Dissection of the Free Energy of Anthracycline Antibiotic Binding to DNA: Electrostatic Contributions

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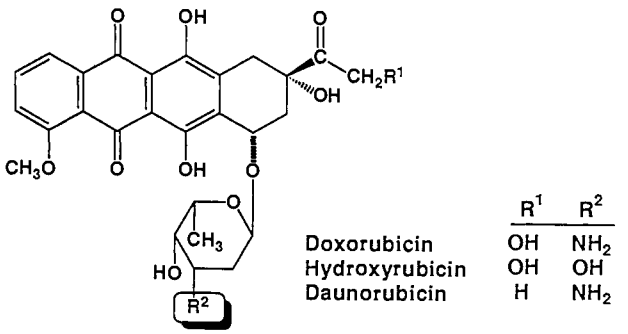
Abstract: Fluorescence titration methods and equilibrium dialysis were used to study the thermodynamics of the interaction of doxorubicin, the β anomer of doxorubicin, daunorubicin, and hydroxyrubicin with DNA. All of these except hydroxyrubicin carry a net charge of +1 at neutral pH, arising from the protonation of the daunosamine moiety. Hydroxyrubicin is a synthetic anthracycline antibiotic in which the amine moiety has been replaced by a hydroxyl group, which is uncharged but polar at neutral pH. The comparative binding studies we describe offer a unique opportunity to evaluate the electrostatic contributions to the DNA binding free energy of these anthracycline antibiotics and to test specific predictions arising from current polyelectrolyte theory as applied to ligand–DNA interactions. We find that the quantity ($\delta \log K/\delta \log [M^+]$) changes from a value of –0.95 for doxorubicin to –0.18 for hydroxyrubicin. The latter value is in excellent agreement with the value of –0.24 predicted by the theory of Friedman and Manning for the interaction of an uncharged intercalating ligand with DNA. The DNA binding free energy decreases from –8.8 kcal mol^{-1} for doxorubicin to –7.2 kcal mol^{-1} for hydroxyrubicin under solution conditions of 200 mM Na^+, pH 7.0, 20 °C. The results we obtain allow us to dissect the DNA binding free energy into its electrostatic and nonelectrostatic components. Knowledge of these values helps to clarify the precise role of the amine group in doxorubicin binding to DNA. In addition, comparison of the DNA binding of doxorubicin with the β anomer of doxorubicin provides a striking example of stereoselective antibiotic binding to DNA.

The rational design of new anticancer antibiotics targeted toward DNA requires a thorough understanding of the specificity and energetics of the DNA binding of existing compounds of proven clinical utility. The anthracycline antibiotics are important in this effort, since they are widely, and actively, used in cancer chemotherapy[1] and are believed to act by mechanisms involving direct interaction with DNA.[2] The physical chemistry of their binding to DNA has been recently reviewed.[3] Daunorubicin (daunomycin), the parent anthracycline antibiotic, has been shown to have a unique DNA binding sequence specificity and preferentially binds to the triplet sequences 5′$\frac{A}{T}$CG and 5′$\frac{A}{T}$GC.[4] Molecular modeling[5–7] and X-ray crystallography[8–10] have provided insights into the energetic and structural basis of the observed DNA binding specificity. Solution, X-ray crystallographic, and theoretical studies have converged to provide a detailed and coherent molecular picture of the daunorubicin–DNA interaction, to the extent that this interaction is, arguably, a paradigm for understanding the sequence-specific binding of antibiotics to DNA.



	R^1	R^2
Doxorubicin	OH	NH_2
Hydroxyrubicin	OH	OH
Daunorubicin	H	NH_2

Figure 1. Structures of doxorubicin, hydroxyrubicin, and daunomycin.

In order to explore the contributions of specific chemical constituent within the anthracycline antibiotic structure to the DNA binding free energy, we have begun to synthesize new compounds with altered substituents and to examine their DNA binding properties. We report here some initial results of this effort. Hydroxyrubicin is a synthetic analog of doxorubicin in which the amine group of the sugar portion has been replaced by a hydroxyl group (Figure 1). At neutral pH, the amine group is protonated, and doxorubicin carries a net charge of +1. Substitution of a hydroxyl group for the amine group results in the loss of this positive charge but retains a polar group at the position. The substitution, importantly, does not add a bulky derivative that might sterically hinder the interaction of the antibiotic with DNA. The theory for the interaction of charged ligands with DNA has been developed by extension of polyelectrolyte theory.[11–14] Our binding studies offer a unique opportunity

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to test these theories, since we can compare the binding of charged and uncharged forms of antibiotics which otherwise have nearly identical structures.

