Daunomycin Binding to Detergent Micelles: A Model System for Evaluating the Hydrophobic Contribution to Drug−DNA Interactions

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The interaction of daunomycin with sodium dodecyl sulfate and Triton X-100 micelles was investigated as a model for the hydrophobic contribution to the free energy of DNA intercalation reactions. Measurements of visible absorbance, fluorescence lifetime, steady-state fluorescence emission intensity, and fluorescence anisotropy indicate that the anthraquinone ring partitions into the hydrophobic micelle interior. Fluorescence quenching experiments using both steady-state and lifetime measurements demonstrate reduced accessibility of daunomycin in sodium dodecyl sulfate micelles to the anionic quencher iodide and to the neutral quencher acrylamide. Quenching of daunomycin fluorescence by iodide in Triton X-100 micelles was similar to that seen with free daunomycin. Studies of the energetics of the interaction of daunomycin with micelles by fluorescence and absorbance titration methods and by isothermal titration calorimetry in the presence of excess micelles revealed that association with sodium dodecyl sulfate and Triton X-100 micelles is driven by a large negative enthalpy. Association of the drug with both types of micelles also has a favorable entropic contribution, which is larger in magnitude for Triton X-100 micelles than for sodium dodecyl sulfate micelles. The thermodynamic profile for the interaction of daunomycin with both types of micelles is characteristic of the "nonclassical" hydrophobic effect. The enthalpy for the interaction of daunomycin with sodium dodecyl sulfate micelles increases nonlinearly with temperature, indicating a positive (and temperature dependent) heat capacity change. The binding isotherm for daunomycin association with sodium dodecyl sulfate micelles was cooperative, with a Hill coefficient of 1.6. The cooperative behavior and the positive heat capacity change suggest that the drug alters micelle size or imposes order on the hydrocarbon interior of the micelle.

The anthracycline antibiotic daunomycin (daunorubicin, Figure 1), a potent anticancer drug, has been investigated extensively.[1−7] X-ray crystallographic studies indicate that daunomycin binds to DNA by intercalation.[8,9] DNase I footprinting studies showed that daunomycin binds preferentially to the triplet DNA sequences 5′(A/T)CG or 5′(A/T)GC.[10,11] This binding specificity is determined by contributions from substituents on both daunomycin and the DNA bases.[9,12,13] Daunomycin and related anthracycline drugs also bind to isolated cellular membranes,[14−16] to phospholipids,[17,18] and to reconstituted lipid vesicles in a manner that is dependent on lipid composition.[19−26] Since daunomycin attached to a polymer bead that does not enter cells is cytotoxic,[27] it is possible that its antitumor activity results in part from interaction with membranes. However, anthracycline antibiotic cytotoxicity is strongly correlated with DNA binding affinity[28−30] and current thinking is that inhibition of topoisomerase II is the primary mechanism of their anticancer activity.[31,32]

As shown in Figure 1, daunomycin consists of an anthraquinone ring and a positively charged sugar moiety, daunosamine. Crystal structures of daunomycin−DNA complexes demonstrate that the anthraquinone ring intercalates into DNA with its long axis nearly perpendicular to the long axis of the DNA bases within the intercalation site.[8,9,33] The daunosamine substituent lies in the minor groove and is stabilized by a variety of molecular interactions. With a pK_a of 8.2, the amine function of daunosamine is positively charged at pH 7 and exerts a favorable polyelectrolyte contribution to the binding free energy.[12] The carbonyl substituent on the A ring of the anthracycline ring forms a hydrogen bond with an adjacent DNA base to stabilize the complex. Van der Waals interactions of the daunosamine substituent within the minor groove and of the anthraqinone ring with the bases also stabilize the complex. Analysis of the structure of the drug−DNA complex and the

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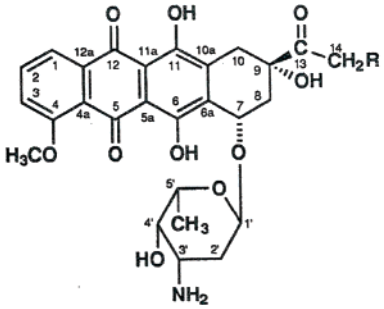

1 - R=OH -- Doxorubicin
2 - R=H -- Daunorubicin

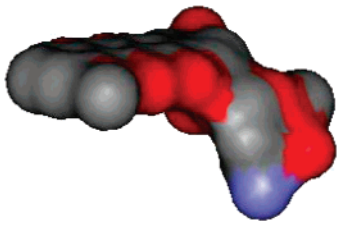

Figure 1. Chemical structure of daunomycin.

energetics of binding suggest that formation of the complex must involve at least three steps: (1) a DNA conformational change to form the intercalation site; (2) hydrophobic transfer of the drug into the site; (3) anchoring of the drug by the formation of noncovalent molecular interactions.[34]

The thermodynamics of daunomycin binding to DNA has been studied in great detail in our laboratory using multiple experimental approaches.[11−13,35−39] Intercalation of daunomycin into DNA at 20 °C is characterized by $\Delta G = -7.9(\pm 0.3)$ kcal mol^{-1}, $\Delta H = -9.0(\pm 0.8)$ kcal mol^{-1}, $-T\Delta S = +1.1(\pm 0.85)$ kcal mol^{-1}, and $\Delta C_p = -160(\pm 33)$ cal mol^{-1} K^{-1}. Intercalation of the drug is driven by a large, negative enthalpy and is opposed by an unfavorable entropy term.

