucleotide sequences [for reviews, see Wang (1993), Kennard and Salisbury (1993), Wang (1992), and Ughetto (1988)]. A wealth of thermodynamic, kinetic, and footprinting data exists, providing detailed evidence for the preferential interaction of daunomycin with certain DNA sequences [for reviews, see

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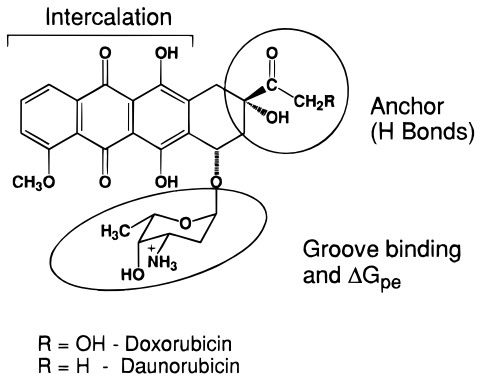

FIGURE 1: Anatomy of an anthracycline antibiotic. Daunorubicin (R = H) and doxorubicin (R = OH) are the parent anthracycline antibiotics. The functional domains important for DNA binding are emphasized in this schematic structure.

Chaires (1990a,b, 1995a,b), Fritzsche and Walter, (1987), and Fritzsche and Berg (1987)]. The underlying basis for this sequence preference has been clarified by molecular modeling studies (Pullman, 1991, 1989). The anthracycline antibiotics thus represent an important model system for understanding how small molecules recognize and bind to DNA. Elucidation of the rules that govern their binding to DNA should help to guide efforts directed toward the rational design of new DNA binding agents.

Figure 1 shows a schematic of daunorubicin that emphasizes its polyfunctional nature. Daunorubicin is more than an intercalator and possesses at least three functional domains. The first of these is the anthraquinone ring system, which indeed intercalates but with a geometry unlike that of simple intercalators like ethidium and proflavin. The long axis of the inserted daunorubicin chromophore is nearly perpendicular to the long axis of the DNA base pairs within the intercalation site, whereas the long axis of the chromophore is nearly parallel to that of the base pairs for the simple intercalators (Williams *et al*., 1992). The second functional domain is the daunosamine, which acts as a minor groove binding agent. Finally, substituents on the A ring define the third domain. These substituents form hydrogen bonds with DNA bases, serving as an anchor to stabilize the complex. It is instructive at this point to compare the binding free energy of daunorubicin with that of a specific protein−DNA interaction. The *lac* repressor (MW 153 400 for the dimer) binds to its specific DNA site with a free energy of −17.3 kcal mol^{-1} (Mathews & van Holde, 1990). The ratio $\Delta G°$/MW is 0.1 cal/Da. For the interaction of daunorubicin (MW 524) with its specific site, a free energy of −8.7 kcal mol^{-1} was found (Chaires, 1995a,b). The ratio $\Delta G°$/MW is 16.5 cal/Da for daunorubicin binding to DNA. The intriguing result of this comparison is that the binding free energy *per unit mass* of ligand is far greater for the small antibiotic than for the protein, by a factor of over 150. The meaning of this finding is clear upon some reflection. Only a small fraction of the total protein mass is in contact with its DNA binding site. In contrast, virtually every atom in the daunorubicin molecule is in contact with its DNA binding site. Daunomycin is buried within the DNA helix, away from the surrounding solvent. The domains shown in Figure 1 all interact in some fashion with the DNA. From this view, daunorubicin represents a rather remarkable streamlined packet of molecular interactions whose design is optimal for DNA binding and for which the maximal binding energy per unit mass is probably obtained. Much may be learned about optimizing binding to DNA from study of this comparatively simple system.

