# The Effect of Water Displacement on Binding Thermodynamics: Concanavalin A

**Zheng Li and Themis Lazaridis***

*Department of Chemistry, City College of CUNY, Convent Avenue & 138th Street, New York, New York 10031*

*Received: May 21, 2004; In Final Form: October 18, 2004*

Interactions at the binding interface of biomolecular complexes are often mediated by ordered water molecules. In this work, we considered two concanavalin A−carbohydrate complexes. In the first, a water molecule is buried at the binding interface. In the second, this water molecule is displaced by a modification of the ligand (Clarke, C.; Woods, R. J.; Gluska, J.; Cooper, A.; Nutley, M. A.; Boons, G. J. *J. Am. Chem. Soc.* **2001**, *123*, 12238−12247). We computed the contribution of this water molecule to the thermodynamic properties using statistical mechanical formulas for the energy and entropy and molecular dynamics simulations. Other contributions to the binding affinity, including desolvation, entropy of conformational restriction, and interaction between the ligand and protein, were also computed. The thermodynamic consequences of displacement of the ordered water molecule by ligand modification were in qualitative agreement with experimental data. The free energy contribution of the water molecule (−17.2 kcal/mol; −19.2 enthalpic and +2 entropic) was nearly equivalent to the additional protein−ligand interactions in trimannoside **2** (−18.9 kcal/mol). The two structural ions interact more strongly with the water than with the hydroxyl of trimannoside **2**, thus favoring trimannoside **1**. The contributions from desolvation and conformational entropy are much smaller but significant, compared to the binding free energy difference. The picture that emerges is that the final outcome of water displacement is sensitive to the details of the binding site and cannot be predicted by simple empirical rules.

## Introduction

Binding between biomolecules is usually accompanied by the displacement of bound water molecules from the binding sites and formation of direct interactions. Whether such interactions are favorable, neutral, or unfavorable to binding affinity is determined by the balance between the direct interactions gained and the solvation interactions lost. In some cases, however, water molecules are not completely removed from the binding interface.[2] For example, in protein kinase CK2,[3] one water molecule mimics the N6 atom of ATP and switches the active site from an ATP- to a GTP-compatible state. Similarly, three water molecules were involved in mimicry of oxygen or nitrogen atoms of ATP in the catalytic subunit of protein kinase A.[4] In the trp-repressor/operator complex, in addition to the direct interactions between phosphates of DNA and the protein, six water-mediated polar contacts to the bases were observed,[5] which are the determinants of specificity in this system.[6] In a survey of 26 ATP−, ADP−, and FAD−protein complexes, it was found that more than one-third of the ribose−protein interactions were mediated by ordered water molecule(s).[7] Many water molecules were observed in or around the interface of a complex of a monoclonal antibody bound to hen egg-white lysozyme,[8] leading to an enthalpic driving force for binding.[9] Crystallographic studies on L-arabinose binding protein (ABP) with two different sugars (D-galactose (Gal) and L-arabinose (Ara)) show one ordered water molecule existing in the ABP−Ara complex, possessing the same position as the $-CH_2OH$ group of Gal in the ABP−Gal complex.[10]

Conflicting results have been obtained on the contribution of interfacial water to binding affinity. Some studies demonstrated that ligands designed to displace the water molecules could exhibit higher affinity.[11−13] For example, cyclic urea inhibitors designed to displace and mimic the interactions of the bound water molecule in the crystal structure of the HIV-1 protease−inhibitor complexes were found to bind more strongly to the protein.[13] Crystallographic studies revealed that two water molecules bound to the tyrosine-82 hydroxyl group in unliganded wild-type FKBP-12 are displaced upon formation of a complex with FK506. In the Y82F mutant of the protein no ordered water molecules are observed. Thermodynamic measurements showed that the enthalpy of ligand binding to the wild type protein is 4.2 kcal/mol less negative than to the mutant. This unfavorable enthalpy change was found to be outweighed by an entropic advantage leading to a slightly more negative binding free energy (0.6 kcal/mol) for the mutant.[11] Crystal structures of OppA−dipeptide complexes revealed several ordered water molecules mediating interactions between the ligand and protein.[14,15] These water molecules are displaced when the ligand changes from dipeptide to tri- and tetrapeptides. Isothermal titration calorimetry measurements of the binding of the peptides with different lengths to OppA indicate that the dipeptide is bound with about 60-fold lower affinity than related tri- and tetrapeptides.[15] The favorable entropy gained for tri- or tetrapeptides versus dipeptides, although partly offset by an unfavorable enthalpy, results in the favorable binding free energy. The favorable entropy was suggested to result from the displacement of the ordered water.[15] In the structure of the antibiotic novobiocin complexed with a resistant mutant of DNA gyrase, the presence of an ordered water molecule at the interface was thought to be responsible for the weaker binding of the drug to the mutant protein.[16] Not only polar atoms in the ligand but also hydrophobic substituents that pack tightly in a protein binding site can displace ordered water molecules and lead to stronger binding. Weber et al. designed analogues of HABA (2-[(4′-dydroxyphenyl)-azo]-benzoate) incorporating

* Corresponding author. Tel. (212) 650-8364, Fax: (212) 650-6107, Email: tlazaridis@ccny.cuny.edu

10.1021/jp0477912 CCC: $30.25 
Published on Web 12/08/2004

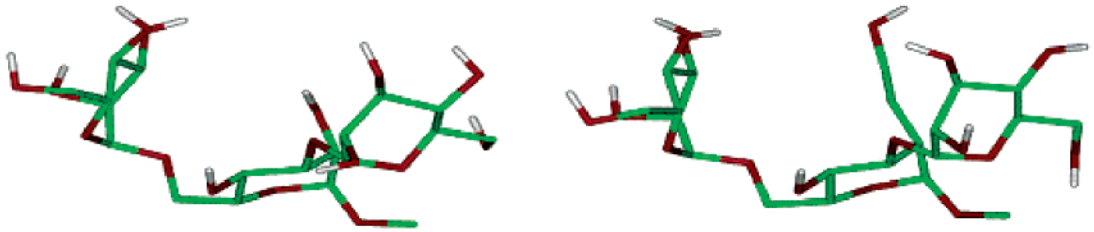

**Figure 1.** Structure of trimannoside **1** and **2**.

some aliphatic groups.[17] Crystallographic studies showed displacement of the water molecules by aliphatic groups and qualitatively similar binding modes for HABA analogues. Isothermal titration calorimetry revealed the gain of −1.98 kcal/mol in binding affinity by adding a single methyl to HABA at the 3′ carbon. This was suggested to be primarily due to the more favorable entropy of water displacement at the binding site.

In other cases, the displacement of ordered water molecules seems to lower the binding affinity. A crystallographic study of cyclosporin A bound to cyclophilin A revealed one tightly bound water molecule which was displaced in going from cyclosporin A- to (5-hydroxynorvaline)-2-cyclosporin. The binding affinity of the analogue was lower by 8−9-fold.[18] The crystal structure of concanavalin A with a trimannoside showed a conserved water molecule bridging the protein and the ligand with several hydrogen bonds and playing a crucial role in molecular recognition.[19] To study the role of this water molecule in binding, an analogue of the trimannoside was designed to replace the hydroxyl at C-2 of the central mannose with a hydroxyethyl group, which was expected to displace and mimic the interactions of this water molecule with the protein.[1] Calorimetric measurements showed that this displacement leads to a more favorable binding entropy, which, however, is offset by an unfavorable enthalpy term. In the structure of a lysozyme−antibody complex, water molecules at the protein−protein interfaces mediate the imperfect surface complementarily via hydrogen bonding.[20] Antibody mutants with Y to F and S to A mutations bound lysozyme with higher enthalpy, higher entropy, and higher free energy (lower affinity). This seems to suggest enhancement of the binding affinity by the bound water molecules.[20] However, these mutations do not displace bound water molecules but rather create an unfavorable gap between them and the antibody. Therefore, this experiment is not directly relevant to the question at hand.