There is great interest in the general problem of understanding the molecular forces that contribute to binding affinity.[40−45] We have attempted to parse drug−DNA binding free energies into terms that account for a variety of molecular contributions.[12,13,34,39,46,47] The observed binding free energy (ΔG_{obs}) contains contributions from at least five component terms:

$$\Delta G_{obs} = \Delta G_{conf} + \Delta G_{r+t} + \Delta G_{pe} + \Delta G_{hyd} + \Delta G_{mol}$$

In this equation, ΔG_{conf} is the contribution from conformational transitions in the DNA and intercalator, ΔG_{r+t} is the free energy cost for the restriction of rotational and translational freedom, ΔG_{pe} is the polyelectrolyte contribution, ΔG_{hyd} is the hydrophobic contribution, and ΔG_{mol} is the contribution of all other molecular interactions (e.g., van der Waals interactions, H-bonding, etc.). The details of the contributing terms and their magnitudes are discussed elsewhere.[34,47] Although this approach is approximate, it provides important insights into the forces driving drug binding to DNA. Previous studies suggested a large hydrophobic contribution to the binding free energy for both intercalation[39] and groove binding.[46,48,49] However, our recent studies raised a quandary that compelled us to consider alternate approaches to evaluating the hydrophobic contribution.

The quandary is as follows. The hydrophobic contribution to drug binding free energy (ΔG_{hyd}) was evaluated using an empirical relationship derived by Spolar and Record[44,50] that relates ΔG_{hyd} to experimental heat capacity changes with $\Delta G_{hyd} = 80(\pm 10)[\Delta C_p]$. This relationship is based on a correlation between ΔC_p and computed values for the removal of nonpolar solvent accessible surface area upon complex formation. For daunomycin, a value of $\Delta G_{hyd} = 80(-160) = -12.8$ kcal mol^{-1} was obtained from such analysis. In the classic view of the hydrophobic effect, water is highly structured around a nonpolar solute.[51,52] Transfer of the nonpolar solute from aqueous solution into its macromolecular binding site (intercalation of the anthraquinone ring into DNA) ought to result in "release" of ordered water molecules. Such is not the case for daunomycin binding to DNA. Using the osmotic stress method developed by Parsegian and co-workers,[53,54] we found binding of daunomycin to DNA results in a net *uptake* of 18(±0.3) water molecules bound to the complex.[38] The water uptake is consistent with high-resolution crystal structures of daunomycin−DNA complexes which show highly ordered, specifically bound water molecules.[55] We cannot reconcile such water uptake with our earlier interpretation and analysis of heat capacity changes as arising from removal of solvent accessible surface area, and now think use of ΔC_p to calculate the hydrophobic contribution to the binding free energy may be inappropriate for intercalation reactions. Apparent ΔC_p values can arise from binding coupled to a conformational change.[56] Since intercalation requires separation of adjacent base pairs, resulting in a lengthening and unwinding of the DNA duplex, it is likely that the heat capacity change results from coupling of binding to this conformational change. For these reasons, we searched for a more appropriate model system that might allow us to evaluate the hydrophobic contribution to the free energy of intercalation reactions. Partitioning of intercalators from aqueous solution into detergent micelles is perhaps such a model system, which we explore here.

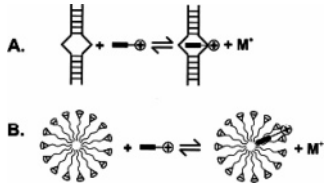

Figure 2. Model for daunomycin intercalation into DNA and for daunomycin partition into micelles: (A) daunomycin intercalation into DNA; (B) daunomycin partition into micelles.

Figure 2 shows the superficial similarities between DNA and the sodium dodecyl sulfate (SDS) micelle as receptors for intercalating drugs. DNA is a cylindrical polyelectrolyte, with a negatively charged exterior (the phosphate backbone) and a hydrophobic interior (the stacked base pairs). An SDS micelle is a spherical polyelectrolyte, with a negatively charged surface (the anionic sulfate head groups) and a hydrophobic interior (the hydrocarbon chains). The depiction of the SDS micelle in Figure 2 is highly schematic. A micelle is, in fact, a highly dynamic structure with bent, tangled hydrocarbon chains within the interior and pronounced deviations from spherical shape; Menger et al. present a far more realistic model.[57] While DNA has specific base substituents that line both the major and minor groove that can participate in specific hydrogen bonding interactions, no such substituents are present in the micelle. SDS micelle formation and the structure of micelles in solution have been studied in depth.[52] Modeling and computational studies provide additional characterization of the properties of SDS micelles.[58−60] Models constructed by Dill and co-workers suggested that crowding of the hydrocarbon chains near the micelle core imposed a degree of order approaching that of a crystal, whereas the chain disorder outside the core, nearer the micelle surface, is more like a liquid.[59,61] Subsequent models

proposing that micelle cores were devoid of internal water[62] are supported by molecular dynamics simulations indicating that the micelle interior is less fluid than a pure alkane and lacks water.[63] These simulations also showed that no stable sodium to sulfate contact pairs formed, consistent with nonspecific, "territorial" sodium binding to the SDS micelle.[63] Intercalator binding to an SDS micelle would capture at least two key features of intercalation reactions: (1) ligand transfer from aqueous solution into an ordered hydrophobic interior; (2) nonspecific polyelectrolyte interactions resulting in the coupled release of territorially bound sodium ions.

The aim of this study is to develop an alternative, more appropriate model for the hydrophobic transfer step (Figure 2) using the partitioning of drug from aqueous solution into detergent micelles. Such an approach has been used in investigations of the thermodynamic transfer properties of biologically important fatty alcohols from aqueous solution to the interior of detergent micelles.[64−66] Analysis of the enthalpies and entropies of the transfer in these studies indicates that the hydrophobic effect is important for these interactions. With this in mind, we have investigated detergent micelles as useful model systems for study of the transfer of DNA-intercalating drugs such as daunomycin from aqueous solution to a hydrophobic binding site. We have examined the interaction of daunomycin with sodium dodecyl sulfate (SDS), an anionic detergent, and Triton X-100, a nonionic detergent, using spectroscopy and calorimetry.