A problem of general current interest concerns the proper thermodynamic analysis of the effects of site-specific modifications on protein interactions and conformational transitions (Jencks, 1981; Ackers & Smith, 1985; Mark & van Gunsteren, 1994; Sharp & Englander, 1994). The problem addressed here is analogous to this more general problem, but the system we study offers several distinct advantages. The primary advantage is that the system is much simpler and, in principle, much more amenable to computational approaches and synthetic manipulations. Further, site-specific changes made in the small antibiotic molecule are less likely to lead to ambiguous global effects than are alterations made in larger, more complex protein molecules. Our experimental studies should provide data of general interest and use in addressing the larger issues concerned with developing appropriate thermodynamic descriptions of site-specific mutations.

Described here are studies of the binding of eight anthracycline antibiotics to DNA over a range of NaCl concentrations. The structures of the compounds studied are shown in Figure 2. Application of polyelectrolyte theory (Record & Spolar, 1990; Record *et al*., 1991) allowed us to partition binding free energies into their nonelectrostatic and polyelectrolyte contributions and then to use the nonelectrostatic part of the binding free energy to evaluate the effect of a specific chemical perturbation on the DNA binding free energy. The advantage of this strategy is that possible contributions from ligand charge are minimized. The resulting comparison allows detailed structure−affinity relationships to be drawn for both charged and uncharged ligands.

MATERIALS AND METHODS

Synthesis of Anthracycline Antibiotics. Daunorubicin and doxorubicin were purchased from Sigma Chemical Co. (St. Louis, MO). 9-Deoxydoxorubicin was provided by the National Cancer Institute (Bethesda, MD). The β anomer of doxorubicin was provided by Dr. F. Zunino (Milan, Italy). Hydroxyrubicin was prepared according to a previously published procedure (Horton *et al*., 1984). Daunomycinone and adriamycinone were obtained by acidic hydrolysis of daunorubicin and doxorubicin, respectively, and were purified by crystallization from methanol. The synthesis of the new daunorubicin analog referred to as WP608 [7-*O*-(4-amino-2,4,6-trideoxy-α-L-*lyxo*-hexopyranosyl)daunomycinone] is described in supporting information. ^{1}H NMR spectra were obtained for all of the synthesized compounds and were in agreement with proposed structures.

DNA Binding Studies. Binding studies utilized sonicated, fractionated calf thymus DNA, prepared as previously described (Chaires *et al*., 1982). BPE buffer, consisting of 6 mM NaH_2PO_4, 2 mM Na_2HPO_4, and 1 mM Na_2EDTA, pH 7.0 (±0.1), was used in all studies, with NaCl added to give the various concentrations indicated in the text. Fluorescence and visible absorbance spectroscopies were used to monitor anthracycline binding, using procedures fully documented elsewhere (Chaires *et al*., 1982, 1993; Chaires, 1985). Analysis of binding isotherms was done by nonlinear least-squares fits of experimental data, as fully described in

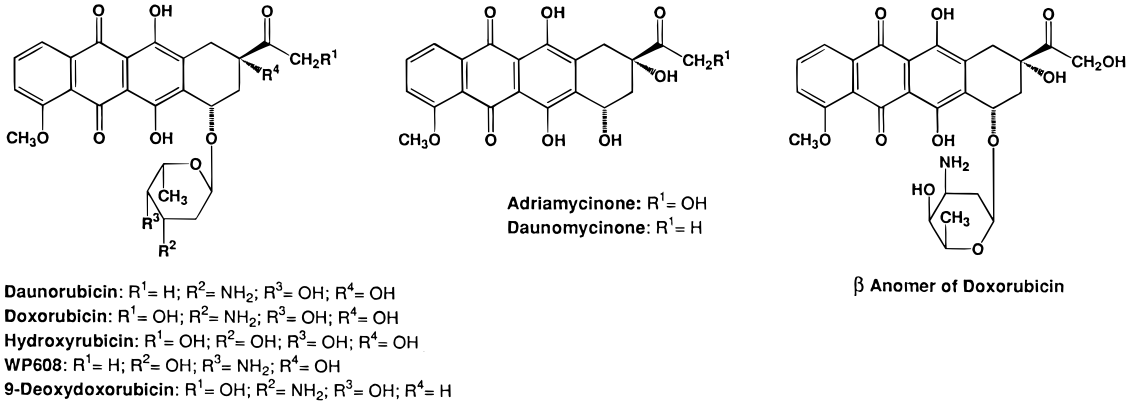

FIGURE 2: Structures of the anthracycline antibiotics studied.