Our results allow us to dissect the free energy of anthracycline antibiotic binding to DNA into its electrostatic and nonelectrostatic components and thus to quantitatively assess the polyelectrolyte contribution to binding. In addition, our data provide perhaps the first quantitative verification of a recent theoretical prediction[13] of the magnitude of the salt dependence of the DNA binding constant for an uncharged, intercalating ligand.

Experimental Procedures

Anthracycline Antibiotics. Doxorubicin (adriamycin) and daunorubicin (daunomycin) were obtained commercially (Sigma Chemical Company, St. Louis, MO) and were used without further purification. Hydroxyrubicin was synthesized as previously described.[15] A molar extinction coefficient of 11 500 M^{-1} cm^{-1} at 480 nm was used for all compounds for concentration determination.

DNA Preparations. Calf thymus DNA (Boerhinger-Mannheim, Indianapolis, IN) was sonicated, phenol extracted, and purified as previously described.[16] A molar extinction coefficient of 12 824 M(bp)$^{-1}$ cm^{-1} at 260 nm was used for DNA concentration determinations. The standard buffer (BPES) used in these studies consisted of 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 0.185 M NaCl, pH 7.0.

Fluorescence Excitation and Emission Spectra. Steady-state fluorescence measurements were obtained on an SLM Model 4800C spectrofluorometer equipped with a thermostated cuvette compartment and interfaced to an IBM PS/2 Model 55 SX computer. Model MC-320 holographic grating monochromators (1500 lines/mm) were used in these studies. Excitation and emission spectra were recorded with an excitation resolution of 8 nm and an emission resolution of 4 nm. In all cases, spectra were corrected for background fluorescence and scatter from buffer or DNA by subtraction of an appropriate blank baseline spectrum.

Fluorescence Titration Experiments. Fluorescence titration experiments were conducted on a Perkin-Elmer 650–40 spectrofluorometer. An excitation wavelength of 480 nm was used, and total fluorescence emission was monitored by collecting all light passing through a 495 nm cutoff filter. Titrations were performed by maintaining a fixed total antibiotic concentration (0.5, 1.0, or 2.0 μM) and increasing the DNA concentration (in base pairs) over the range 1 nM to 1 mM. Temperature was maintained at 20 °C.

Equilibrium Dialysis Experiments. SpectraPor 2 dialysis tubing (Spectrum Medical Industries, Los Angeles, CA) with MWCO 12–14 000 was used for dialysis experiments. Volumes of 1 mL of DNA (0.1 mM bp) were dialyzed against 15 mL of antibiotic solution of varied concentration. After reaching dialysis equilibrium (24–48 hr), free antibiotic concentrations (C_f) were determined from the dialysate by visible absorbance measurements at either 480 nm (E^{M}_{480} = 11 500 M^{-1} cm^{-1}) or 540 nm (E^{M}_{540} = 5100 M^{-1} cm^{-1}). Total antibiotic (C_t) in the retentate was determined by absorbance measurements at the isosbestic wavelength, 540 nm. Bound antibiotic (C_b) was then determined by difference, $C_b = C_t - C_f$.

Data Analysis. Binding data from equilibrium dialysis experiments were cast into the form of a Scatchard plot of r versus r/C_f, where r is the binding ratio (mol antibiotic bound/mol DNA bp). Data were fit by nonlinear least squares analysis to the neighbor exclusion model of McGhee and von Hippel[17]

$$r/C_f = K(1-nr)\{(1-nr)/(1-[n-1]r)\}^{n-1} \quad (1)$$

where K is the binding constant for the interaction of antibiotic with an isolated DNA site and n is the neighbor exclusion parameter expressed in base pairs. Fluorescence titration data were fit directly to obtain association constants in terms of molar base pair concentrations.[18] These are lower than the constants obtained from the neighbor exclusion model by the factor of n, the number of base pairs per binding site. The software FitAll (MTR Software, Toronto, Canada) was modified and used for nonlinear least squares analysis of experimental binding data.