Experimental Section

Materials. Daunomycin obtained from the Sigma Chemical Co. (St. Louis, MO) was used without further purification. The concentration of daunomycin solutions was determined from absorbance at 480 nm ($\epsilon_{480} = 11\,500\ \mathrm{cm^{-1}\ M^{-1}}$). Calf thymus DNA was purchased from Pharmacia and was sonicated and purified as described previously.[67] DNA solutions were dialyzed against BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 0.185 M NaCl, pH 7.0) for 24 h. Lauryl sulfate (sodium salt (SDS), high purity) and Triton X-100 (protein grade detergent, 10% solution) were from CALBIOCHEM (La Jolla, CA). Acrylamide (3× recrystallized electrophoresis grade) was from Research Organics, Inc. (Cleveland, OH). Experiments were performed in BPES buffer except as noted.

Instrumentation. Absorbance spectra were recorded using a Cary Varian 3E UV−visible spectrophotometer in 1 cm path cells. Steady-state fluorescence data was recorded using an ISS Greg 200 fluorometer. An ISS K2 multifrequency cross-correlation phase and modulation fluorometer with a xenon arc lamp was used for fluorescence lifetime determinations using a 1 cm path cell; a scattering solution of glycogen (0.5 mg/mL) was used as a reference. Samples used for spectral titration and solute quenching experiments were prepared separately with different molar ratios of daunomycin and SDS or Triton X-100. Absorbance and fluorescence measurements were carried out at room temperature. Isothermal titration calorimetric data were obtained using a model 4200 ITC microtitration calorimeter (Calorimetric Sciences Corp., Spanish Fork, UT) interfaced to a Gateway 2000PC (P4D-66) for data acquisition and analysis.

Fluorescence Measurements. For steady-state fluorescence, samples were excited at 480 nm and emission was measured at 590 nm; anisotropy measurements were made using the L-format. For fluorescence lifetime measurements, excitation was at 480 nm and emitted light passing through a 495 nm cutoff filter was measured. Lifetime measurements were performed at 15 frequencies in a 1−200 MHz range, and data were collected until the standard deviation of each measurement of phase and modulation was, at most, 0.20 and 0.004, respectively. In sodium iodide quenching experiments, ionic strength was maintained constant at 0.2 M with NaCl. Data were analyzed using a nonlinear least-squares fit using the hyperbolic form of the Stern−Volmer equation[68] but are presented as

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \tag{1}$$

where F_0 is the intensity of fluorescence in the absence of quencher, F is the fluorescence intensity in the presence of quencher, and K_{sv} is the Stern−Volmer quenching constant. Cases with a significant static component were analyzed using the expression

$$\left(\frac{F_0}{F} - 1\right)/[Q] = (K_s + K_d) + [K_s K_d][Q] \tag{2}$$

where K_s is the static quenching constant.

Determination of Binding Constants. Binding isotherms were obtained by measuring fluorescence of a fixed concentration of daunomycin with varying concentrations of SDS or Triton X-100 or at a fixed surfactant concentration with varying concentrations of daunomycin. Fluorescence binding data were analyzed using the relationship

$$F = F_f(C_t - C_b) + F_b C_b \tag{3}$$

where F is observed fluorescence, F_f is the fluorescence intensity of free ligand, F_b is the fluorescence intensity of bound ligand, C_t is total ligand concentration, and C_b is the concentration of bound ligand. At the concentrations of daunomycin employed self-association of the drug is negligible.[69] Nonlinear least-squares fits of this function to data were made by employing the program FitAll (MTR Software, Toronto, Canada). Values for binding constants were obtained by fitting eq 4 or eq 5 to the data.

$$r = \frac{NK[L]}{1 + K[L]} \tag{4}$$

$$r = \frac{NK^n[L]^n}{1 + K^n[L]^n} \tag{5}$$

In eqs 4 and 5, N is the number of sites, L is the free ligand, and r is bound ligand; in eq 5, n is the Hill coefficient. Fluorescence measurements were corrected for inner filter effect using the relationship $F = F_{obs}(10e^{\epsilon cl})$, where ϵ is the molar extinction coefficient, c is molar concentration, and l is the cell path length in cm. SDS micelle concentration was calculated from the relationship [micelles] = ([C_s] − cmc)/N, where [C_s] is the bulk concentration of surfactant, N (101 for SDS) is the mean aggregation number, and cmc is the critical micelle concentration.

Isothermal Titration Calorimetry (ITC). Calorimetric titrations were performed in BPES buffer at 25.00 ± 0.01 °C or at the temperatures indicated for determination of the heat capacity change associated with daunomycin binding to SDS. We used the "model free" ITC protocol[47] in which micelles were in excess over the course of the titration such that all drug added at each step partitioned into the micelle. The approach allows determination of enthalpy values by simple evaluation of peak area without curve fitting to assumed binding models. Serial 3 μL injections of 1 mM ligand were made at 300 s