Table 1: Energetics of Anthracycline Antibiotic Binding to DNA[a]

compound	$K/10^6$ (M^{-1})	$-\Delta G°$	$-\delta \log K/\delta \log [Na^+]$	$-\Delta G_{pe}$ (kcal/mol)	$-\Delta G_t$ (kcal/mol)
doxorubicin	29.6	9.9_8	0.97	2.3	7.7
daunorubicin	11.6	9.4_4	1.08	2.6	6.8
WP608	8.0	9.2^0	1.28	3.1	6.1
9-deoxydoxorubicin	4.2	8.8_6	0.91	2.3	6.5_6
hydroxyrubicin	0.3_5	7.4	0.18	0.4	7.0
β anomer doxorubicin	0.1_1	6.7	0.91	2.2	4.5
adriamycinone	0.05_2	6.3	0.26	0.6	5.7
daunomycinone	0.02_2	5.8			5.2

[a] Binding constants (K) and standard free energy changes ($\Delta G°$) refer to solution conditions of 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 1 mM Na_2EDTA, pH 7.0 at 20 °C. The polyelectrolyte contribution to the standard free energy change was calculated from the relation $\Delta G_{pe} = (SK)RT \ln [NaCl]$, where $SK = \delta \log K/\delta \log [Na]$. The thermodynamic free energy change was calculated by difference, $\Delta G_t = \Delta G° - \Delta G_{pe}$. We estimate the average error in $\Delta G°$ values to be ± 0.1 kcal mol^{-1} and the average error in ΔG_{pe} to be approximately 0.2 kcal mol^{-1} for the charged compounds and 0.05 kcal mol^{-1} for hydroxyrubicin and the aglycons. The propagated uncertainty in ΔG_t is approximately 0.3 kcal mol^{-1}.

Correia and Chaires (1994). The salt dependence of the DNA binding of selected compounds was examined by the "salt back-titration" method described by Lohman and Mascotti (1992).

RESULTS AND ANALYSIS

Figure 3 shows the results of equilibrium binding studies of the interaction of anthracycline antibiotics with calf thymus DNA. Binding constants were obtained over a range of NaCl concentrations in order to apply polyelectrolyte theory to dissect the binding free energy into its nonelectrostatic and polyelectrolyte contributions.

Table 1 summarizes the binding constants obtained in 16 mM Na^+, along with the salt dependence of the binding constants ($\delta \log K/\delta \log [Na^+]$) obtained by linear least-squares fits of the data in Figure 3.

The binding constant (K) was used to calculate the Gibbs free energy ($\Delta G°$), using the standard relation

$$\Delta G° = -RT \ln K \quad (1)$$

where R is the gas constant and T is the temperature in Kelvin. The salt dependence of the binding constant defines the slope

$$SK = \delta \log K/\delta \log [Na^+] \quad (2)$$

Record and co-workers (Record & Spolar, 1990; Record *et al.*, 1991) have shown that SK may be used to calculate the polyelectrolyte contribution (ΔG_{pe}) to $\Delta G°$ at a given NaCl concentration by the relation

