Hydration of Drug−DNA Complexes: Greater Water Uptake for Adriamycin Compared to Daunomycin

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Received July 7, 2008

Abstract: Water is an integral part of DNA, and the conserved water molecules at the binding sites can modulate drug binding to DNA or protein. We report here that anthracycline antitumor antibiotics, adriamycin (AM) and daunomycin (DM), binding to DNA is accompanied by different hydration changes, with AM binding resulting in the uptake of about twice as many water molecules as DM. These results indicate that water is playing an important role in drug binding to DNA.

The anthracycline antitumor antibiotics, adriamycin (doxorubicin, AM[a]) and daunomycin (daunorubicin, DM), have been widely used in the clinic for the treatment of a variety of cancers. Their antitumor activity has been attributed to their interactions with DNA, which can inhibit both DNA replication and RNA transcription or inhibit topoismerase II.[1−6] Analysis of the structure of the drug−DNA complex and the energetics of binding[3] suggest that formation of the complex involves at least three steps: (1) a DNA conformational change to form the intercalation site, (2) hydrophobic transfer of the drug into the site, and (3) anchoring of the drug by the formation of noncovalent molecular interactions. The only structural difference between DM and AM (Figure 1) is the additional hydroxyl at C14 of AM. Thermodynamic studies demonstrated that AM binding to DNA is tighter than DM by about 1 kcal mol^{-1}. It is not yet entirely clear what causes the difference. The DM and AM DNA complexes are essentially *isostructural* but are not *isoenergetic*.[7] The molecular interactions of the two compounds that can be visualized in high-resolution structures are identical, yet the thermodynamic profiles for binding of the two drugs are distinctly different.[7] Understanding drug−DNA interactions at the molecular level is important and of general interests for rational drug design. We report here that AM and DM binding to DNA is accompanied by different hydration changes, with AM binding uptake about twice as many water molecules than DM. The role of water in the formation of drug−DNA and protein−DNA complexes is underappreciated and poorly understood. This report emphasized the importance of thermodynamically coupled hydration changes in DNA binding reactions.

Water is an integral part of DNA structure.[8,9] There are at least two hydration layers surrounding duplex DNA, the first of which consists of about 20 water molecules per nucleotide.[8] Water can also mediate interactions between the ligand and DNA.[10] Hydration plays an important role in the binding affinity and specificity of small molecule−DNA[11] or protein−DNA interactions.[12,13] Osmotic stress method has been widely used as a direct in vitro probe to quantify hydration changes accompanying drug binding to DNA,[14−16] ligand binding to protein,[17] and DNA−protein interactions.[12,13] We have studied the hydration changes accompanied some typical intercalator binding to DNA,[14,15] and recently we reported that water is important for metal ions binding to amyloid Aβ[18] and for human telomeric i-motif formation induced by carbon nanotube.[19]

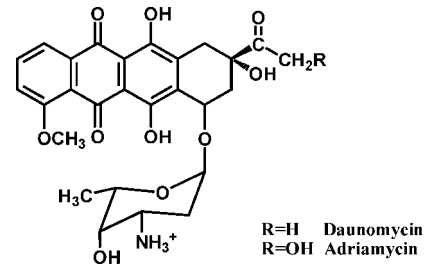

Figure 1. Chemical structures of daunomycin and adriamycin.

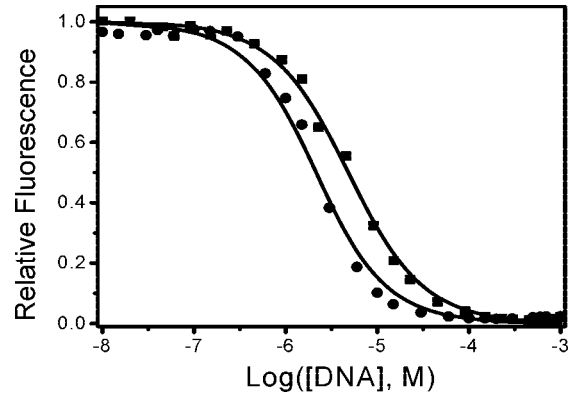

Figure 2. DNA binding isotherms for the interaction of daunomycin (squares) and adriamycin (circles) in BPES buffer. The normalized fluorescence response is shown as a function of total DNA concentration. In these titrations, the ligand concentration was kept constant at 1 μM, while the DNA concentration was varied between 1 mM and 0.01 μM bp. Data fitting and determination of binding parameters were carried out using nonlinear least-squares analysis. The solid lines through the data show the best fitting curves. Experimental details were as described previously.[23]

Previous studies on the thermodynamics[20,21] and dynamics[22] of DM and AM binding to DNA demonstrate that AM binding is tighter than DM, although their dynamic primary processes for both drugs are of a similar nature, showing the importance of the additional hydroxyl group for their interaction with DNA. Under our experimental conditions, nonlinear least-squares analyses of the binding isotherms for the interaction of DM and AM with DNA indicate that the binding affinity of AM with DNA is more than 2-fold higher than that of DM (Figure 2), consistent with the previously reported values determined by isothermal titration calorimetry (ITC) method.[21]

Note that our method of direct fitting to fluorescence data yields an association constant with units of M^{-1}, with reference to base pairs. This association constant will differ from those obtained by fits using neighbor exclusion models by a factor

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[a] Abbreviations: DM, daunomycin; AM, adriamycin.

