Contrasting Hydration Changes for Ethidium and Daunomycin Binding to DNA

Xiaogang Qu and Jonathan B. Chaires*

Department of Biochemistry
University of Mississippi Medical Center
2500 North State Street Jackson, Mississippi 39216-4505

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Water is an integral part of DNA structure.[1] There are at least two hydration layers surrounding duplex DNA, the first of which consists of about 20 water molecules per nucleotide.[1a] Water and cations may, in addition, bind in complicated, specific ways to particular DNA sequences.[2] Recent structural and thermodynamic studies show that water is an important contributor to both the affinity and specificity of protein−DNA interactions.[3] The participation of water in small-molecule binding to DNA, in contrast, has not been well-characterized. One notable exception is the application of the osmotic stress method [4] to examine coupled hydration changes in the binding of a netropsin analogue to DNA.[5] The surprising finding in that study was that interaction of that groove binder with DNA was accompanied by the net *uptake* of 50−60 water molecules. We report here the application of the osmotic stress method to the study of the DNA binding of two intercalators, ethidium and daunomycin. Contrasting behavior was found for these two molecules. No water uptake or release was found for the simple intercalator ethidium, while significant water uptake was found for the complex intercalator daunomycin.

The osmotic stress method offers a simple, elegant means of evaluating the participation of water in biochemical reactions.[4] In one version of the osmotic stress method, osmolytes (in the form of neutral solutes or cosolvents) are added directly to the solution containing the macromolecules and ligands being studied, thereby altering water activity in the solution. It is assumed that the added osmolytes do not interact with any of the reactants under study, an assumption that is usually verified by using a variety of neutral solutes whose size and physicochemical properties differ. In the present study, sucrose, betaine, and triethylene glycol were used as osmolytes. The osmotic stress method using these osmolytes was recently used to study water release in DNA duplex and triplex melting reactions.[6]

Figure 1 shows binding isotherms,[7] cast in the form of a Scatchard plot, for the interaction of ethdium and daunomycin with calf thymus DNA in the absence and presence of an osmolyte (sucrose) that perturbs water activity. From these primary data, the qualitative effect is clear. The presence of sucrose significantly alters the interactions of daunomycin with DNA, decreasing its apparent affinity. In contrast, sucrose has little affect on ethidium binding to DNA.

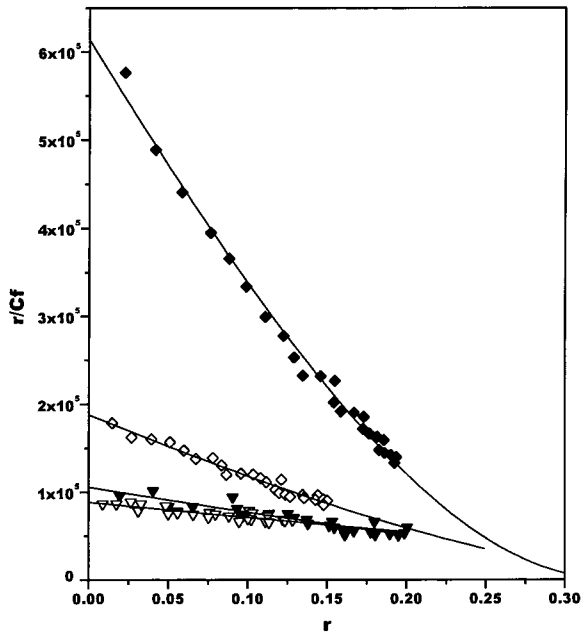

Figure 1. Binding isotherms for the interaction of daunomycin and ethidium with calf thymus DNA in the absence and presence of sucrose. Nonlinear least-squares fits (solid line) of the data for daunomycin in the absence of sucrose (♦) yields a binding constant of 6.1×10^5 M^{-1}. In presence (◇) of added sucrose (3.27 *O*), the daunomycin binding constant is reduced to 1.9×10^5 M^{-1}. Binding data for ethidium in the absence (▼) and presence (▽) of 3.15 *O* sucrose yields binding constants of 1.0×10^5 M^{-1} and 0.9×10^5 M^{-1}, respectively.

The results of more extensive binding studies are shown in Figure 2. Binding constants for the interaction of both ethidium and daunomycin were determined for three different osmolytes (sucrose, betaine, and triethylene glycol) at several different osmolalities. Figure 2 shows that all of the osmolytes exert similar effects on the binding constant, from which it may be concluded that their effect is due to changes in water activity rather than from direct interaction with either DNA or daunomycin or from changes in the solvent dielectric constant. Figure 2 shows that as osmolyte concentration increases (and water activity decreases), the daunomycin−DNA binding constant decreases. In contrast, osmolyte concentration does not alter the ethidium−DNA binding constant in any systematic way within experimental error.

From the slopes of the least-squares lines through the data in Figure 2, it is possible to quantify the involvement of water in the DNA binding of ethidium and daunomycin. Assuming that there is no direct interaction of the osmolytes with DNA, the intercalators, or the complexes, the change in hydration is given by the equation

$$d[\ln(K)]/d[\mathrm{Osm}] = -\Delta n_w/55.5$$

where *K* is the DNA binding constant, Osm is the osmolality of

* To whom correspondence should be addressed.