$$\Delta G_{pe} = (SK)RT \ln [Na^+] \quad (3)$$

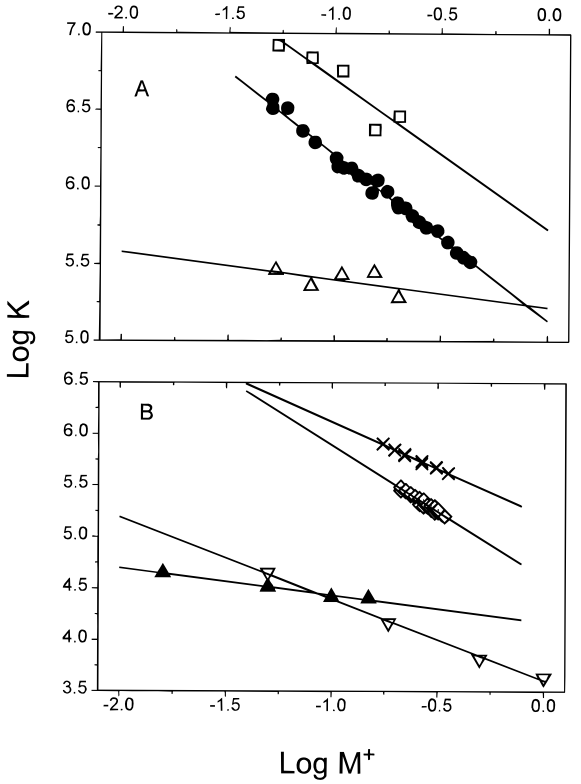

FIGURE 3: NaCl concentration dependency of anthracycline antibiotic DNA binding constants: (A) doxorubicin (open squares), daunorubicin (solid circles), and hydroxyrubicin (open triangles); (B) 9-deoxydoxorubicin (crosses), WP608 (open diamonds), adriamycinone (solid triangles), and the β anomer of doxorubicin (open down triangles). The slopes of these plots define the quantity $SK = \delta \log K/\delta \log [Na^+]$ listed in Table 1.

ΔG_{pe} is the portion of the binding free energy that is in excess over the value of the free energy a 1 M Na^+. The difference between the Gibbs free energy and ΔG_{pe} defines the "nonelectrostatic" free energy contribution (ΔG_t):

$$\Delta G_t = \Delta G^\circ - \Delta G_{pe} \quad (4)$$

The quantity ΔG_t refers to that portion of the binding free energy that is independent of salt concentration and which contains a minimal of contributions from polyelectrolyte effects such as coupled ion release. ΔG_t is useful for the comparison of the binding strength of charged and uncharged ligands, since it contains but a small contribution from ligand charge.

Equations 1−4 were used to partition the binding free energy into its nonelectrostatic and polyelectrolyte contributions. Table 1 summarizes all estimates of ΔG°, ΔG_t, and ΔG_{pe} derived from the experimental data shown in Figure 3.

Error estimates were made for computed free energy values using appropriate expressions for the propagation of error (Taylor, 1982). Uncertainties in the primary experimental values K and SK were estimated to be about 15% and 10%, respectively, based on the statistics on least-squares fits of data.

DISCUSSION

The data described here for eight anthracycline antibiotics enable us to analyze in detail the structure−DNA affinity relationships among the members of the group.

Energetic Contribution of Charge. Application of polyelectrolyte theory permits dissection of the DNA binding free energy of the series of anthracyclines into their nonelectrostatic and polyelectrolyte components, a procedure that offers a rigorous thermodynamic approach for the comparison of charged and uncharged ligands. Of the compounds we have studied, all except hydroxyrubicin and the aglycons are positively charged under the conditions of our binding studies. Table 1 shows the polyelectrolyte contribution to the binding free energy (ΔG_{pe}) for the charged compounds is about 2−3 kcal mol^{-1} in 16 mM Na^+. For the uncharged hydroxyrubicin and the aglycons, ΔG_{pe} is about 0.5 kcal mol^{-1}. The polyelectrolyte contribution of these uncharged intercalators to the binding free energy is due to ion release arising from changes in the phosphate spacing that results from intercalation (Mueller & Crothers, 1968; Friedman & Manning, 1984).