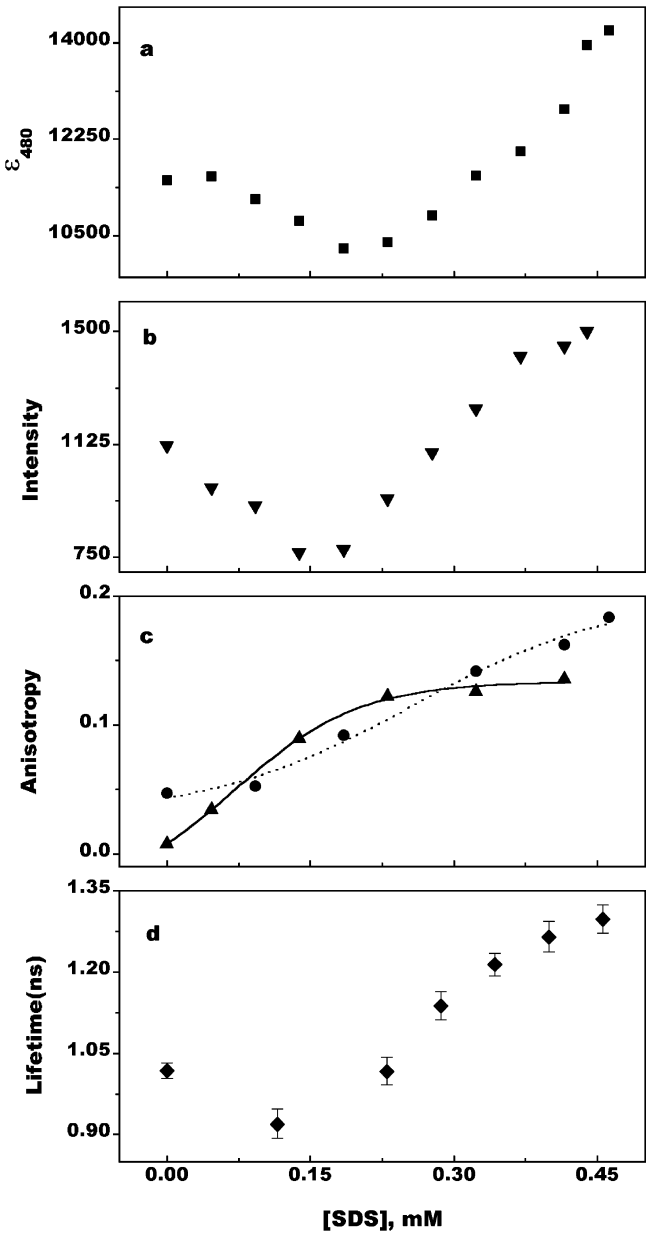

Figure 3. Effect of SDS on the optical properties of daunomycin: (a) absorbance at 480 nm; (b) fluorescence intensity at 590 nm; (c) excitation anisotropy at 490 nm (solid circles); emission anisotropy at 590 nm (solid upper triangles); (d) fluorescence lifetime. Daunomycin concentration was 10 μM for the experiment shown in panel a and 1 μM for the experiments shown in panels b−d.

intervals into 323 μM SDS or 91 μM Triton X-100 micelles. Peaks were integrated to determine the heat output/injection. Heats of dilution for drug were determined by titrating into buffer; enthalpy of drug detergent interaction was calculated after subtraction of the heats of dilution. Dilution of concentrated daunomycin solutions is accompanied by a significant endothermic effect, reflecting dissociation of the drug. This endothermic process decreases during the titration as a result of self-association of the drug at higher concentrations. Consequently, the heat of dilution was estimated by extrapolation to infinite dilution from titrations into BPES buffer as described previously.[67] Two experiments of 20 incremental additions were performed to determine the enthalpy changes for each drug−micelle and drug−DNA interaction or for dilution heats. The 40 enthalpy estimates were averaged to obtain the mean value and the standard deviation and were plotted as a histogram which was fit to a Gaussian distribution. The experimental error was less than 10%. The calorimeter was calibrated by titration of a standard solution of 0.1036 M NaOH with a standard solution of 0.0995 M HCl at the temperatures employed.

Results and Analysis

Effect of Daunomycin on Micelle Formation. To analyze the association of daunomycin with SDS, we examined the spectra of the drug in the presence of the detergent to determine what changes were associated with binding. Under the ionic and thermal conditions of our studies, SDS has a cmc of 1 mM and a micelle number of 100.[70−73]

Figure 3a,b shows the change in absorbance (measured at 480 nm) and fluorescence (measured at 590 nm with excitation at 480 nm) of daunomycin in the absence and presence of sodium dodecyl sulfate (SDS). Initially, absorbance and fluorescence intensity decreased as SDS concentration increased until minimum values for both were reached at 0.2 mM SDS. We interpret the initial decrease in the fluorescence as quenching by monomeric SDS. Analysis of the quenching of fluorescence by low concentrations of SDS gave a Stern−Volmer constant of 3.2 M^{-1}. Above 0.2 mM SDS, absorbance and fluorescence intensity increased. The changes in absorbance and fluorescence were accompanied by a 10 nm red shift in the 560 nm emission band and a 5 nm blue shift in the 590 nm emission band at SDS concentrations greater than 0.2 mM, consistent with a change in the dielectric constant of the environment of the fluorophor. In the presence of Triton X-100 there was no change in the 560 nm band and a 5 nm blue shift in the 590 nm band.

To determine if daunomycin altered the critical micelle concentration (cmc) the anisotropy of daunomycin was measured at different SDS concentrations as shown in Figure 3c. Both the excitation and emission anisotropies increased with increasing SDS with a midpoint near 0.2 mM SDS, suggesting the cmc was lowered from its value of approximately 1.0 mM[70,71] in the absence of daunomycin. Fluorescence lifetime measurements (Figure 3d) showed a reduction in the value of τ from 1.02 ns in the absence of SDS[74] to 0.92, followed by an increase above 0.2 mM SDS reaching a plateau at 1.32 ns.

Analysis of Daunomycin Binding to Detergent Micelles by Solute Quenching. To understand how daunomycin associates with detergent micelles, we examined the accessibility of the fluorophor to two quenchers, iodide and acrylamide. Figure 4 shows the quenching of daunomycin fluorescence by iodide

TABLE 1: Quenching Constants for Daunomycin, in SDS or Triton X-100 Micelles[a]

	iodide				acrylamide	
species	K_d (M^{-1})	K_s (M^{-1})	τ_0 (ns)	K_d/τ_0 (M^{-1} ns^{-1})	K_d (M^{-1})	K_d/τ_0 (M^{-1} ns^{-1})
daunomycin	14.5	1.8	1.02	14.2	1.92	1.9
daunomycin−SDS	0.3_5			0.3	0.5_5	0.5
daunomycin−Triton X100	12	1.7		11.8	0.5	0.5

[a] K_d and K_s are the dynamic and static quenching constants, respectively. Experiments were performed in BPES for iodide and 5 mM sodium cacodylate (pH 7.2), 0.1 M NaCl, and 3 mM Na_2EDTA for acrylamide. τ_0 is fluorescence lifetime of daunomycin in BPES. Experiments were performed at 298 K.