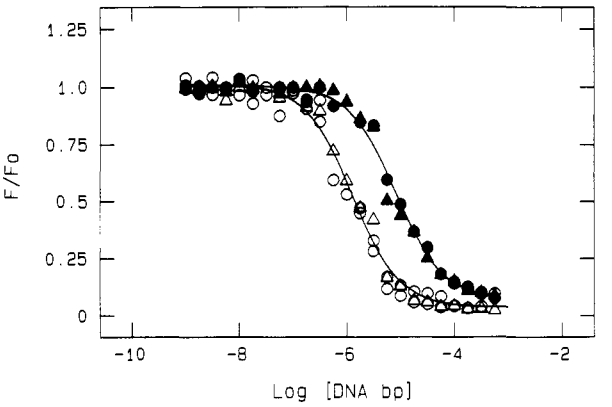

Figure 2. Fluorescence titration experiments. Fixed concentrations of either doxorubicin (adriamycin) (open symbols) or hydroxyrubicin (filled symbols) were titrated with increasing concentrations of calf thymus DNA. The triangles and circles refer to separate titration experiments. Solutions contained BPES buffer, consisting of 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 185 mM NaCl, pH 7.0, and experiments were conducted at 20 °C. For each compound, separate experiments at 0.5, 1.0, and 2.0 μM total concentration were conducted.

Molecular Modeling Studies. The electrostatic potentials of doxorubicin and hydroxyrubicin were calculated by solving the nonlinear Poisson–Boltzmann equation using the molecular modeling program Delphi[19] (Biosym Technologies, Inc.). Doxorubicin crystal coordinates[10] were obtained from the Nucleic Acid Database[20] and were incorporated into the molecular modeling program Discover/InsightII (Biosym Technology, Inc.). Hydroxyrubicin coordinates were obtained by using the Builder program of InsightII to replace the 3′ amino group on doxorubicin with a hydroxyl group. The AMBER force field was used to assign atomic potentials to the compounds. Atomic charges were assigned by MOPAC after assigning a net charge of +1 to doxorubicin and a net charge of 0 to hydroxyrubicin. Parameters used for the Delphi program included an ion exclusion radius of 2.0 Å and a surrounding solvent mapped into a 65 × 65 × 65 point cubic lattice. The midpoint of each grid line joining the two lattice points was assigned a dielectric constant of 78.5 (to approximate an aqueous solution). A solvent condition of 0.185 M ionic strength and pH 7.0 was used to mimic the conditions used in actual experiments.

Results

Fluorescence excitation and emission spectra for free and DNA-bound hydroxyrubicin were found to be identical to those reported[16] for daunorubicin (data not shown). Hydroxyrubicin fluorescence emission is nearly completely quenched upon binding to DNA, behavior that was previously observed for doxorubicin and daunorubicin.[16]

Figure 2 shows the results from fluorescence titration experiments using doxorubicin and hydroxyrubicin. The titration midpoints of these curves differ for each antibiotic, a direct indication that their DNA binding affinities differ. The shape and position of these titration curves were found to be independent of the total antiobiotic concentration over the range 0.5–2.0 μM, an observation that indicates that these antibiotic concentrations are approximately equal to, or less than, the reciprocal of the antibiotic binding constants for DNA.[18] Nonlinear least squares analysis of the titration curves shown in Figure 2 yielded binding constants of 1.5 (±0.1) × 10^5 (M bp)$^{-1}$ and 1.0$_5$ (±0.1) × 10^6 (M bp)$^{-1}$ for hydroxyrubicin and doxorubicin, respectively. Substitution of the hydroxyl group for the amine group thus results in the reduction of the binding constant by a factor of 7 under the ionic conditions of this titration experiment. Note that these