Comparison of Binding Free Energies: Use of $\Delta\Delta G_t$. To evaluate the free energy contribution to DNA binding of specific substituents, we compared the quantity $\Delta\Delta G_t$, the difference in the nonelectrostatic portion of the binding free energy relative to the parent compound, doxorubicin. The rationale for this approach requires some discussion. The binding free energy for a bimolecular interaction contains several components (Williams *et al*., 1993), including terms arising from the differences in the conformational free energies of the reactants, molecular interactions occuring upon complex formation, the hydrophobic transfer of ligand from solution to the binding site, and contributions from coupled processes like ion and water release. In our procedure, we first compute ΔG_t for all of the anthracycline binding interactions, which allows us to separate polyelectrolyte effects and thereby minimize differences arising from ligand charge. Next, the difference in ΔG_t between doxorubicin and the other compounds is computed. Since the free energy contributions arising from reactant conformational changes and the loss of rotational freedom are likely to be similar for all compounds studied, this step narrows down the free energy differences to contributions arising primarily from molecular interactions within the complexes and from the hydrophobic transfer from solution into the binding site. A positive value of $\Delta\Delta G_t$ indicates a *less favorable* binding free energy relative to doxorubicin. From the appropriate propagation of error (Taylor, 1982), the uncertainty in $\Delta\Delta G_t$ values is estimated to be at most ±0.5 kcal mol^{-1}.

The $\Delta\Delta G_t$ value for a particular compound reflects primarily differences in molecular interactions within the binding sites resulting from substituent alteration, along with any differences in energy for transferring the ligand from solution into the binding site. Further separation of these two effects is not yet possible, although some assessment of the importance of the latter contribution is possible for some of the compounds by considering phase partition data. The partition coefficient (P) in a H_2O−octanol system is 0.5 and 2.8 for doxorubicin and daunorubicin, respectively (Satyanarayana and Chaires, unpublished data). For the uncharged hydroxyrubicin, the partition coefficient is 15−20 in the same system (Burke *et al*., 1993). These values correspond to free energies ($\Delta G_{tr} = -RT \ln P$) for the transfer from water to octanol of +0.4 and −0.6 kcal mol^{-1} for doxorubicin and daunorubicin, respectively, and of approximately −1.6 kcal mol^{-1} for hydroxyrubicin. If it is assumed that the transfer between water and octanol approximates the hydrophobic transfer from solvent to the DNA binding site, these numbers suggest that $\Delta\Delta G_t$ could contain favorable contributions of approximately 1 and 2 kcal mol^{-1} for daunorubicin and hydroxyrubicin, respectively. $\Delta\Delta G_t$ values cannot, therefore, be interpreted solely in terms of microscopic free energy changes resulting from changes in the molecular interactions within the drug binding site.

In spite of this caveat, we believe that our approach provides the most rigorous and thermodynamically sound evaluation of substituent effects on drug−DNA interactions attempted thus far. Calculated $\Delta\Delta G_t$ values are collected in Table 2 and are shown graphically in Figure 4. These data provide the basis for further discussion of substituent effects.

Contributions of the Daunosamine. Perhaps the most striking result to emerge from our studies is a quantitative evaluation of the contribution of the carbohydrate portion of doxorubicin, the daunosamine. While the role of carbohydrates in sequence-specific drug−DNA interactions has attracted increasing attention (Chen *et al*., 1985; Hawley *et al*., 1989; Li *et al*., 1994), the energetic contribution of such moieties has not been experimentally evaluated. Comparison of the $\Delta\Delta G_t$ values in Table 2, however, does allow such an evaluation. Comparison of doxorubicin and its aglycon, adriamycinone, reveals a 2.0 kcal mol^{-1} energetic penalty for removal of the daunosamine, a value that represents over 20% of the total binding free energy. A 2 kcal mol^{-1} free energy loss corresponds to about a 100-fold decrease in the binding constant. A similar difference in binding free energy between daunorubicin and its aglycon, daunomycinone, may be calculated. The daunosamine thus contributes a substantial portion of the total binding free energy. The DNA