From a theoretical point of view, the role of water-mediated interactions is poorly understood. Most theoretical work on ordered water molecules has so far focused on free energies. Wade et al.[21] found that the free energy of transferring water molecules from bulk solvent into protein cavities is positive for empty cavities and negative for cavities containing water molecules. A free energy perturbation study found that the transfer of two water molecules to specific locations in the bacteriorhodopsin transmembrane channel was strongly favorable.[22] Ben-Naim proposed that ordered water molecules forming a hydrogen-bond bridge between a functional group on one binding partner and a functional group on another lowers the free energy substantially.[23] This assertion was confirmed by calculation of the potential of mean force of two polar groups approaching each other at fixed orientation in the bulk.[24]

Dunitz estimated that the inclusion of water molecules in a binding interface can cost up to 7 cal/mol K (about 2 kcal/mol at 298 K) in entropy.[25] Similar estimates for the enthalpy of water ordering gave −3.8 kcal/mol[2]. Therefore, the inclusion of a single water molecule in a protein−ligand interface could decrease the free energy by 1.8 kcal/mol. These estimates, however, do not consider the specifics of the interface and the interactions that the bound water experiences with the binding partners, and thus cannot provide much guidance as to whether water displacement is favorable or unfavorable in specific cases.

More empirical ("bioinformatic") approaches to this problem have also recently appeared. Garcia et al. performed a multivariate regression analysis to establish a statistical correlation between the structural properties of water molecules in the binding site of a free protein crystal structure, with the probability of observing the water molecules in the same location in the crystal structure of the complexes.[26] The temperature B-factor, the solvent contact surface area, and total hydrogen bond energy and the number of protein water contacts were found to correlate with the conservation of an ordered water molecule upon complex formation. Wang et al.[27] used the number of bridging water molecules in a QSAR-type predictor of the binding affinity of tyrosine phosphatase inhibitors.

In previous work, we applied the inhomogeneous fluid solvation theory[28,29] over the region occupied by a bound water molecule in a HIV-1 protease−inhibitor complex (KNI-272).[30] We found that the entropic penalty of ordering was large but was outweighed by the favorable water−protein interactions. A large negative contribution from the bound water molecule to the heat capacity was also obtained. This work showed that the theory can yield the thermodynamic contributions of ordered water molecules and qualitatively estimate which bound water molecules would be most favorable to displace. However, no connection to *binding* thermodynamics could be made in that work because the inhibitor that displaced the ordered water molecule (DMP450) was entirely different from KNI-272. This complicates the attribution of the binding free energy difference to one or a few factors. An ideal system for making this connection is the Con A−trimannoside complex introduced above, where the structures of the ligands (trimannoside **1** and **2**) and their binding to the protein are very similar (Figure 1). Therefore, only a few contributions to binding affinity will be different for the two ligands, namely, the contribution of the ordered water molecule in the Con A−trimannoside **1** complex, the direct interactions $\Delta E_{L-P}$ of **1** and **2** with the protein, the desolvation entropy and enthalpy of the ligands, and the conformational entropy of the additional hydroxyethyl side chain in trimannoside **2**. All these contributions are calculated to obtain an estimate of the difference in binding enthalpy and entropy. We find that the above inventory of contributions is consistent with experimental data and provides insights into the sensitive balance of factors that determine whether water displacement is favorable or not.

## Methods

In this work, we use a statistical mechanical theory that combines expressions for the energy and entropy as functionals

of the molecular correlation functions with an inhomogeneous view of infinitely dilute solutions. This view considers the solute as fixed at the origin and uses spatially dependent correlation functions, rather than the standard homogeneous correlation functions that are averages over the entire body of the fluid. This allows one to focus on the vicinity of the solute where most of the contributions to the solvation thermodynamics originate. The expression for the entropy is an infinite series which cannot be calculated exactly. Usually, the contribution of three- and higher-particle correlations is neglected. This is equivalent to the Kirkwood superposition approximation (KSA), i.e., $g^{(3)}_{1,2,3} = g^{(2)}_{1,2}\, g^{(2)}_{1,3}\, g^{(2)}_{2,3}$. This approximation has given good results for the entropy in simple fluids.[31] However, for cases where triplet correlations are important, the results of this approach should be viewed with caution. Obviously, for isolated water molecules, only one-body distribution functions are involved. This theory has been applied to several systems: the excess entropy in pure liquid water;[32] solvation thermodynamics in simple Lennard-Jones and hard sphere fluids;[31] and the solvent reorganization energy and entropy of hydration of methane.[29]

In this approach, ordered water molecules in the binding interface of biomolecular complexes are considered as part of the solvent. Their contributions to the solvation energy and entropy are decomposed into the solute−solvent terms ($E_{sw}$, $S_{sw}$) and solvent reorganization terms ($\Delta E_{ww}$, $\Delta S_{ww}$),[28,29] which can be expressed as integrals of the solute−solvent and solvent−solvent correlation functions ($g_{sw}(\mathbf{r},\omega)$ and $g_{ww}(\mathbf{r},\mathbf{r}',\omega,\omega')$) over the regions occupied by them. This decomposition of the energy is exact for a pairwise-additive force field, whereas the decomposition of the entropy is based on the neglect of higher than two-particle correlations. $S_{sw}$ can be expressed as an integral over the solute−solvent correlation function $g_{sw}(\mathbf{r},\omega)$:

$$S_{sw} = -k\rho/\Omega \cdot \int g_{sw}(\mathbf{r},\omega) \ln g_{sw}(\mathbf{r},\omega)\, d\mathbf{r}\, d\omega \qquad (1)$$

where $k$ is Boltzmann's constant, $\rho$ is the density of bulk solvent, and $\Omega$ is the integral over $\omega$ (the orientation of the solvent with respect to the solute).

Using the identity

$$g_{sw}(\mathbf{r},\omega) = g^{tr}_{sw}(\mathbf{r}) g^{or}_{sw}(\omega|\mathbf{r}) \qquad (2)$$

the above integral can be decomposed into a translational and an orientational contribution:

$$S_{sw} = -k\rho \int g^{tr}_{sw}(\mathbf{r}) \ln g^{tr}_{sw}(\mathbf{r})\, d\mathbf{r} - k\rho/\Omega \int d\mathbf{r}\, g^{tr}_{sw}(\mathbf{r}) \int g^{or}_{sw}(\omega|\mathbf{r}) \ln g^{or}_{sw}(\omega|\mathbf{r})\, d\omega \qquad (3)$$

If we further assume that $g^{or}_{sw}(\omega|\mathbf{r})$ is independent of $\mathbf{r}$ within a certain region of space $v$

$$g^{or}_{sw}(\omega|\mathbf{r}) \approx g^{or}_{sw}(\omega)$$

and restrict the range of integration within $v$, eq 3 becomes:

$$S^{v}_{sw} = -k\rho \int_v g^{tr}_{sw}(\mathbf{r}) \ln g^{tr}_{sw}(\mathbf{r})\, d\mathbf{r} - kN_{wat}/\Omega \int g^{or}_{sw}(\omega) \ln g^{or}_{sw}(\omega)\, d\omega \qquad (4)$$

where $N_{wat}$ is the number of water molecules in that region of space ($N_{wat} = \rho \int_v g_{sw}{}^{tr}(\mathbf{r})\, d\mathbf{r}$). The region occupied by the ordered water molecule in concanavalin A is so small (see below) that this assumption is justified.