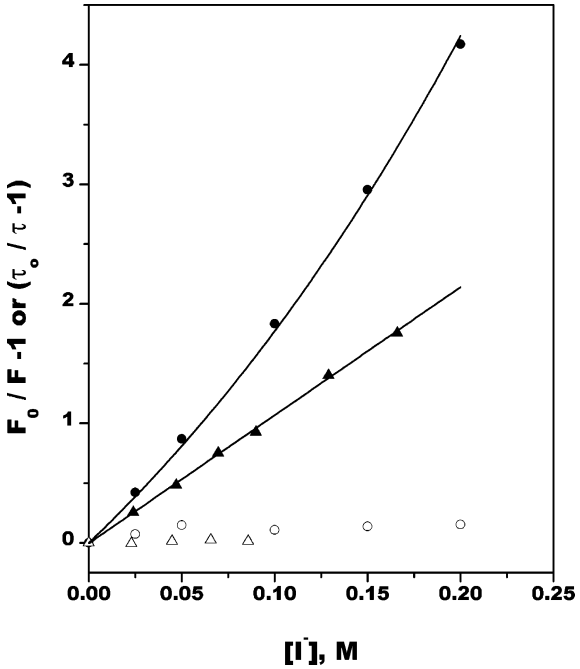

Figure 4. Quenching of daunomycin fluorescence by iodide anions. Steady-state fluorescence is shown by circles; lifetime is shown by triangles. Key: daunomycin in SDS micelles (open symbols); daunomycin in BEPS buffer (solid symbols).

in the absence and presence of SDS micelles. The fluorescence of free daunomycin is strongly quenched by iodide (Figure 4, closed circles) while in the presence of 0.5 mM SDS (Figure 4, open circles), quenching of steady-state fluorescence was not significant. Similar results were obtained measuring fluorescence lifetime (Figure 4, closed and open triangles). Curvature in the Stern−Volmer plots of steady-state fluorescence in the absence of SDS indicates a static component to the quenching process similar to what has been observed in studies of adriamycin.[75] There was excellent agreement for K_d determined from steady-state and lifetime measurements. Values for K_d, K_s, τ_0, and K_d/τ_0 calculated from fits of the Stern−Volmer equation to the data are summarized in Table 1. The observation that the iodide quenching curve monitored by lifetime measurements is linear, while the steady-state curve is nonlinear, is diagnostic of static quenching.[68]

Quenching of daunomycin fluorescence by nonionic acrylamide was also examined in the absence and presence of SDS as shown in Figure 5. Compared to iodide, acrylamide was less effective as a quencher for free daunomycin but was about as effective as iodide for daunomycin complexed with SDS. There was no static component in quenching by acrylamide. Values for K_d,and K_d/τ_0 calculated from fits of the Stern−Volmer equation to the data are summarized in Table 1.

To extend the studies of interaction of daunomycin with a non ionic detergent, we examined quenching of daunomycin fluorescence in the presence of Triton X-100. Unlike the results obtained in SDS, the fluorescence of daunomycin in the presence of Triton X-100 micelles was significantly quenched by iodide ($K_d = 12\ M^{-1}$). However, when acrylamide was used as quencher, the results for daunomycin with Triton X-100 micelles were similar to those obtained with SDS micelles (Table 1). The different results with Triton X-100 and SDS in the case of iodide indicate that the daunomycin fluorophor is still accessible to this quencher; the result may be explained by charge repulsion of the iodide by the negative charged sulfate of SDS, a result not expected for uncharged acrylamide.

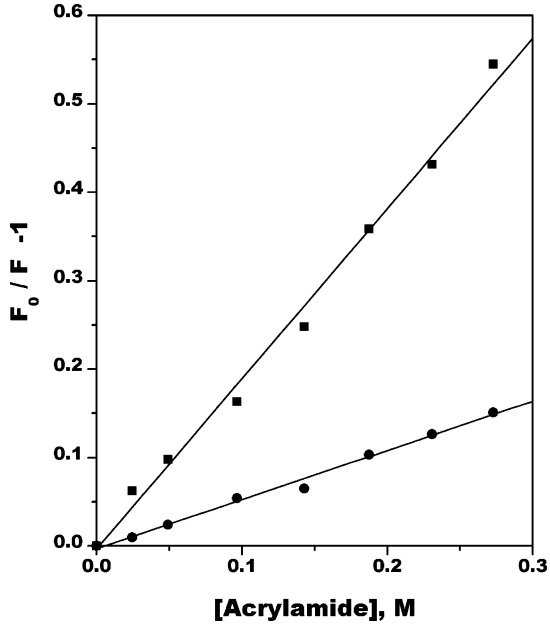

Figure 5. Fluorescence quenching of daunomycin by acrylamide: (1) daunomycin (squares); (2) daunomycin−SDS micelles (circles) in 5 mM sodium cacodylate, containing 0.1 M NaCl and 3 mM Na_2EDTA at 25 °C.

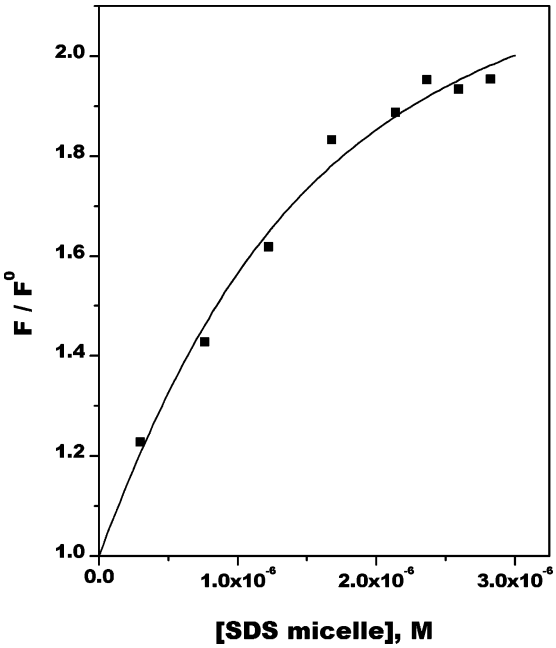

Figure 6. Fluorescence titration of daunomycin with SDS.