Table 2: Energetic Cost of Structural Alterations in Anthracycline Antibiotics[a]

compound	structural alteration	$\Delta\Delta G_t$ (kcal/mol)
doxorubicin	reference compound	0.0
hydroxyrubicin	3′-NH_2 → 3′-OH	0.7
daunorubicin	14-OH → 14-H	0.9
9-deoxydoxorubicin	9-OH → 9-H	1.1
WP608	3′-NH_2 → 3′-OH; 4′-OH → 4′-NH_2; 14-OH → 14-H	1.6
adriamycinone	sugar removed	2.0
daunomycinone	sugar removed; 14-OH → 14-H	2.5
β anomer doxorubicin	sugar orientation	3.2

[a] The quantity $\Delta\Delta G_t$ refers to the difference in the thermodynamic binding free energy relative to the reference compound, doxorubicin. In all cases, $\Delta\Delta G_t$ is positive, indicating a *less favorable* binding interaction resulting from the structural alteration. The maximum error in $\Delta\Delta G_t$ is estimated to be 0.5 kcal mol^{-1}.

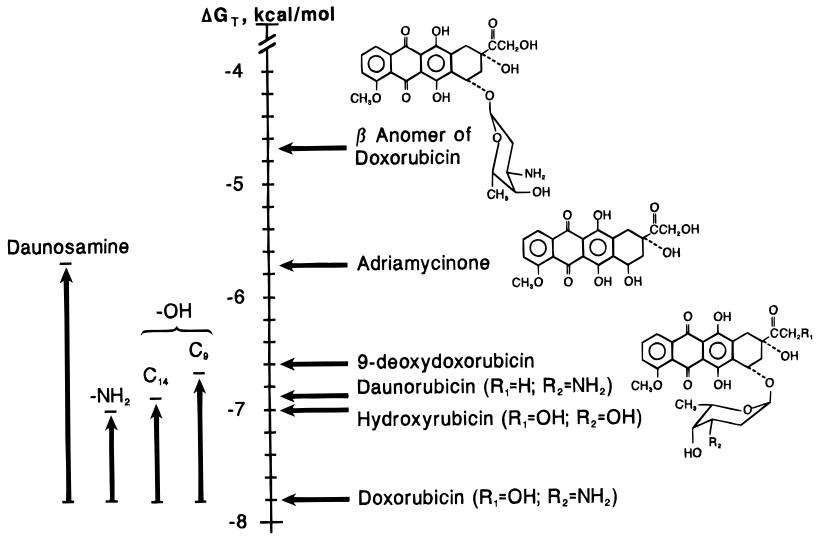

FIGURE 4: Free energy diagram showing the effect of structural alterations on the nonelectrostatic portion of the binding free energy. The magnitude of ΔG_T (kcal mol^{-1}) is shown on the axis. The structures of the compounds are shown on the right side of the figure. The arrows on the left side of the figure indicate the magnitude of the free energy difference ($\Delta\Delta G_T$) resulting from a specific chemical alteration.

binding of the β anomer of doxorubicin, in which the daunosamine is attached differently, is found to be less favorable than the binding of the aglycon when the binding free energy is corrected for polyelectrolyte effects. This is a striking and unexpected result because it indicates that the energetic cost of having the daunosamine positioned in a stereochemically unfavorable way is greater than not having it present at all. Since the daunosamine moieties of both doxorubicin and daunorubicin normally fit snugly in the minor groove, the energy penalty must arise from steric clashes of the unfavorably positioned sugar moiety in the β anomer.

Energetic Contribution of the Amine. Hydroxyrubicin is an uncharged compound in which the 3′-amine found in doxorubicin has been replaced by a hydroxyl group. Table 2 shows, however, that $\Delta\Delta G_t$ is approximately +1 kcal mol^{-1}, indicating that hydroxyrubicin DNA binding affinity is *decreased* more than is expected from just the loss of the polyelectrolyte contribution from the positively charged amine. The amine substituent is therefore important for DNA binding for more than just its positive charge. Some, but not all, high-resolution crystal structures of anthracycline−DNA complexes show that the amine participates in hydrogen-bonding interactions with DNA bases. The loss of such an interaction is perhaps what is indicated by the observed $\Delta\Delta G_t$ value.