The solute−solvent energy can also be written as an integral:

$$E_{sw} = \rho/\Omega \int g_{sw}(\mathbf{r},\omega)\, u_{sw}(\mathbf{r},\omega)\, d\mathbf{r}\, d\omega \qquad (5)$$

where $u_{sw}$ is the potential energy, but it is more easily evaluated directly from a simulation.

The solvent terms, $\Delta E_{ww}$ and $\Delta S_{ww}$ can also be expressed as integrals over the space around the solute. Thus, the contribution of specific regions of space to the solvation properties can be determined. This approach can be applied over regions occupied by bound water molecules in biomolecular complexes, (e.g., HIV-1 protease-KNI 272 complex[30]) to provide a rigorous estimate of the contribution of such molecules to the thermodynamic functions. In this case, the bound water molecule can be considered as part of the solvent, i.e., upon insertion of the protein and the ligand into the solvent, one bulk solvent molecule will stay at the interface of the protein−ligand complex and act as a bound water molecule.

**Construction of Initial Structures and Force Field.** For the first system, the initial structure was constructed based on the X-ray structure of the protein Con A complexed with trimannoside **1** (methyl-3, 6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranoside) (pdb code 1ONA[19]). This crystal structure shows that three of the Con A subunits (I, II, and III) are almost identical, while subunit IV is a little different. Here, we select subunit I and the trimannoside bound to it from the X-ray crystal structure. The bound water molecule bridging trimannoside **1** and Con A, trimannoside **1**, and the ions ($Ca^{2+}$ and $Mn^{2+}$) were included according to the crystal structure. No bonds of any kind were established between the ions and protein atoms. The second system was constructed based on the first one by removing the bound water and replacing the hydroxyl group in trimannoside **1** with a hydroxyethyl group.

The CHARMM22 force field[33] was used for the protein and the TIP3P model for water. Parameter sets for the two ligands (trimannoside **1** and **2)** were obtained from the CHARMM carbohydrate force field.[34,35] The Lennard-Jones parameters used for calcium were $R_{min} = 1.71$ Å and $\epsilon = -0.12$ kcal/mol as included in the CHARMM22 force field.[33] The same parameters were used for $Mn^{2+}$. This assumption will not affect the results because of the large distance between $Mn^{2+}$ and the ligands (>10 Å). Partial charges for the trimannosides were obtained with the program QUANTA (Accelrys, Inc.) using CHARMM template charges.

**MD Simulations.** Molecular dynamics (MD) simulations were carried out with the program CHARMM, version c30a1. A 15 Å sphere of TIP3P water molecules was added around the active site and subjected to spherical stochastic boundary conditions.[36] Solvent molecules overlapping with the protein, trimannosides, or crystal water molecule were deleted. SHAKE was used for the bonds involving hydrogen. The integration time step of the MD simulations was 2 fs. A cutoff distance (30 Å) was applied for the computation of nonbonded interactions. The computational procedures on both systems were as follows. First, energy minimizations were performed on the initial systems using the ABNR method. Next, MD simulations at 300 K were performed, starting from the energy-minimized system, lasting for 8 ns with the protein kept fixed and the ligands free. The protein was kept fixed in order to reduce the statistical uncertainties in the computed contributions to the binding free energy and to avoid possible structural shifts due to force field inaccuracies. This simplification may lead to somewhat more favorable protein−ligand interactions, but the qualitative picture should not change. To calculate the contribution of the ordered

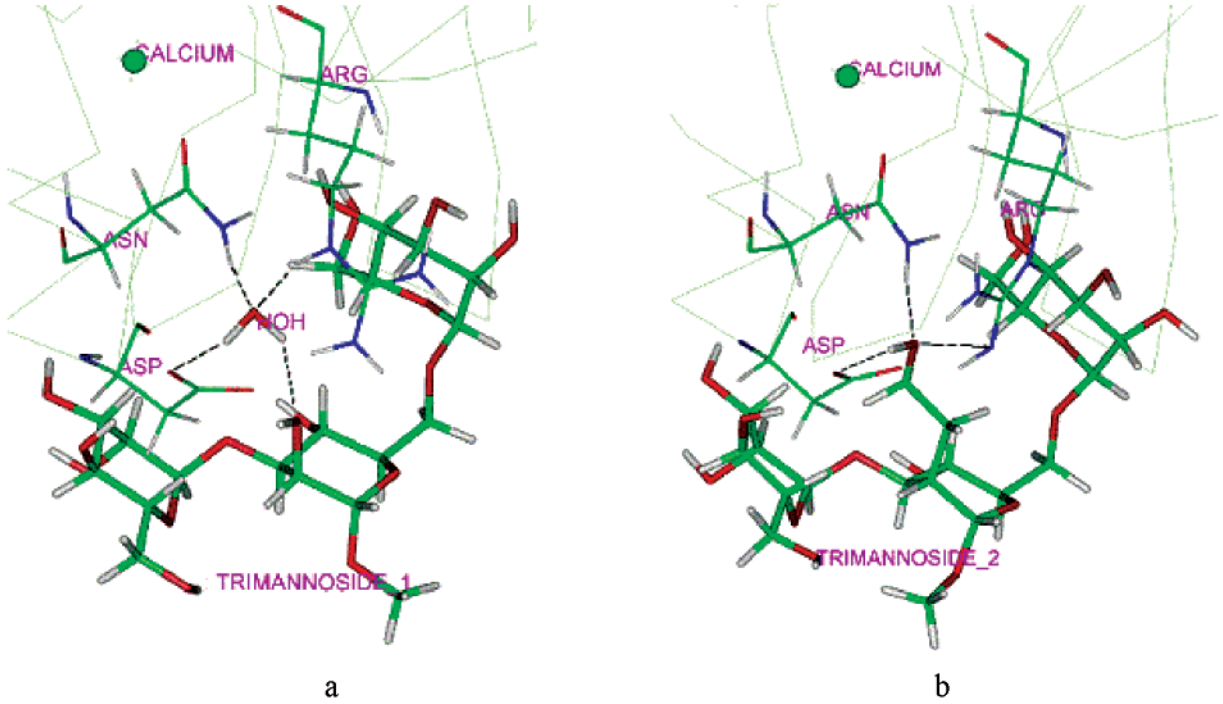


**Figure 2.** Binding of trimannoside **1** and **2** to Con A (structures obtained after 8 ns MD simulation). (a) Ordered water molecule bridging trimannoside **1** (in thicker sticks) and the 3 residues (Asp14, Asn16, and Arg228 in thinner sticks) at the binding site of Con A with 4 hydrogen bonds (dashed black line). (b) Similar hydrogen bonds (three hydrogen bonds in dashed black line) of the C2 hydroxyl in trimannoside **2** with the protein. Oxygen atoms are in red, nitrogen atoms in blue, carbon atoms in green and hydrogen atoms in gray; calcium is shown as a sphere.

water molecule to the heat capacity, we repeated the MD simulations at 330 K.

**Calculation of Euler Angles.** According to Euler's orientation theorem, any orientation may be described using three Euler angles. Any rotation $A$ can be written as

$$A = BCD = \begin{pmatrix} \cos\phi & \sin\phi & 0 \\ -\sin\phi & \cos\phi & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\theta & \sin\theta \\ 0 & -\sin\theta & \cos\theta \end{pmatrix} \begin{pmatrix} \cos\psi & \sin\psi & 0 \\ -\sin\psi & \cos\psi & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (6)$$

where $\theta$, $\varphi$, $\psi$ are the Euler angles.

If X and X′ are the coordinates of any point in the original and final coordinate systems before and after the rotation $A$, respectively, they are related by

$$\mathrm{X} = A^{-1}\mathrm{X}' \quad (7)$$

To simplify the calculations, the oxygen atoms of the ordered water molecule in each frame were first translated to its original position. Next, a body-fixed transformation was performed with the $y$-axis on the bisector of HOH, the $z$-axis perpendicular to $y$-axis on the plane of water molecule, and the $x$-axis perpendicular to this plane. Six equations were obtained for the coordinates the two hydrogen atoms in each frame. Solving these equations, we obtained the Euler angles ($\theta$, $\varphi$, $\psi$).