Thermodynamics of Daunomycin Binding to Detergent Micelles. To analyze the thermodynamics of daunomycin binding to SDS and Triton X-100 micelles, association of the drug with the detergents was monitored by examining the increase in steady-state fluorescence. To determine the maximum fluorescence change associated with daunomycin binding, detergent micelles were added to a fixed concentration of daunomycin as shown in Figure 6 for SDS. Fluorescence increases with increasing SDS micelles with a maximum fluorescence enhancement of 2.4 at infinite concentration of SDS micelles; a similar value was obtained with Triton X-100. The enthalpy of binding measured by titration calorimetry and the derived thermodynamic parameters (ΔG^0 and ΔS^0) are summarized in Table 2 for SDS, Triton X-100, and calf thymus DNA (Figure 7). Daunomycin binding constants for micelles

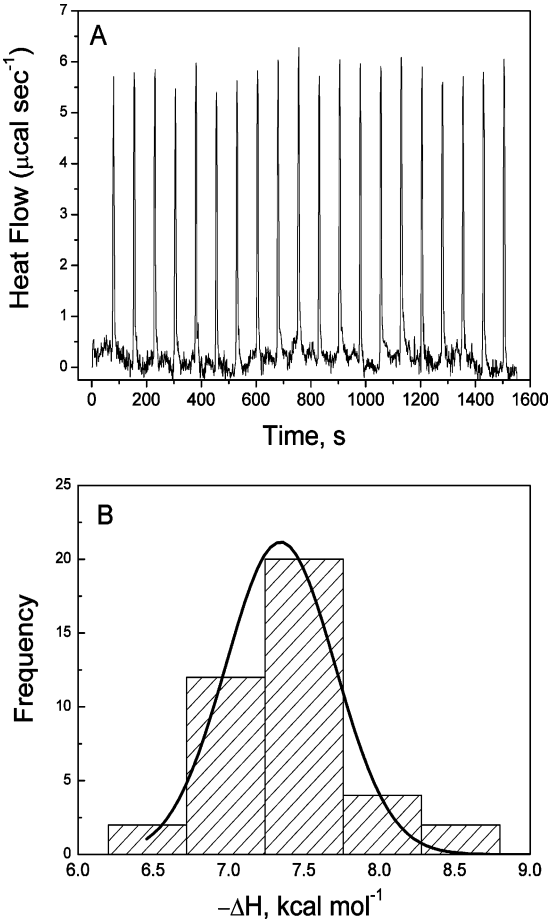

Figure 7. Determination of the enthalpy of partitioning of drug into SDS micelles at 298 K. (A) Shown is a representative titration using the "model-free ITC" protocol.[47] Micelles were in excess over the course of the titration to ensure that all added drug partitions into the micelle. Each peak yields an estimate of the partition enthalpy without recourse to curve fitting to an assumed model. The partitioning heats are exothermic; the Calorimetric Sciences ITC employed displays exothermic heats as positive values. (B) Shown are results obtained for the partitioning of daunomycin into SDS micelles. Data from two "model-free" titration experiments providing 20 enthalpy estimates each are shown as a histogram with a fit to a Gaussian distribution. The 40 enthalpy estimates provide $\Delta H = -7.4(\pm 0.4)$ kcal mol^{-1}. Key: panel A, representative titration of daunomycin into SDS; panel B, distribution of ΔH for daunomycin binding to SDS micelles.

TABLE 2: Thermodynamic Parameters for Binding of Daunomycin SDS Micelles, Triton X-100 Micelles, and Calf Thymus DNA[a]

param	SDS	Triton X-100	DNA
$10^{-5}K$ (M^{-1})	11.9	0.7	6.6
ΔG^0 (kcal mol^{-1})	−8.1	−6.5	−7.9
ΔH^0 (kcal mol^{-1})	−7.4	−3.3	−10.8
ΔS^0 (cal mol^{-1} deg^{-1})	2.5	10.9	−9.7

[a] Binding constants were calculated from fluorescence titration experiments. Values for ΔH^0 were obtained from isothermal titration calorimetry (ITC); errors were ±10−15%. ΔS^0 was calculated using the relationship $\Delta G^0 = \Delta H^0 - T\Delta S^0$. Experiments were performed at 298 K.

of both detergents are similar to the value for binding to calf thymus DNA. Although the values for ΔG^0 are similar in all cases, binding to Triton X-100 has a significant, favorable entropic component while binding of the drug to DNA has a large negative entropic contribution that is offset by the enthalpy. Binding to SDS is largely enthalpically driven with only a small contribution from the entropy term. Although we focused most

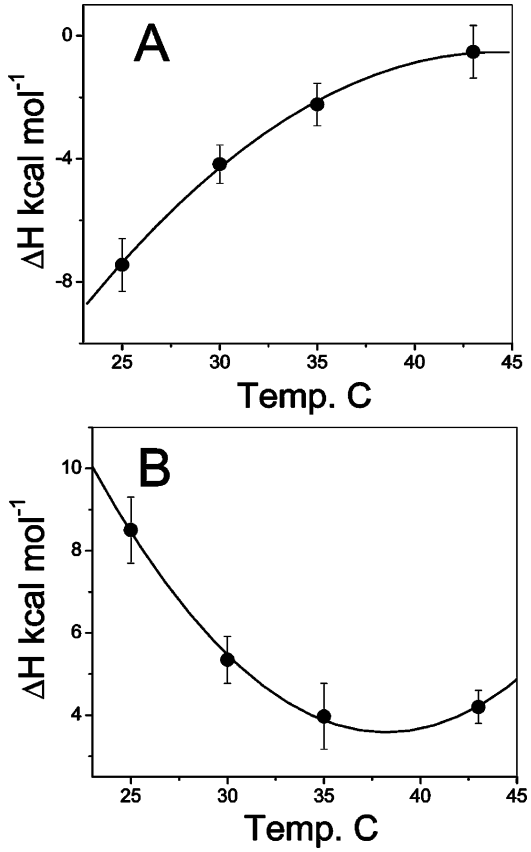

Figure 8. Temperature dependence of daunomycin binding to SDS and heat of dilution. Values for ΔH for daunomycin binding to SDS were determined by titration of daunomycin into excess SDS micelles (panel A). Values of ΔH for the heat of dilution resulting from dissociation of self-associated daunomycin (panel B) were determined by titration into BPES buffer and extrapolating to 0 addition of daunomycin.