Energetic Contributions of OH at the C-9 and C-14 Positions. Hydroxyl groups attached at the C-9 and C-14 positions have been identified by crystallographic and vibrational spectroscopic studies as key participants in hydrogen-bonding interactions that stabilize the drug−DNA complex (Pohle *et al*., 1990; Wang, 1992, 1993). The $\Delta\Delta G_t$ values observed for daunorubicin and 9-deoxydoxorubicin indicate a substantial energetic penalty for alteration of either of these key hydroxyl groups (Table 2). Daunorubicin lacks the hydroxyl at C-14 and loses 0.9 kcal mol^{-1} in binding free energy relative to doxorubicin. 9-Deoxydoxorubicin lacks the hydroxyl at C-9 and loses 1.1 kcal mol^{-1} in binding free energy. Estimates for the free energy of hydrogen bond formation range from −1 to −4 kcal mol^{-1}, so the observed $\Delta\Delta G_t$ values are generally consistent with the loss of hydrogen-bonding interactions.

Combined Substituent Effects in WP608. WP608 offers an opportunity for evaluation of combined substituent effect. In WP608, the amine has been moved from the C-3′ to the C-4′ position and an OH group added at C-3′. The aglycon is identical to that found in daunorubicin, so the removal of the C-14 OH may be evaluated. Interestingly, the effect of substituent changes in WP608 on binding energy appears to be essentially additive. The loss of the C-14 OH costs 0.9 kcal mol^{-1} (daunorubicin *versus* to doxorubicin, Table 2). Replacement of 3′-NH_2 by OH costs 0.7 kcal mol^{-1} (hydroxyrubicin *versus* doxorubicin, Table 2). The sum of these two changes, 1.6 kcal mol^{-1}, equals (perhaps somewhat fortuitously) exactly the 1.6 kcal mol^{-1} difference in binding energy observed between WP608 and doxorubicin (Table

2). The energetics of WP608 binding to DNA also indicate that substituents at C-4′ probably are not involved in important molecular interactions with DNA, after correction for possible polyelectrolyte contributions. This conclusion is consistent with structural studies (Wang, 1992, 1993; Kennard & Salisbury, 1993; Ughetto, 1988) that show that substituents at C-4′ are directed out from the minor groove, away from close contact with the DNA.

Comparison with Predictions from Computational Studies. Comparative DNA binding affinities have been computed for several anthracycline derivatives (Chen *et al*., 1986; Gresh *et al*., 1989). Two of these cases may be compared to the experimental data described here. The DNA binding energies of 4-demethyoxydaunorubicin and its β anomer were calculated. The interaction of the β anomer was destabilized by 24.6 kcal mol^{-1}, or by 18% of the total interaction energy of 4-demethoxydaunorubicin (Chen *et al*., 1986). On a percentage basis, the computed effect of alteration of the sugar orientation is less than experimentally observed here, since the ΔG_t for the binding of the β anomer of doxorubicin was found to be 55% of that observed for doxorubicin. The DNA interaction of 9-deoxydoxorubicin was computed to be only about 1% lower than that for doxorubicin (Gresh *et al*., 1989). Again, the computed energetic effect is substantially lower than was experimentally observed here, where ΔG_t for 9-deoxydoxorubicin was found to be 15% lower than the value for doxorubicin. The origin of quantitative differences between the computed and experimental effects is not immediately evident.

Comparison with Previous Experimental Work. Hundreds of anthracycline derivatives have been synthesized and their biological and chemical properties compared with the parent compounds, doxorubicin and daunorubicin (Weiss, 1992). Comparison of structure−affinity relationships of all of these anthracyclines is difficult for a variety of reasons, including differences in the solution conditions used in DNA binding studies in different laboratories and differences in the methods used to analyze and interpret binding isotherms. The dissection procedure we employ is unique in studies of drug−DNA interactions and offers a significant advantage in that comparison of the nonelectrostatic portion of the free energy extracts a quantity that is independent of variations in salt concentrations. We are thus able to reevaluate the results of several recent studies to provide meaningful comparisons with our data for some of the compounds we have studied.