## Results

The MD simulations performed on Con A−trimannoside **1** and **2** complexes gave very similar conformations for the two ligands, consistent with the results of Clarke et al.[1] (see Figure 2, the RMS differences between the 8ns time-averaged MD simulation structures and the experimental structures for ligand **1** and **2** were 0.6 Å and 0.5 Å, respectively).

We first calculated the contribution of the ordered water molecule to the energy, entropy, and heat capacity of solvation using the inhomogeneous fluid theory.[28,29] From the MD simulations of the Con A−trimannoside **1** complex, the translational and orientational correlation functions were obtained. We first calculated the translational correlation function $g_{sw}^{tr}(\mathbf{r}) = g_{sw}^{tr}(r,\theta',\phi')$, where $r$, $\theta'$, and $\phi'$ are spherical coordinates of the water oxygen with respect to its average position. This function gives the local density relative to bulk water. The radial distribution function $g_{sw}^{tr}(r)$ ($g_{sw}^{tr}(\mathbf{r})$ averaged over $\theta'$ and $\phi'$), $g_{sw}^{tr}(\theta')$ ($g_{sw}^{tr}(\mathbf{r})$ averaged over $r$ and $\phi'$), and $g_{sw}^{tr}(\phi')$ ($g_{sw}^{tr}(\mathbf{r})$ averaged over $r$ and $\theta'$) during different portions of the trajectory at 300 K are shown in Figure 3. The similarity of the distributions over the first and second half of the simulation suggests good convergence of these functions in 8 ns. We next calculated the orientation of the water molecule in each frame, described by three Euler angles, and from these the average orientational correlation function $g_{sw}^{or}(\theta,\phi,\psi)$ over the region occupied by this water molecule. Figure 4 shows the distribution of each angle.

The calculation of the integrals in eq 4 was done using either the full three-dimensional functions $g_{sw}^{tr}(r,\theta',\phi')$ and $g_{sw}^{or}(\theta,\phi,\psi)$ or the factorization approximations $g_{sw}^{tr}(r,\theta',\phi') = g_{sw}^{tr}(r)\, g_{sw}^{tr}(\theta')\, g_{sw}^{tr}(\phi')$, and $g_{sw}^{or}(\theta,\phi,\psi) = g_{sw}^{or}(\theta)\, g_{sw}^{or}(\phi)\, g_{sw}^{or}(\psi)$. The results are similar. For example, with bin sizes $dr = 0.06$ Å, $d\theta' = \pi/6$, $d\phi' = \pi/3$ for integration of $g_{sw}^{tr}(r,\theta',\phi')$, we obtained $S_{sw}^{r} = -11.2$ cal/mol K using the factorization approximation and $-10.4$ cal/mol K without it, and with bin sizes $d\theta = \pi/36$, $d\phi = \pi/36$, $d\psi = \pi/36$ for integration of $g_{sw}^{or}(\theta,\phi,\psi)$, we obtained $S_{sw}^{or} = -10.9$ cal/mol K using the factorization approximation and $-11.6$ cal/mol K without it. The values of the thermodynamic parameters discussed below were obtained using the factorization approximations and the bin sizes $dr = 0.06$ Å, $d\theta' = \pi/6$, $d\phi' = \pi/3$ and $d\theta = \pi/36$, $d\phi = \pi/36$, $d\psi = \pi/36$.

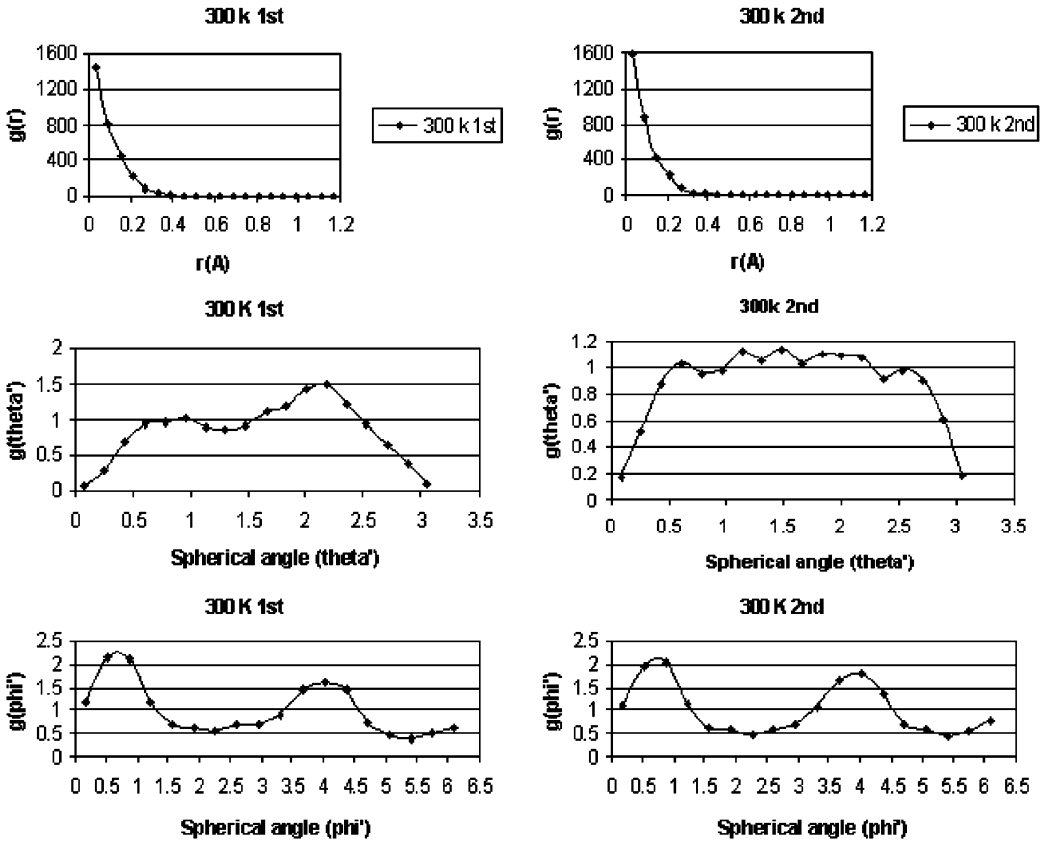


**Figure 3.** Translational distribution function $g^{r}_{sw}(r)$, $g^{tr}_{sw}(\theta')$, and $g^{tr}_{sw}(\phi')$ during different portions (first and second half) of the simulations. Here, $r$ is the distance of the bound water molecule from its average position.

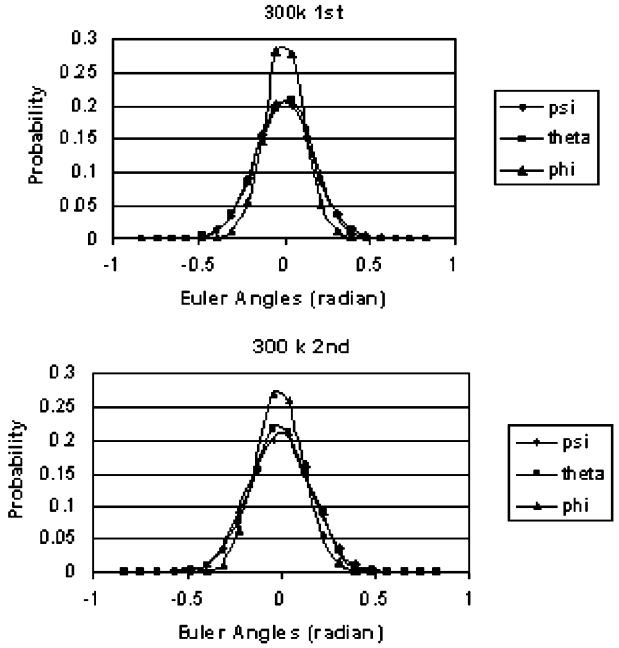


**Figure 4.** Probability distribution of the Euler angles around their average values at 300 K, during different portions (first and second half) of the simulations.