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(7) DNA binding constants were determined by fluorescence titration as described in: Qu, X.; Chaires, J. B. *Methods Enzymol.* **1999**, in press. Briefly, fixed concentrations of calf thymus DNA (Sigma Chemical Co., St. Louis) in BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 0.185 M NaCl, pH 7.0) were titrated with increasing drug concentrations using the Aviv, Inc. (Lakewood, NJ) model ATF 105 automated titration spectrofluorometer. Data were fit to the McGhee−von Hippel neighbor exclusion model as described in Correia, J. J.; Chaires, J. B. *Methods Enzymol.* **1994**, *240*, 593−614. Solution osmolalities were measured using a Wescor, Inc., model 5520 vapor pressure osmometer.

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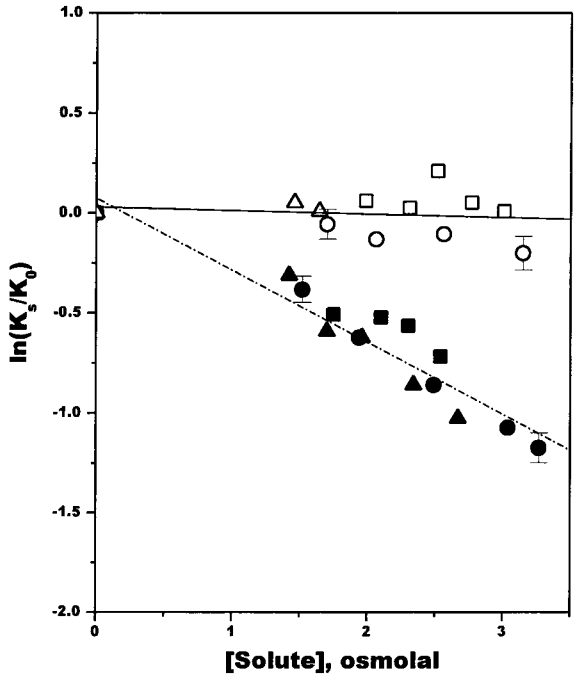

Figure 2. Dependence of binding constants on osmolyte concentration. The natural logarithm of the ratio of the binding constant at a given osmolality relative to the binding constant in BPES buffer[7] is shown as a function of solution osmolality.[4,5] Data for ethidium binding are shown as open symbols. Data from daunomycin binding are shown as filled symbols. The different symbols indicate different osmolytes: sucrose (●, ○); betaine (■, □); triethylene glycol (▲, △). Error bars, (determined by Monte Carlo analysis[8]) representative of the precision of the measurements, are shown for selected data points.

the solution, and Δn_w is the difference in the number of bound water molecules between the complex and the free reactants.[4] A positive sign for Δn_w indicates the uptake of water upon complex formation. For ethidium, a global least-squares fit to the data for all of the osmolytes shown in Figure 2 yields a slope of -0.0046, from which we calculate $\Delta n_w = +0.25$ (± 0.31). The error estimate is derived from a thorough Monte Carlo investigation of the data.[8] For ethidium, there appears to be no net water uptake or release upon binding to DNA within experimental error. In contrast, the linear fit to the dauomycin data yields a slope of -0.3242, from which we calculate $\Delta n_w = +18.0$ (± 0.3). Daunomycin binding to DNA is accompanied by the uptake of 18 water molecules.

At first glance, the magnitude of Δn_w for daunomycin binding is surprisingly large. It is important to remember that Δn_w is the net difference in thermodynamically bound water between the drug−DNA complex and the hydrated reactants. Crystal studies[9] have shown a large number of apparently specifically bound water molecules within the daunomycin−DNA complex. These include a water molecule simultaneously hydrogen-bonded to the drug O13 substituent and to a cytosine on the upper side of the intercalation site, 3−4 water molecules interacting with a sodium ion and with drug and DNA substituents in the major groove and several water molecules that form a "minispine" of hydration in the minor groove in the vicinity of the amine group on the daunosamine moiety. Apart from these apparently specifically bound waters in the complex, Frederick and co-workers [9a] have mapped 15−20 water molecules in the first-layer solvent shell whose positions appear to be conserved over three different anthracycline crystal structures. We do not claim at all that these waters that are observed in crystal structures are the very ones counted by the osmotic stress technique. We do note, however, that their number is generally consistent with the magnitude of Δn_w and that the uptake of 18 water molecules should not be considered unreasonable.

The findings reported here suggest that hydration changes may be another distinguishing trait between simple and complex intercalators. Simple intercalators, as exemplified by ethidium and proflavin, typically have a planar aromatic ring system that inserts between DNA base pairs but have few substituents that interact with the minor groove. Simple intercalators generally have small (2 bp) site sizes, show little sequence selectivity, and their DNA complexes have comparatively short lifetimes. In contrast, complex intercalators, like daunomycin and actinomycin, possess not only an intercalating ring system but also bulky substituents that interact extensively with the DNA minor groove. Complex intercalators have, in comparison to simple intercalators, larger site sizes (3−6 bp), generally show pronounced sequence selectivity, and have comparatively longer bound lifetimes. Differences in hydration might well be a heretofore unknown contributor to the functional differences between the simple and complex intercalators. Interestingly, ion release from the polyelectrolyte effect is generally the same for both simple and complex intercalators of the same charge.[10]

The rational design of new small molecules that can bind selectively and with high affinity to particular DNA targets requires a thorough understanding of both the structures involved and the underlying energetics of binding. Recent studies have clarified the energetic contributions to drug−DNA interactions, and have provided a framework for correlating thermodynamic and structural data.[11] The hydration changes described here that accompany some intercalation reactions are yet another factor that must be considered in attempts to parse binding free energies for these systems. The osmotic stress method provides a simple, yet powerful, way to examine hydration changes that accompany drug−DNA interactions. The osmotic stress method complements acoustic and densimetric methods for probing the influence of drug binding on DNA hydration.[12]

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