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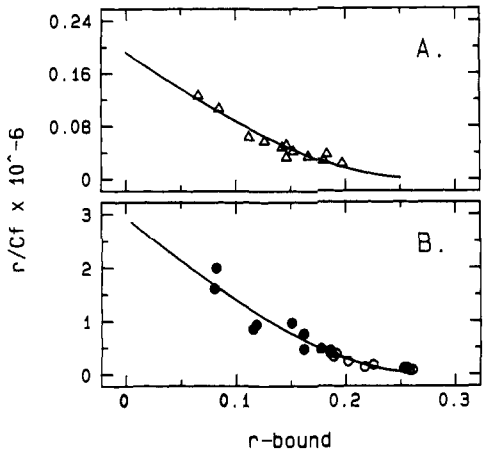

Figure 3. Scatchard plots of the binding of hydroxyrubicin (A) and doxorubicin (B) to calf thymus DNA. Data were obtained by equilibrium dialysis. The solid lines indicate the best nonlinear least squares fit of the data to the simple neighbor exclusion model of McGhee and von Hippel, yielding the binding parameters listed in Table I.

Table I. Summary of Thermodynamic Binding Parameters for the Interaction of Doxorubicin, Daunorubicin, and Hydroxyrubicin with Calf Thymus DNA[a]

compound	$K_{eq}/10^5$ (M^{-1})	n (bp)	$\delta \log K/\delta \log [M^+]$
doxorubicin	29.0 ± 2.0	3.4 ± 0.1	−0.96 ± 0.1
daunorubicin[b]	6.9 ± 0.2	3.4 ± 0.1	−1.25 ± 0.1$_5$
hydroxyrubicin	1.9 ± 0.1	3.5 ± 0.1	−0.18 ± 0.1$_5$
β anomer of doxorubicin[c]	0.15	4.3 ± 0.5	−0.91 ± 0.1

[a] Data refer to solutions containing 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 185 mM NaCl, pH 7.0, at a temperature of 20 °C. [b] Data from ref 16 and: Chaires, J. B. *Biopolymers* **1985**, *24*, 403. [c] Data from Britt et al. *Mol. Pharm.* **1986**, *29*, 74.

binding constants, expressed with units of $(M\ bp)^{-1}$, will differ from binding constants obtained using the neighbor exclusion model by a factor of n, the number of base pairs per binding site.

Binding constants for the two antibiotics were also evaluated using data obtained from equilibrium dialysis (Figure 3). Nonlinear least squares fits of these data to the simple neighbor exclusion model of McGhee and von Hippel (eq 1) yielded the binding parameters shown in Table I. Values for K (referring to the interaction of antibiotic with an isolated DNA site) of 29.0 (±2.0) × 10^5 M^{-1} and 1.0 (±0.1) × 10^5 M^{-1} were found for doxorubicin and hydroxyrubicin, respectively. These values are in very good agreement with those obtained independently by the fluorescence titration method, when the number of base pairs per site is taken into consideration. The exclusion parameter is essentially the same for doxorubicin and hydroxyrubicin (3.5 bp), indicating that the two antibiotics remove the same number of base pairs as potential binding sites upon their interaction with the DNA lattice.

The salt dependencies of the binding constants of doxorubicin and hydroxyrubicin are shown in Figure 4, along with data taken from previous publications from this laboratory for other anthracycline antibiotics of interest. Data are presented in the form of double logarithmic plots of log K vs log $[M^+]$, according to the theory of Record and co-workers.[11] The slopes of all anthracyclines *except* hydroxyrubicin are seen in Figure 4 to be approximately −1. In contrast, hydroxyrubicin displays a markedly reduced slope of −0.18.

Discussion

The primary aim of our studies is to dissect the free energy for anthracycline antibiotic binding into its nonelectrostatic and electrostatic components. Application of polyelectrolyte theory to the binding data of Table I will allow us to make these estimates. Data obtained for the DNA binding of hydroxyrubicin, a new,