The contributions to solvation free energy from the ordered water molecule are listed in Table 1. At 300 K, the translational contribution to the solute−solvent entropy is −10.4 cal/mol K and the orientational contribution −11.6 cal/mol K, giving $S_{sw}$ = −22.0 cal/mol K. The solute−solvent energy $E_{sw}$ was calculated directly from the simulation as −30.2 kcal/mol, of which −23.6 kcal/mol is due to H-bonds and −6.6 kcal/mol is due to longer range interactions (beyond first neighbors). The latter includes the interaction of water with the two ions, which is −9.2 kcal/mol, showing that the ions enthalpically stabilize the bound water, but other long-range interactions destabilize it. The ions also lower the entropy of the bound water, but this reduction is much smaller than the favorable interaction energy (data not shown). The solvent reorganization energy is the water−water interaction energy in the complex plus the enthalpy of removing a water molecule from bulk water at 300 K, i.e., $\Delta E^{0}_{ww}$ = +10.1 kcal /mol. For the calculation of the solvent reorganization entropy, we neglect the contribution of water−water correlations in the complex (indeed, the calculated interaction energy $E_{ww}$ is only +0.9 kcal/mol at 300 K).

**TABLE 1: Contributions to Solvation from the Ordered Water Molecule in Con A−Trimannoside 1 Calculated from the MD Simulations at Different Temperature**[a]

| | 300 K | 330 K |
|---|---|---|
| $E_{sw}$ | −30.2 ± 0.1 | −30.0 ± 0.2 |
| $E_{w-ions}$[b] | −9.2 ± 0.02 | −9.2 ± 0.02 |
| $E_{ww}$ | +0.9 ± 0.1 | +0.9 ± 0.2 |
| $S^{or}_{sw}$ | −11.6 ± 0.01 | −11.4 ± 0.01 |
| $S^{tr}_{sw}$ | −10.4 ± 0.1 | −9.9 ± 0.1 |
| $S_{sw} = S^{or}_{sw} + S^{tr}_{sw}$ | −22.1 ± 0.1 | −21.3 ± 0.1 |
| $\Delta E_{solv} = E_{sw} + E_{ww} + \Delta E^{0}_{ww}$ | −19.2 ± 0.1 | −19.5 ± 0.2 |
| $\Delta S_{solv} = S_{sw} + \Delta S^{0}_{ww}$ | −6.8 ± 0.1 | −7.8 ± 0.1 |
| $\Delta G_{solv} = \Delta E_{solv} - T\Delta S_{solv}$ | −17.2 ± 0.1 | −16.9 ± 0.1 |
| $\Delta C_p$ | −10 ± 3 | |

[a] Units for enthalpy and free energy are kcal/mol. Units for entropy and heat capacity are cal/mol K. All of the error bars listed in Table 1 and other tables were calculated by averaging the values of each thermodynamic parameter over 2 ns portions of the MD trajectories, and then taking the standard deviation of the 4 samples. [b] $E_{w-ions}$ is included in $E_{sw}$.

Therefore, $\Delta S_{ww}$ is simply the entropy cost of removing a water molecule from bulk water, $\Delta S^0_{ww} = +15.2$ cal/mol K.[32] Thus, $\Delta E_{solv} = -19.2 \pm 0.1$ kcal/mol and $\Delta S_{solv} = -6.8 \pm 0.1$ cal/mol K. This value is very close to the upper bound (7 cal/mol K) estimated by Dunitz.[25] Noting that the $P\Delta V$ term is negligible, the contribution of the ordered water molecule to the solvation free energy at 300 K is

$$\Delta G_{solv} = E_{sw} + \Delta E_{ww} - T(S_{sw} + \Delta S_{ww}) = -17.2 \pm 0.1 \text{ kcal/mol} \quad (8)$$

Clearly, the negative value of $\Delta G_{solv}$ originates mainly from the interaction of the bound water molecule with the protein and ligand.

To calculate the contribution of this water molecule to the heat capacity, we repeated the MD simulation at 330 K. We obtained $S_{sw} = -21.3$ cal/mol K, $E_{sw} = -30.0$ kcal/mol, and $E_{ww} = +0.9$ kcal/mol. From the properties of bulk water, $\Delta E^0_{ww} = +9.6$ kcal/mol and $\Delta S^0_{ww} = +13.5$ cal/mol K.[32] Therefore, $\Delta E = -19.5 \pm 0.1$ kcal/mol, $\Delta S = -7.8 \pm 0.1$ cal/mol K. The contribution of the water molecule to the heat capacity of solvation from simulation was calculated as[29]

$$\Delta C_p(a) = \left(\frac{d\Delta H}{dT}\right) \cong \frac{\delta E_{sw}}{\delta T} + \frac{\delta \Delta E_{ww}}{\delta T} = -10 \pm 3 \text{ cal/mol K} \quad (9)$$

An alternative calculation gives the same result:

$$\Delta C_p(a) = T\left(\frac{d\Delta S}{dT}\right)_p = -10 \pm 1 \text{ cal/mol K} \quad (10)$$

The negative value of $\Delta C_p$ is consistent with the proposal that ordered water molecules contribute to the negative heat capacity of binding in some complexes.[37] Apparently, the decrease of the energy and entropy with temperature of the protein−water interaction in the Con A−trimannoside **1** complex is slower than that of the solvent reorganization energy and entropy. In other words, the interactions of the water in the binding site are less susceptible to temperature than bulk water.

The contribution of the ordered water molecule is only one of the factors affecting binding. Other factors include the desolvation of ligand (L) and protein (P), the translational, rotational, and vibrational entropy of L and P, the direct interactions between L and P, etc. In the case of Con A binding trimannosides **1** and **2**, the protein was found to undergo no conformational change.[38] NMR and MD simulations show that these two ligands have very similar conformational properties and the same binding modes of both complexes except for the substituent on the central mannose.[1] Because the two ligands are very similar, the difference in free energy of binding can be decomposed into four terms

$$\Delta\Delta G = \Delta\Delta G_{desolv} + \Delta\Delta G_{int} + \Delta E_{L-P} + \Delta\Delta G_{solv}(\text{ordered water}) \quad (11)$$

where $\Delta\Delta G_{desolv}$ is the difference of the desolvation free energy of the two ligands, $\Delta\Delta G_{int}$ is the "internal" entropic contributions (changes in the translational, rotational, and vibrational degrees of freedom of the protein and ligands), $\Delta E_{L-P}$ is the difference of the direct interactions between the two ligands and the protein, and $\Delta\Delta G_{solv}$ (ordered water) is the contribution of the ordered water molecule to the solvation free energy.