Roche and co-workers (Roche *et al*., 1994) recently reported binding constants for several daunorubicin analogs in 0.1 M Na^+.[1] Among the compounds studied were the aglycon (daunomycinone) and a compound in which the 3′-amine was replaced by a hydroxyl group. The latter is analogous to hydroxyrubicin studied here. Using the reported binding constants for the interaction of these compounds with their "GC" deoxyoligonucleotide, we have computed ΔG_t values, assuming that values for the salt dependence of the binding constant are similar to what we report here. The results of our calculation provide a corresponding daunorubicin set for comparison with the doxorubicin series described here. For daunomycinone, a $\Delta\Delta G_t$ value of 2.5 kcal mol^{-1} was calculated, in excellent agreement with the value of 2.0 reported for adriamycinone (Table 2). For 3′-deamino-3′-hydroxydaunorubicin, a $\Delta\Delta G_t$ of 1.5 kcal mol^{-1} was calculated, a value in fair agreement with the value of 0.7 kcal mol^{-1} observed for hydroxyrubicin (Table 2). The agreement between the corresponding daunorubicin and doxorubicin series reinforces our conclusions concerning the energetic contributions of the daunosamine moiety and the 3′-amino group.

Several groups have reported binding constants for the interaction of doxorubicin and 9-deoxydoxorubicin to DNA. Rizzo *et al* (1989) studied the binding of these two compounds and daunorubicin to the hexanucleotide 5′-d(CGTACG) in 1 M NaCl. Relative to doxorubicin, we calculate from their data $\Delta\Delta G_t$ of 0.5 kcal mol^{-1} for *both* daunorubicin and 9-deoxydoxorubicin, values slightly lower than the values near 1 kcal mol^{-1} we observed (Table 2). From the data of Gresh *et al*. (1989), $\Delta\Delta G_t$ values of 0.8 and 1.1 kcal mol^{-1} may be calculated for the interaction of daunorubicin and 9-deoxydoxorubicin with poly[d(GC)], respectively, values that are in much closer agreement with the values in Table 2. Finally, Cera *et al*. (1991) report binding constants for the interaction of anthracyclines with calf thymus DNA in 0.104 M NaCl that may be used to compute $\Delta\Delta G_t$ values of 0 and 0.3 kcal mol^{-1} for daunorubicin and 9-deoxydoxorubicin, respectively. Although the quantitative agreement of these diverse studies with our data is only fair, the general trend reinforces our conclusions concerning the hydroxyl groups at the C-9 and C-14 positions.

SUMMARY

By using data experimental studies of the NaCl concentration dependence of the DNA binding of eight anthracycline antibiotics, it is possible to partition their DNA binding free energies into polyelectrolyte and nonelectrostatic components. Use of the latter quantity provides a means of rigorously evaluating structure−affinity relationships among these antibiotics. The results of the quantitative analysis show a striking and large contribution to the DNA binding energy from the daunosamine moiety. Specific substituents, 3′-NH_2, 9-OH, and 14-OH, each contribute approximately 1 kcal mol^{-1} to the DNA binding free energy, probably by participating in specific molecular interactions. The results of these studies clarify and quantify the contributions of specific anthracycline substituents to DNA binding and provide a foundation for the rational design of new DNA binding agents.

ACKNOWLEDGMENT

Thanks to Dr. Susan Wellman for comments on the manuscript.

SUPPORTING INFORMATION AVAILABLE

A description of the synthesis of compound WP608 (3 pages). Ordering information is given on any current masthead page.

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[1] The article by Roche *et al*. (1994) contains typographical errors in which the structures of daunomycin and analog 1 are misidentified. In Figure 1 of the paper and throughout the text, daunomycin and analog 1 are incorrectly said to have OH groups at the C-2′ (R3) position. In both cases, R3 should be simply an H. The error has been pointed out to the authors, and an erratum has been submitted (C. Roche and D. Crothers, personal communication).

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