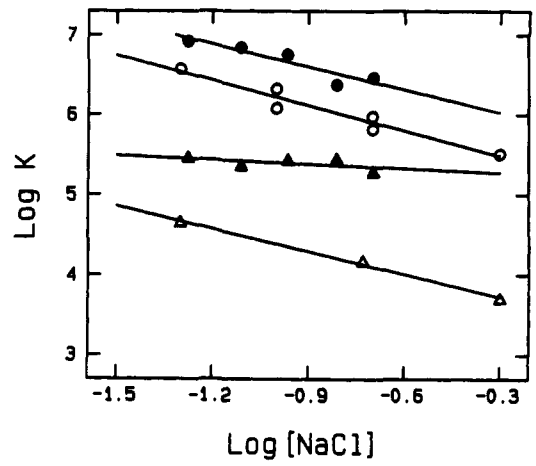

Figure 4. Dependency of equilibrium binding constants on NaCl concentration for doxorubicin (closed circles), daunorubicin (open circles), hydroxyribicin (closed triangles), and the β anomer of doxorubicin (open triangles). Data are presented as a double logarithmic plot according to the theory of Record *et al.* The linear least squares fits to the data are shown by the solid lines, from which the slopes ($\delta \log K/\delta \log [NaCl]$) = $-S$ are obtained, yielding for doxorubicin a slope of 0.96 and for hydroxyrubicin a slope of 0.18.

uncharged anthracycline antibiotic, will provide a unique test of specific predictions of polyelectrolyte theory.

Polyelectrolyte theory has been extended to explicitly and quantitatively describe the thermodynamic linkage between cation and charged ligand binding to the DNA lattice.[11-14] Qualitatively, the picture is as follows. Cations are condensed around the DNA in order to neutralize the highly negatively charged lattice.[12] These cations are nonspecifically ("territorially") bound and are mobile. Binding of a positively charged ligand to the DNA results in the release of bound cation, since the positive charge on the ligand may serve to neutralize the DNA in place of the cation. Cation and ligand binding are thus thermodynamically linked, and the binding of one influences the binding of the other. The dependency of the apparent ligand binding constant on cation concentration is a manifestation of the thermodynamic linkage.

The binding data obtained for doxorubicin and hydroxyrubicin are summarized in Table I, along with previously published data for two other anthracycline antibiotics, daunorubicin and the β anomer of doxorubicin. Both of these latter compounds possess the protonated daunosamine group and are charged at neutral pH. The β anomer of doxorubicin is a stereoisomer of the parent compound, with an altered orientation at the C-1′ adriamycinone. Table I shows that values of the slope ($\delta \log K/\delta \log [M^+]$) range from −0.91 to −1.25 for anthracycline antibiotic binding to DNA, with the notable exception of hydroxyrubicin, which has a value of −0.18. For the binding of a *monovalent* intercalator to DNA, the quantity ($\delta \log K/\delta \log [M^+]$) is predicted to be −0.88 by Record and co-workers[11,14a] and −1.24 by Friedman and Manning.[13] We note that the value predicted by Record and co-workers was not derived to include contributions arising from conformational transitions in the DNA. Wilson and Lopp[14b] have extended the theory of Record and co-workers to include the effects of intercalation, producing expressions for ($\delta \log K/\delta \log [M^+]$) that predict slopes near the Manning–Friedman values for monovalent intercalators. A detailed discussion of the origin of the different predicted values may be found in ref 13. We note that the experimental values obtained for doxorubicin, daunorubicin, and the β anomer of doxorubicin are all in excellent agreement with the theoretical predictions and tend toward the higher values predicted by Friedman and Manning[13] and Wilson and Lopp.[14b] Friedman and Manning[13] predicted that, for the binding of an *uncharged* intercalator to DNA, ($\delta \log K/\delta \log [M^+]$) = −0.24. The value of −0.18 obtained for hydroxyrubicin is in excellent agreement with the predicted value. This slight

Table II. Free Energy of Anthracycline Antibiotic Binding to Calf Thymus DNA[a]

compound	R^1	R^2	$\Delta G_{obs}°$	$\Delta G°$	$\Delta G_{el}°$	$\delta\Delta G$
doxorubicin	OH	NH_2	−8.8	−7.8	−1.0	0
daunorubicin	H	NH_2	−7.9	−6.9	−1.0	0.9
hydroxyrubicin	OH	OH	−7.2	−7.0	−0.2	0.8
β anomer of doxorubicin	OH	NH_2	−5.7	−4.7	−1.0	3.1