It is reasonable to assume that, in this case, the $\Delta\Delta G_{desolv}$ originates only from the difference in desolvation free energy of the hydroxyethyl group in **2** and the hydroxyl group it replaced in **1**. We also assume that $\Delta\Delta G_{int}$ originates only from the entropy cost ($-T\Delta S_{config}$) of constraining the hydroxyethyl moiety of trimannoside **2** in the complex. As a result, with eq 11, the $\Delta\Delta G$ can be expressed as

$$\Delta\Delta G = \Delta\Delta H - T\Delta\Delta S \quad (12)$$

where

$$\Delta\Delta H = \Delta H_{solv}(\text{ordered water}) + \Delta E_{L-P} + \Delta\Delta H_{desolv} \quad (13)$$

$$\Delta\Delta S = \Delta S_{solv}(\text{ordered water}) + \Delta S_{config} + \Delta\Delta S_{desolv} \quad (14)$$

We further assume that only the central mannose contributes to the interaction energy difference between trimannoside **1** and **2**. The difference in interaction energy of the C-2 substituents of the two ligands with the protein ($\Delta E_{L2OH-P}$) is −18.4 kcal/mol. The interaction energies of the ions ($Ca^{2+}$ and $Mn^{2+}$) with the C-2 substituents of the two ligands $\Delta E_{L2OH-Ions}$ were also calculated. This interaction is more favorable for trimannoside **2** by 2.3 kcal/mol (Table 2). This value is much smaller than the interaction of the ions with the ordered water in the first complex (−9.2 kcal/mol).

Another contribution to the difference in binding free energy is the different desolvation enthalpy and entropy of trimannoside **2** relative to trimannoside **1**. We assume that the solvation effects of polar and apolar groups of trimannoside **1** and **2** are additive and proportional to their solvent accessible surfaces:[39]

$$\Delta S^{dehy}_{apl} = \sum_i \widehat{\Delta S^{dehy}_{apl,i}} \Delta ASA_{apl,i} \quad (15)$$

$$\Delta S^{dehy}_{pol} = \sum_k \widehat{\Delta S^{dehy}_{pol,k}} \Delta ASA_{pol,k} \quad (16)$$

where $\widehat{\Delta S^{dehy}_{apl}}$ and $\widehat{\Delta S^{dehy}_{pol}}$ are the dehydration entropies of the apolar and polar groups per unit of surface area. Similar equations apply to the dehydration enthalpies and free energies. The analytic surface area method in CHARMM was used to calculate the polar (oxygen) and apolar (carbon) accessible surface area (ASA) of mannose 2 of the two ligands in the complexed and free states. Table 3 shows that trimannoside **2** buries not only more apolar but also more polar surface area than trimannoside **1**. This is because the hydroxyl group in trimannoside **2** is more solvent exposed than that in trimannoside **1**. For the normalized dehydration enthalpies and entropies we

**TABLE 2: Interaction Energies (kcal/mol) of the C2 Substituents of Trimannoside 1 and Trimannoside 2 with Con A and $Ca^{2+}$ and $Mn^{2+}$ Obtained Directly from the MD Simulations at 300 K**

| | Con A−trimannoside **1** | Con A−trimannoside **2** | Δ**1**, **2** |
|---|---|---|---|
| $E_{L2OH-P}$ | −9.4 ± 0.1 | −27.8 ± 0.02 | −18.4 ± 0.1 |
| $E_{L2OH-Ions}$ | −10.0 ± 0.08 | −12.3 ± 0.03 | −2.3 ± 0.1 |

**TABLE 3: Apolar and Polar Accessible Surface Areas (Å$^2$) of Mannose 2 in Trimannoside 1 and 2 in the Complex (final structure after 8 ns MDs) and Free States[a]**

| | complex | | free | | ΔASA | |
|---|---|---|---|---|---|---|
| | polar | apolar | polar | apolar | polar | apolar |
| trimannoside **1** | 7.5 | 136.8 | 65.6 | 168.2 | 58.1 | 31.4 |
| trimannoside **2** | 10.5 | 125.2 | 77.3 | 179.8 | 66.8 | 53.6 |

[a] Calculated with water probe radius 1.5Å.

**TABLE 4: Contributions to the Binding Enthalpy, Entropy, and Heat Capacity[a]**

| | Con A−trimannoside **1** | | Con A−trimannoside **2** | | Δ **1**,**2** | |
|---|---|---|---|---|---|---|
| | 300 K | 330 K | 300 K | 330 K | 300 K | 330 K |
| $E_{L2OH-P}$ | −9.4 ± 0.1 | −9.3 ± 0.03 | −27.8 ± 0.02 | −27.6 ± 0.05 | −18.4 ± 0.1 | −18.3 ± 0.05 |
| $\Delta E_{solv}$ (ordered water) | −19.2 ± 0.1 | −19.5 ± 0.1 | 0 | 0 | +19.2 ± 0.1 | +19.5 ± 0.1 |
| $\Delta S_{solv}$ (ordered water) | −6.8 ± 0.1 | −7.8 ± 0.1 | 0 | 0 | +6.8 ± 0.1 | +7.8 ± 0.1 |
| $\Delta H_{desolv}$[b] | +15.4 | +15.7 | +18.3 | +19.3 | +2.9 | +3.6 |
| $\Delta S_{desolv}$[b] | +18.0 | +18.9 | +23.1 | +26.4 | +5.1 | +7.5 |
| $\Delta S_{config}$ | 0 | 0 | −2.9 | −2.9 | −2.9 | −2.9 |
| $\Delta E$ (total)[c] | −13.2 ± 0.1 | −13.1 ± 0.1 | −9.5 ± 0.02 | −8.3 ± 0.05 | +3.7 ± 0.1 | +4.8 ± 0.1 |
| $\Delta S$ (total) | +11.2 ± 0.1 | +11.1 ± 0.1 | +20.2 | +23.5 | +9.0 ± 0.1 | +12.3 ± 0.1 |
| $\Delta G$ (total) | −16.6 ± 0.1 | −16.8 ± 0.1 | −15.6 ± 0.02 | −16.1 ± 0.05 | +1.0 ± 0.1 | +0.7 ± 0.1 |
| $\Delta C_p$[c] | 3 ± 3 | | 40 ± 1 | | +37 ± 3 | |

[a] Units for enthalpy and free energy are kcal/mol. Units for entropy and heat capacity are cal/mol K. [b] $\Delta H_{desolv}$ stands for the contribution of enthalpies of dehydration from the hydroxyl group in trimannoside **1** and from the hydroxyethyl group in trimannoside **2**; similarly for $\Delta S_{desolv}$. [c] $\Delta E$ (total) = $E_{L2OH-P}$ + $\Delta E_{solv}$ (ordered water) + $\Delta H_{desolv}$, and $\Delta G$ (total) = $\Delta E$ (total) − $T\Delta S$ (total). [d] $\Delta C_p$ calculated as $\Delta\Delta E$ (total)/$\Delta T$.

**TABLE 5: Experimental and Calculated Differences (trimannoside 2 − trimannoside 1) in binding between the two ligands[a]**

| | Δ (trimannoside **1**, **2**) from calc. | Δ (trimannoside **1**, **2**) from experimental data[1] |
|---|---|---|
| | 300 K | 298.15 K |
| ΔΔE (kcal/mol) | +3.7 ± 0.1 | +2.3 ± 0.9 |
| ΔΔS (cal/mol K) | +9.0 ± 0.1 | +3.7 ± 3.0 |
| ΔΔG (kcal/mol) | +1.0 ± 0.1 | +1.2 ± 0.1 |
| ΔΔCp (a) (cal/mol K) | +37 ± 4 | +17.0 ± 14 |

[a] Units for enthalpy and free energy are kcal/mol; Units for entropy and heat capacity are cal/mol K.

used the values given by Makhatadze and Privalov for aliphatic surface and the polar parts of the serine side chain, i.e., 0.03 and 0.25 kcal/mol Å$^2$, respectively, for the enthalpy; 0.14 and 0.24 cal/mol K Å$^2$, respectively, for the entropy; and −0.01 and 0.18 kcal/mol Å$^2$, respectively, for the free energy.[39,40] The differences in desolvation entropy, enthalpy, and free energy of mannose 2 for the two ligands were calculated as +5.1 cal/mol K, +2.9 kcal/mol, and +1.4 kcal/mol, respectively. The positive value of these quantities is due to the larger amount of polar surface area buried for trimannoside **2**. Employing eqs 9, 10 and the heat capacity of dehydration of −OH and −$CH_2$−$CH_2$−OH as −9 cal/mol K[32] and −33.4 cal/mol K,[41,42] respectively, we calculated the desolvation entropy and enthalpy of the two ligands at 330 K as $\Delta\Delta H_{desolv}$(330 K) = +3.6 kcal/mol and $\Delta\Delta S_{desolv}$ (330 K) = +7.5 cal/mol K (Table 4).