TABLE 3: Enthalpy of Binding of Adriamycin, Ethidium Bromide, Propidium Iodide, and Actinomycin D to SDS Micelles or Triton X-100 Micelles[a]

compd	SDS	Triton X-100
adriamycin	−7.0	−3.6
ethidium bromide	−8.8	−3.5
propidium iodide	−9.4	−1.7
actinomycin D	+3.8	+2.9

[a] Values for ΔH^0 (kcal mol^{-1}) were obtained from isothermal titration calorimetry (ITC). Errors were ±10−14%. Experiments were performed at 298 K.

of our attention on daunomycin, for comparison, we also examined the enthalpy of binding of adriamycin, ethidium bromide, propidium iodide, and actinomycin D to SDS and Triton X100 micelles, summarized in Table 3. Adriamycin, ethidium bromide, and propidium iodide had negative binding enthalpies with the value being significantly more negative (−7.0 to −9.4 kcal mol^{-1}) for binding to SDS compared to Triton X100 (−1.7 to −3.6 kcal mol^{-1}). Actinomycin D differed from the other compounds in giving positive values for binding to both SDS (3.8 kcal mol^{-1}) and Triton X100 (2.9 kcal mol^{-1}). To extend the thermodynamic analysis of daunomycin binding to SDS, ΔH was determined at four temperatures; the results of these experiments are shown in Figure 8. While the binding of daunomycin to SDS was exothermic, the value of ΔH became more positive with increasing temperature as shown in Figure 8A, indicative of a positive heat capacity change associated with binding. The dilution of daunomycin into buffer was endother-

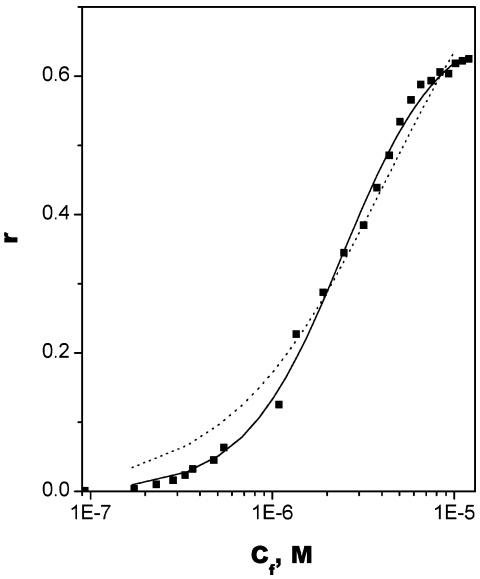

Figure 9. Binding isotherm for daunomycin with SDS micelles. C_f and r were calculated with fluorescence titration data. The data was fit with a hyperbolic binding isotherm (dotted line) or with the Hill equation (solid line).

mic, but ΔH exhibited the opposite behavior becoming more negative at higher temperatures as shown in Figure 8B. Daunomycin binding to SDS and its heat of dilution into buffer exhibited nonlinear behavior.

The binding constant and binding stoichiometry was then determined by adding increasing concentrations of daunomycin to a fixed concentration of detergent micelles as shown in Figure 9 for SDS. Fitting the expression for an uncoupled binding isotherm (eq 4) to the data gave values for SDS and Triton X-100 respectively of 3.4×10^5 and 4.4×10^5 M^{-1} for the association constants and 0.9 and 1.1 per micelle for the binding stoichiometry. The pattern of residuals in the fit indicated that binding of the drug to micelles was not adequately described by a simple uncoupled binding isotherm and that a more complex model was required. A fit of the Hill equation (eq 5) gave a value of 4.2×10^5 for K and a Hill coefficient of 1.6. The results of fitting the two transformations to the data are shown in Figure 9. While the Hill equation adequately describes the binding isotherm, it should be noted that detergent micelles do not have fixed ligand binding sites. It may be more appropriate to consider K more as a parameter related to partitioning of daunomycin into the detergent micelle rather than binding, as has been considered for the partitioning of detergents into phospholipid vesicles.[76,77] Nonetheless, the form of the equation used to describe the portioning is the same mathematical form as a hyperbola used to describe simple Langmuir binding. If K (expressed as an association constant) and the concentration of free detergent (C_{Df}) are raised to a power in eqs 3 and 4 in the work of Heerklotz and Seelig,[77] one essentially has the Hill equation. Studies in the work of Heerklotz and Seelig have the additional complication that SDS associates into micelles; there is no significant self-association of daunomycin at the concentrations we employed.

Polyelectrolyte Effect on Daunomycin Binding Contributions to SDS Micelles. Binding of daunomycin to DNA has a significant polyelectrolyte contribution to its binding free energy. To determine the polyelectrolyte contribution for the daunomycin interaction with SDS micelles, binding constants were determined at different NaCl concentrations and plotted as log K vs log [Na^+] (data not shown). The slope SK = (δ log K/δ log [Na^+]) was found to be 0.94 ± 0.09 for daunomycin−micelle interactions, compared to a value of 1.08 ± 0.1 for daunomycin binding to DNA. These values may be used to calculate the polyelectrolyte contribution (ΔG_{pe}) to ΔG^0 at a given NaCl concentration from the relation[78−81]

$$\Delta G_{pe} = (\mathrm{SK})RT \ln [\mathrm{Na}^+] \quad (6)$$

The "nonelectrostatic" contribution (ΔG_t) to free energy[12] was calculated from

$$\Delta G_t = \Delta G^0 - \Delta G_{pe} \quad (7)$$

The results of this analysis for daunomycin binding to SDS micelles and for its binding to calf thymus DNA are summarized in Table 4. While the electrostatic component for binding to SDS is slightly lower, the results for daunomycin binding to DNA and to the detergent are remarkably similar.