[a] Units for all values are kcal mol^{-1}. $\Delta G_{obs}°$ is the observed free enrgy under in a solution consisting of 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 185 mM NaCl, pH 7.0, at a temperature of 20 °C. $\Delta G°$ is the nonelectrostatic free energy component, referring to a standard state of ≈1 M monovalent salt concentration. $\Delta G_{el}°$ is the polyelectrolyte contribution to the binding free energy, evaluated at 0.2 M [NaCl]. The quantity $\delta\Delta G$ is the difference in $\Delta G°$ relative to the value obtained for doxorubicin.

salt dependence of the binding constant arises from the change in phosphate spacing resulting from the lengthening of the DNA helix caused by intercalation, and the concomitant alteration of the charge density of the lattice.[13] The data obtained for hydroxyrubicin provide perhaps the first quantitative verification of the Friedman–Manning prediction for this case.

The observed free energy of antibiotic binding is defined as $\Delta G_{obs}° = -RT \ln K_{obs}$. Values calculated from the binding constants (Table I) are shown in Table II. The observed binding free energy may be partitioned into two contributions[14]

$$\Delta G_{obs}° = \Delta G° + \Delta G_{el} \quad (2)$$

where $\Delta G°$ is the nonelectrostatic contribution to ΔG_{obs} (referring to a standard state of ≈1 M monovalent salt) and ΔG_{el} is the polyelectrolyte contribution to ΔG_{obs}. The latter term may be estimated from the experimentally determined quantity ($\delta \log K/\delta \log [M^+]) = S$. Record and co-workers[14] have shown that the driving force from the polyelectrolyte effect (relative to [MX] = 1 M) may be estimated as $\Delta G_{el} = SRT \ln [MX]$, where [MX] is the monovalent salt concentration. If an average value of $S = 1.04$ is assumed for doxorubicin, daunorubicin, and the β anomer of doxorubicin, a value of ΔG_{el} of approximately −1.0 kcal mol^{-1} may be calculated for these compounds in a solution containing 0.2 M NaCl. This value means that, under the solution conditions of our study, these compounds thus bind to DNA with a *favorable* electrostatic contribution. A value of $\Delta G_{el} \approx -0.2$ kcal mol^{-1} may be calculated for hydroxyrubicin, indicating a lesser polyelectrolyte contribution to its DNA binding free energy. By taking the difference $\Delta G_{obs}° - \Delta G_{el}$, $\Delta G°$ may be calculated. Table II shows the separate free energy contributions estimated for anthracycline antibiotic binding to DNA. For a purely electrostatic binding interaction, $\Delta G°$ is expected to be 0. For all of the anthracycline antibiotics, large, negative values of $\Delta G°$ are found (Table II). These values are consistent with the notion that these antibiotic–DNA complexes are stabilized predominantly by hydrogen bonding and van der Waals interactions. Such interactions have been observed in the high-resolution structures of the complexes of doxorubicin and daunorubicin with DNA.[8-10] In addition to the stacking interactions between the planar anthracycline ring system and DNA base pairs at the intercalation site, these structures reveal that several specific hydrogen bonds are formed between the antibiotic and the DNA. The values of $\Delta G°$ in Table II support the fact that these interactions stabilize the complex.

Table II shows that the nonelectrostatic component of the free energy of doxorubicin binding to DNA is −7.8 kcal mol^{-1}. The observed binding free energy of hydroxyrubicin is −7.2 kcal mol^{-1}. This value is less, by 0.6 kcal mol^{-1}, than the value expected for hydroxyrubicin from the simple loss of favorable electrostatic interactions. A possible explanation of the observed effect is as follows. Electrostatic calculations show dramatic differences in the electrostatic potentials surrounding doxorubicin and hydroxyrubicin (data not shown[21]). While the hydroxyl group in hydroxyrubicin that replaces the amine is uncharged, the *electrostatic potential* around the group is negative. Since the minor groove of B form DNA is calculated to have a negative electrostatic potential,[22] it is possible that the sugar portion of hydroxyrubicin experiences an unfavorable repulsive interaction with the minor groove. The result would be a binding free energy that is less favorable than that expected from only the loss of the polyelectrolyte contribution, exactly as we observe. Preliminary energy minimization calculations, using as a starting structure the coordinates of the doxorubicin–$(CGTACG)_2$ complex,[10] show minimized total energies of 85.2 and 79.4 kcal for hydroxyrubicin and doxorubicin, respectively. The trend resulting from these calculations is generally consistent with the trend in the experimental free energy differences we have measured and indicates that doxorubicin binding to DNA is more favorable than is hydroxyrubicin binding to DNA.