We also calculated the entropy of conformational restriction of the hydroxyethyl side-chain of trimannoside **2**. Empirical scales give an average value of conformational entropy of about 1.5 cal/mol K per rotatable bond.[43−48] In this work, the conformational entropy change for the hydroxyethyl side-chain of ligand **2** upon protein−ligand binding was estimated by comparing the distributions of the dihedral angles in the complex and in the free ligand. We performed rigid rotations of the three dihedral angles in the hydroxyethyl side-chain of ligand **2** and one dihedral angle of ligand **1** and obtained the probability ($p$) from the Boltzmann expression. Then we used the integrals of $p\ln(p)$ to estimate the entropy change, which was $\Delta S_{config}$ = −2.9 cal/mol K, consistent with the empirical scales.

Table 4 lists all contributions involved in the binding enthalpy and entropy that are different between the two ligands. The calculated difference in thermodynamic parameters of binding and the corresponding experimental values are summarized in Table 5. The entropy contribution of the displacement is +9.0 cal/mol K, qualitatively comparable with the experimental data (+3.7 cal/mol K). The enthalpy contribution (+3.7 kcal/mol) also compares favorably with the experimental data (+2.3 kcal/mol). The overestimates of the enthalpy and entropy compensate to give a difference in binding affinity very close to experiment (+1.0 kcal/mol, vs +1.2 kcal/mol from experiment). The positive value for $\Delta\Delta C_p$, which is also in agreement with experiment,[1] is largely due to the negative contribution of the ordered water molecule in trimannoside **1**.

**TABLE 6: Thermodynamic Parameters of the Ordered Water Molecules Obtained in the HIV-1 Protease−KNI-272 Complex and Con A-Trimannoside 1 Complex[a]**

| | $E_{sw}$ | $\Delta E_{solv}$ | $-T\Delta S_{solv}$ | $\Delta G_{solv}$ |
|---|---|---|---|---|
| HIV-1 protease | −28.2 | −18.1 | +2.9 | −15.2 |
| Con A | −30.2 | −19.2 | +2.0 | −17.2 |

[a] Units for the energy are kcal/mol.

## Discussion

Using the inhomogeneous fluid solvation theory,[28,29] we first obtained the contribution of the ordered water molecule in Con A−trimannoside **1** complex to binding thermodynamics. The contribution of the ordered water molecule to the solvation enthalpy and entropy shows a large entropic penalty of ordering of the water but also a water−protein/ligand interaction that is much stronger than water−water interactions in bulk solvent. To put these results into perspective, it is useful to compare to previous results on the ordered water molecule in the HIV-1 protease−KNI-272 complex.[30] There are some small differences in these two cases (see Table 6). First, the interaction energy of the water molecule with the protein/ligand is lower in trimannoside **1**−Con A complex by 2 kcal/mol. The interaction energy of the ordered water molecule with the ligands cannot explain this favorable interaction because there are two hydrogen bonds of the ordered water molecule to KNI-272, whereas there is only one hydrogen bond to trimannoside **1**, and the interaction energy of this water with the C2 hydroxyl was about 4 kcal/mol. Instead, the ordered water molecule binds strongly with the protein Con A. Three hydrogen bonds were observed between the water molecule and the protein. The interaction with the ions was also found to stabilize the bound water molecule. Second, a larger entropic penalty ($-T\Delta\Delta S_{solv}$ = 0.9 kcal/mol) of ordering was obtained for the water molecule at HIV-1 protease−KNI-272 complex, suggesting that this water molecule is more ordered than that in the trimannoside **1**−Con A complex. This is consistent with the more structured radial distribution function and orientational correlation functions. It

is interesting to note that enthalpy−entropy compensation is not observed in the values listed in Table 6. That is, lower energy does not correlate with lower entropy. This may be due to the significant contribution of long range interactions in Con A. When the water molecule is already quite ordered, any additional long range interactions do not increase the ordering substantially (this is akin to dielectric saturation).

The elimination of the contribution of the ordered water molecules is the main origin of the loss of enthalpy by the displacement. This large contribution is almost compensated by the stronger direct interaction of trimannoside **2**, leading to a small change in binding enthalpy. Another small contribution to the more positive enthalpy in binding of trimannoside **2** to Con A is the more positive desolvation enthalpy of trimannoside **2** relative to trimannoside **1**.[49] This value is much smaller than the other two large contributions: $\Delta E_{L-P}$ and $\Delta E_{solv}$ (ordered water), but its contribution to the total change of enthalpy is not negligible.

The entropy of releasing the ordered water molecule in Con A−trimannoside **1** to bulk solvation is favorable and it is only partly offset by the constraint of the hydroxyethyl moiety of trimannoside **2** in the complex. The positive difference of the desolvation entropy of the two ligands adds to the overall favorable entropy change. But this favorable entropy change is outweighed by the unfavorable enthalpy change, leading to the unfavorable free energy change $\Delta\Delta G = +1.0$ kcal/mol. The final outcome on binding affinity is a sensitive result of compensation of large contributions. Therefore, it is difficult to predict a priori whether water displacement will be favorable or not; it depends on structural details.

The above analysis differs in some ways from that proposed by Clarke et al.[1] In that work, the loss of enthalpy from trimannoside **1** to trimannoside **2** was proposed to result from differences in hydrogen bonding between the interactions of the two ligands with Con A and was based on several assumptions, some of which do not agree with our findings. First, the desolvation enthalpy of protein and the two ligands in their work was assumed to be the same for the two complexes, which is not what we find (although our estimate of this quantity is also very approximate). Second, counting hydrogen bonds neglects long range contributions to the difference in ligand−protein interactions, which we find to be substantial, especially the interaction with the ions. Third, the interaction energy of the water molecule in complex **1** with the protein was assumed to be identical to the interaction energy of the hydroxyl group in the hydroxyethyl moiety of trimannoside **2**, but our calculation gave an energy difference of 2.4 kcal/mol, showing a stronger interaction of the ordered water molecule with the protein. Finally, their assumption that the fractional occupancy of the hydrogen bond of an additional, dynamic water molecule with the C2 hydroxyl was the same as that of hydrogen bonds in bulk water is also in conflict with our simulations. The dynamic water appeared very scarcely in our simulations (the fractional occupancy of the additional hydrogen bond was about 0.01, very different from 0.7 in bulk water). In addition, the interaction energy of the C2 hydroxyl in trimannoside **1** and **2** with the bulk water was unfavorable (+1.0 and +2.1 kcal/mol, respectively).

Although this work is more rigorous, it also involves some significant approximations, especially the consideration of only a few contributions to binding affinity and the assumption that all others are the same between the two ligands. For example, the reorganization energy and entropy of Con A upon association may not be exactly the same for the two trimannosides, despite the widely accepted picture of "preformed" lectin binding sites. We are also neglecting the ligand reorganization energy and possible changes in the translational/rotational entropy cost of binding. As mentioned above, the estimation of desolvation enthalpy and entropy is also highly approximate. In addition, there are the standard uncertainties related to use of a classical force field, lack of polarizability, etc. Thus, the true uncertainties are larger than the statistical error bars in Tables 1−5. However, the qualitative picture should be correct.