10.1021/jm800826y CCC: $40.75
Published on Web 09/13/2008

Table 1. Summary of Thermodynamic Parameters for Daunomycin and Adriamycin Binding to Calf Thymus DNA in BPES Buffer[a]

drug	F_b/F_0	$K/10^5$, M^{-1}	$\Delta G°_{25}$ kcal mol^{-1}	Δn_w
DM	0.043	2.3 ± 0.12	−7.3	17.8 ± 0.6
AM	0.030	6.05 ± 0.58	−7.9	35.8 ± 2.2

[a] $G°_{25} = -RT \ln K$; $T = 298$ K; $\Delta n_w = -55.5 \times \partial \ln(K_s/K_0)/\partial[\text{Osm}]$.

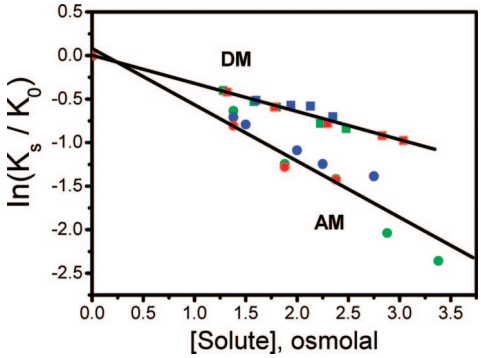

Figure 3. Dependence of DNA binding constants on osmolyte concentration. The natural logarithm of the ratio of the binding constant at a given osmolyte concentration (K_s) relative to the binding constant in BPES buffer (K_0) is shown as a function of solution osmolality. The colors indicate data obtained using a particular osmolyte: betaine (blue), sucrose (red), or triethylenglycol (green). Different symbols indicate data for different intercalators. Square points: daunomycin; Circle points: adriamycin.

determined by the number of base pairs in a ligand binding site. For AM and DM, this factor is 3−4 because there are 3−4 base pairs in a drug binding site. The calculated thermodynamic parameters are summarized in Table 1. In addition to favorable interactions such as hydrophobic interactions, van der Waals, electrostatic interactions, and water contribution should not be neglected.[11,14−16] As indicated in crystal structure,[4] in the vicinity of the additional hydroxyl group of AM, water molecules form a network that is different from the case of DM, and this can be the reason why AM binding to DNA uptakes more water molecules. Water bridged hydrogen bonding can further stabilize AM−DNA complex and enhance their binding.[11] That notion is tested here.

We chose three commonly used osmolytes (sucrose, betaine, and triethylene glycol) whose size and physicochemical properties differ to perturb water activity.[14,15] Because the change in the binding constant at low osmolality of the solution (<1 osmolal) is small and difficult to measure accurately, it was not used for the analysis. Fluorescence titrations were used to calculate the DNA−drug binding constants.[14,15] The presence of osmolytes significantly decreases the apparent DNA binding affinity of both AM and DM. Each individual osmolyte exerts a similar effect on the drug binding constants. Assuming that there is no direct interaction of the osmolytes with DNA or drugs,[15] the change in hydration is given by the equation

$$\partial \ln(K_s/K_0)/\partial[\text{Osm}] = -\Delta n_w/55.5$$

where $\ln(K_s/K_0)$ is the change in binding free energy, "Osm" is the osmolality of the solution, and Δn_w is the difference in the number of bound water molecules between the complex and free reactants. A positive sign for Δn_w indicates the uptake of water upon complex formation. The negative slopes of the best-fit lines in Figure 3 indicate that Δn_w is positive and that additional water is bound upon complex formation. Within experimental errors, the Δn_w values are +17.8 ± 0.6 for DM and +35.8 ± 2.2 for AM, showing that AM binding to DNA results in the uptake of about twice as many water molecules than DM binding.

We and others have shown that hydration changes are related to ligand properties such as size and structure.[14−16] Different intercalator binding to DNA acquires different number of water molecules.[14,15] It is unexpected and surprising that the AM−DNA complex binds twice as many water molecules than the DM complex because these two drug molecules share similar DNA binding preference and have the same chemical structure except the additional hydroxyl at C14 of adriamycin. There were some common apparently specifically bound water molecules within the DM−DNA and AM−DNA complex.[4−6] These include 3−4 water molecules interacting with a sodium ion and with ligand 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. However, in the region of the distinguishing C14, there is only one water molecule hydrogen-bonded to the daunomycin O13 substituent and to a cytosine on the intercalation site. While for AM, besides this bridging water molecule, the O13 substituent also employs two bridging water molecules to form another hydrogen bond to O3′ of the terminal base pair. In addition, the O14 of AM stretches out bridging interacted to phosphate groups of the proximal DNA strand through water networks.[4] It is similar to the previously reported minor groove binder Hoechst 33258 and its analogue (*meta*-hydroxyl).[24] Crystal structures of their DNA binding complexes indicated a network of approximately 30 water molecules associated with the altered hydroxyl.

One cautionary note needs to be mentioned. Our binding constants are macroscopic quantities that are averaged over drug binding sites with different sequences.[25] Because hydration of DNA is sequence dependent,[26] the number of water taken up upon binding may vary for different sequences along the DNA lattice.

In summary, different hydration changes accompany AM and DM binding to DNA. The greater water uptake by AM may enhance drug binding to DNA and may be the reason why AM binding is stronger than DM. Water is an integral part of DNA, and the conserved water molecules at the binding sites can modulate drug binding to DNA. How to identify and evaluate the role of water molecules in biomolecular recognition is important for thorough understanding of drug−DNA, drug−protein, or protein−DNA interactions. In this sense, our work provides new insight on drug binding to DNA and the role of water molecules in biomolecular recognition.

Acknowledgment. This work was supported by NSFC and Funds from CAS and Jilin Province. J.B.C. was supported by grant CA35635 from the National Cancer Institute.

Supporting Information Available: Experimental section and bioassays. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM800826Y