Early models for the daunorubicin–DNA complex proposed a specific electrostatic interaction between the charged amine group and DNA phosphates (reviewed in[23]). High-resolution single-crystal results, however, do not show such a specific interaction.[8-10] Instead, the daunosamine is seen in these structures to fit snugly into the minor groove, resulting in favorable van der Waals contacts, but with no specific electrostatic bonding of the amine group to DNA. The contribution of the protonated amine to the binding free energy arises, instead, from nonspecific polyelectrolyte effects, as our analysis shows. Specific amine–phosphate interactions need not be invoked to explain the salt dependence of anthracycline binding to DNA, as was previously supposed.[23] The results for the β anomer of doxorubicin (Table II) strongly reinforce this view. The stereochemistry of the daunosamine in the β anomer precludes its proper fit into the minor groove. The ΔG_{el} contribution to the binding free energy, however, is identical to that observed for doxorubicin and daunorubicin. The β anomer binding free energy is over 3 kcal *less* favorable than that observed for doxorubicin, most probably because of the loss of favorable van der Waals contacts in the minor groove. Doxorubicin and its β anomer provide a striking example of *stereoselectivity* in antibiotic binding to DNA. The difference in DNA binding affinity of these two anthracycline isomers is far more pronounced than the difference observed for the enantiomers of tris(*o*-phenanthroline)ruthenium(II),[24] which have generated considerable excitement as stereoselective "probes" of DNA structure.

Recently, the contribution of the charged amine to anthracycline binding to DNA has been investigated by comparative studies using an N-acetyl derivative of doxorubicin.[25] *N*-acetyldoxorubicin was reported to bind to DNA with a 2.3 kcal mol^{-1} *less favorable* binding free energy when compared to doxorubicin. This free energy difference is larger in magnitude by 0.7 kcal than the difference we observe between doxorubicin and hydroxyrubicin and is over twice the expected loss to the binding free energy resulting from the loss in ΔG_{el}. The free energy of *N*-acetyldoxorubicin binding to DNA is about 1 kcal less favorable than that of binding of hydroxyrubicin. We attribute this

(21) A photograph showing the calculated electrostatic potentials of doxorubicin and hydroxyrubicin was provided as supplementary material for use by the editor and reviewers of this manuscript in support of the arguments made in this paragraph. Interested readers may obtain this material upon request.

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difference to steric hindrance from the bulky N-acetyl group. The amine group in doxorubicin lies snugly in the minor groove, and its replacement by a more bulky group would surely be energetically unfavorable. Hydroxyrubicin has a decided advantage over *N*-acetyldoxorubicin as a model compound for investigating polyelectrolyte contributions in anthracycline binding in that the change from the amine to the hydroxyl is less likely to sterically hinder its binding to DNA.

The binding of the uncharged antibiotic actinomycin to DNA has been studied previously as a function of NaCl concentration,[26] but without quantitative analysis of the salt dependency of the binding constant. Mueller and Crothers[26a] observed a 5-fold increase in the actinomycin binding constant upon a decrease in NaCl concentration from 0.2 to 0.01 M. They offered a qualitative explanation for the results that is the essence of the later quantitative treatment developed by Friedman and Manning, namely that intercalation increases the DNA phosphate spacing and thereby reduces its charge density. Winkle and Krugh[26b] also noticed a salt dependence of actinomycin binding to DNA but did not quantitatively characterize that dependence. From the combined data of these two studies, the quantity ($\delta \log K/\delta \log [Na^+]$) may be estimated to range from –0.36 to –0.54, in fair agreement with the predictions of the Friedman–Manning theory.

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Supplementary Material Available: Calculated three-dimensional electrostatic potential maps for doxorubicin and hydroxyrubicin and text describing them (1 page). Ordering information is available on any current masthead page.