TABLE 4: Energetics of Daunomycin Binding to Calf Thymus DNA and SDS Micelles in BPES Buffer[a]

parameter	DNA	SDS micelles
$10^{-5}K$ (M^{-1})	6.6	11.9
ΔG^0 (kcal mol^{-1})	−7.9	−8.1
SK	−1.08	−0.94
ΔG_{pe} (kcal mol^{-1})	−1.1	−0.9
ΔG_t (kcal mol^{-1})	−6.8	−7.2

[a] The polyelectrolyte contribution to Gibbs free energy change was calculated from the relation $\Delta G_{pe} = (\mathrm{SK})RT \ln [\mathrm{Na}^+]$, where SK = ($\delta$ log K/δ log [Na^+]). The thermodynamic free energy change was calculated by difference, $\Delta G_t = \Delta G^0 - \Delta G_{pe}$. Experiments were performed at 298 K.

Discussion

We have examined the binding of daunomycin to detergent micelles as an alternative to a model for the hydrophobic component of its binding to DNA. The anthracycline ring inserts into SDS and Triton X-100 micelles as indicated by changes in absorbance, increased fluorescence, and a blue shift in the emission spectrum, effects that can be explained by the chromophor being in a more hydrophobic environment. Similar changes in the absorbance and fluorescence spectra occur when anthracycline drugs bind to various phospholipid vesicles,[14,82] reflecting interaction of the anthracycline ring with the hydrocarbon of the bilayer. The polar head group of SDS appears to exclude negatively charged iodide from interacting with the anthracycline ring and quenching its fluorescence,[24] an effect not seen in the interaction of daunomycin with Triton X-100. A similar effect is observed with doxorubicin binding to lipid vesicles, rendering the drug inaccessible to iodide quenching.[24] The static component of iodide quenching seen with the free drug may arise from formation of a complex of I^- with the NH_3^+ of the daunosamine substituent of daunomycin, an effect that would be eliminated by interaction of the NH_3^+ of daunosamine with the sulfate of SDS. As expected, acrylamide, being uncharged and slightly hydrophobic, was equally effective as a quencher of daunomycin fluorescence when the drug was bound to Triton X-100 or SDS. Daunomycin also associates with detergent monomers as indicated by effects on the fluorescence lifetime and quenching of fluorescence below the cmc. Association of the drug with monomers may indicate that the drug can be incorporated into micelles as a drug−detergent complex as well as the free drug.

Daunomycin binding to detergents shows evidence of positive cooperativity as indicated by the Hill coefficient of 1.6 and yet

binds with a stoichiometry of approximately 1/micelle. Similar values for the Hill coefficient were observed for doxorubicin binding to various acidic phospholipid vesicles with Hill coefficients ranging from 1.5 to 1.8.[24] The apparent discrepancy between the binding stoichiometry and the Hill coefficient for the binding of daunomycin to SDS can be reconciled if daunomycin binding to a micelle makes subsequent binding more favorable and a fraction of the micelles do not bind the drug; the stoichiometry for a population of micelles could coincidentally have a value near unity at saturation. An alternate explanation is that the micelles become larger as the drug binds so that the concentration of the micelles is lower; binding of daunomycin to the larger micelles would be more favorable and thus account for the Hill coefficient. These explanations require that incorporation of the drug into micelles altered their properties such that the affinity for drug is increased, which is what was proposed for the binding of doxorubicin to phospholipid vesicles.[24] Consistent with such structural alterations induced by drug binding, adriamycin changes the distribution of cardiolipin in dioleoylphosphatidylcholine−cardiolipin liposomes as revealed by ^{31}P NMR and differential scanning calorimetry.[20] The positive heat capacity change when daunomcyin binds to SDS also suggests altered micelle structure. The nonlinearity of ΔH with temperature may be related to temperature-dependent changes in the structure of the SDS micelle.

The binding affinity for daunomycin to SDS and Triton X-100 micelles is only slightly less than for natural DNA, as is the case for binding of other anthracycline drugs to phospholipids.[19,24] While the free energy of binding was similar, the contributions from entropy and enthalpy to binding were significantly different. Daunomycin binding to DNA has a large enthalpic contribution which offsets an unfavorable entropic contribution, while its binding to detergents has either a small entropic contribution (SDS) or a significant favorable entropic contribution (Triton X-100). While daunomycin binding to DNA exhibits a negative heat capacity change,[39] its binding to SDS exhibits a nonlinear heat capacity change of the opposite sign (Figure 8). Binding of anthracycline drugs, including daunomycin, to phospholipid vesicles is exothermic with no significant temperature dependence of ΔH, consistent with a small heat capacity change.[19] Free energy parsing demonstrates that the polyelectrolyte and nonpolyelectrolyte contributions to binding are similar for daunomycin interacting with DNA or SDS.

Our original idea in examining daunomycin interaction with SDS micelles, that it would be a model for the hydrophobic component of DNA binding, appears to be rather simplistic, and more complex models are likely required to explain the energetics of binding. In particular, the large negative ΔH led us to consider the nonclassical hydrophobic effect[83−86] to account for the binding to SDS. The hydrophobic effect as originally proposed by Tanford[52,87] is an entropic process with ΔH near zero; it does not include significant contributions from van der Waals interactions between the hydrophobic components. Inclusion of these interactions results in a significant negative ΔH term and a negative heat capacity change (ΔC_p). While the binding of daunomycin to SDS does not conform to the more conventional view of the hydrophobic effect since there is a significant enthalpic contribution to binding, the positive value for ΔC_p does not conform to the nonclassical model for the hydrophobic effect. However, the binding of the antibacterial peptides, magainins, to small unilemellar vesicles,[86] which is considered "nonclassical", shows a large negative ΔH (−15 to −18 kcal mol^{-1} at 30 °C), a significant, positive ΔC_p (130 cal mol^{-1}), and a nonlinear dependence of ΔH on temperature, attributes that are similar to daunomycin binding to SDS.

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