The inhomogeneous fluid solvation theory is applicable to not only highly ordered, isolated water molecules but to also clusters of water molecules, or water molecules that are not fully buried. Such calculations are in progress. This approach offers unprecedented detail in the calculation of contributions to binding affinity and may help us identify simplified correlations between structure and thermodynamic properties. Our approach may also be helpful in rational drug design by identifying which water molecules would be most favorable to displace.

**Acknowledgment.** We are grateful to Dr. Enrique Garcia-Hernandez for bringing concanavalin A to our attention and for help with the carbohydrate force field. This work was supported by the National Science Foundation (MCB-031667). Computational resources were provided by an RCM1 grant from NIH (SG12RR0003060).

## References and Notes

(1) Clarke, C.; Woods, R. J.; Gluska, J.; Cooper, A.; Nutley, M. A.; Boons, G. J. *J. Am. Chem. Soc.* **2001**, *123*, 12238−12247.
(2) Ladbury, J. E. *Chem. Biol.* **1996**, *3*, 973−980.
(3) Niefind, K.; Putter, M.; Guerrra, B.; Issinger, O. G.; Schomburg, D. *Nature Struct. Biol.* **1999**, *6*, 1100−1103.
(4) Zheng, J.; Trafny, E. A.; Knighton, D. R.; Xuong, N.; Taylor, S. S.; Sowadski, J. M. *Acta Crystallogr. D* **1993**, *49*, 362−365.
(5) Otwinowski, Z.; Schevitz, R. W.; Zhang, R.-G.; Sigler, P. B. *Nature* **1988**, *335*, 321−329.
(6) Joachimiak, A.; Haran, T. E.; Sigler, P. B. *EMBO J.* **1994**, *13*, 367−372.
(7) Babor, M.; Sobolev, V.; Edelman, M. *J. Mol. Biol.* **2002**, *323*, 523−532.
(8) Bhat, T. N.; Bentley, G. A.; Fishmann, T. O.; Boulot, G.; Poljak, R. J. *Nature* **1990**, *347*, 483−485.
(9) Bhat, T. N.; Poljak, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1089−1093.
(10) Quiocho, F. A.; Wilson, D. K.; Vyas, N. K. *Nature* **1989**, *340*, 404−407.
(11) Connelly, P. R.; Aldape, R. A.; Wilson, K. P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1964−1968.
(12) Watson, K. A.; Papageorgiou, A. *Biochemistry* **1994**, *19*, 5745−5758.
(13) Lam, P. Y. S.; Jadhav, P. K.; Erickson-Viitanen, S. *Science* **1994**, *263*, 380−384.
(14) Tame, J. R. H.; Murshudov, G. N.; Dodson, E. J.; Neil, T. K.; Wilkinson, A. J. *Science* **1994**, *264*, 1578−1581.
(15) Sleigh, S. H.; Tame, J. R. H.; Dodson, E. J.; Wilkinson, A. J. *Biochemistry* **1997**, *36*, 9747−9758.
(16) Holdgate, G. A.; Tunnicliffe, A.; Ward, W. H. J.; Timms, D. *Biochemistry* **1997**, *36*, 9663−9673.
(17) Weber, P. C.; Pantolianno, M. W.; Simons, D. M. *J. Am. Chem. Soc.* **1994**, *116*, 2717−2724.
(18) Mikol, V.; Papageorgiou, C.; Borer, X. *J. Med. Chem.* **1995**, *38*, 3361−3367.
(19) Loris, R.; Maes, D.; Poortmans, F.; Wyns, L.; Bouckaert, J. *J. Biol. Chem.* **1996**, *271*, 30614−30618.
(20) Yokota, A.; Tsumoto, K.; Shiroishi, M.; Kondo, H.; Kumagai, I. *J. Biol. Chem.* **2003**, *278*, 5410−5418.
(21) Wade, R. C.; Mazor, M. H.; McCammon, J. A.; Quiocho, F. A. *Biopolymers* **1991**, *31*, 919−931.
(22) Roux, B.; Nina, M.; Pomes, R.; Smith, J. C. *Biophys. J.* **1996**, *71*, 670−681.
(23) Ben-Naim, A. *J. Chem. Phys.* **1990**, *93*, 8196−8210.
(24) Durell, S. R.; Brooks, B. R.; Ben-Naim, A. *J. Phys. Chem.* **1994**, *98*, 2198−2202.
(25) Dunitz, J. D. *Science* **1994**, *264*, 670.

(26) Garcia-Sosa, A. T.; Mancera, R. L.; Dean, P. M. *J. Mol. Model.* **2003**, *9*, 172−182.
(27) Wang, J.; Chan, S.; Ramnarayan, K. *J. Comput.-Aided Mol. Des.* **2003**, *18*, 495−513.
(28) Lazaridis, T. *J. Phys. Chem. B* **1998**, *102*, 3531−3541.
(29) Lazaridis, T. *J. Phys. Chem. B* **2000**, *104*, 4964−4979.
(30) Li, Z.; Lazaridis, T. *J. Am. Chem. Soc.* **2003**, *125*, 6636−6637.
(31) Lazaridis, T. *J. Phys. Chem. B* **1998**, *102*, 3542−3550.
(32) Lazaridis, T.; Karplus, M. *J. Chem. Phys.* **1996**, *105*, 4294−4316.
(33) MacKerell, A. D.; Bashford, D.; Bellott, M.; Dunbrack, R. L., Jr.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuzcera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Straub, T.; Watanabe, M.; Wiórkiewicz-Kuczera, J.; Yin, D.; Karplus, M. *J. Phys. Chem. B* **1998**, *102*, 3586−3616.
(34) Ha, S. N.; Giammona, A.; Field, M.; Brady, J. W. *Carbohydrate Res.* **1988**, *180*, 207−221.
(35) Kuttel, M.; Brady, J. W.; Naido, K. J. *J. Comput. Chem.* **2002**, *23*, 1236−1243.
(36) Brooks, C. L.; Karplus, M. *J. Chem. Phys.* **1983**, *79*, 6312−6325.
(37) Morton, C. J.; Ladbury, J. E. *Protein Sci.* **1996**, *5*, 2115−2118.
(38) Weis, W. I.; Drickamer, K. *Annu. Rev. Biochem*. **1996**, *65*, 441−473.
(39) Makhatadze, G. I.; Privalov, P. L. *J. Mol. Biol.* **1993**, *232*, 639−659.
(40) Privalov, P. L.; Makhatadze, G. I. *J. Mol. Biol.* **1993**, *232*, 660−679.
(41) Plyasunov, A. V.; Shock, E. L. *Geochim. Cosmochim. Acta* **2000**, *64*, 439−468.
(42) Cabani, S.; Gianni, P.; Mollica, V.; Lepori, L. *J. Solution Chem.* **1981**, *10*, 563−595.
(43) Wang, J.; Szewczuk, Z.; Yue, S.-Y.; Tsuda, Y.; Konishi, Y.; Purisima, E. O. *J. Mol. Biol.* **1995**, *253*, 473−492.
(44) Luo, R.; Gilson, M. K. *J. Am. Chem. Soc.* **2000**, *122*, 2934−2937.
(45) Pickett, S. D.; Sternberg, M. J. E. *J. Mol. Biol.* **1993**, *231*, 825−839.
(46) Nicholls, A.; Sharp, K. A.; Honig, B. *Protein Sci.* **1991**, *11*, 281−296.
(47) Creamer, T. P. *Protein Sci.* **2000**, *40*, 443−450.
(48) D'Aquino, J. A.; Freire, E.; Amzel, L. M. *Protein Sci.* **2000**, *Suppl 4*, 93−107.
(49) Garcia, H. E.; Hernadez, A. A. *Protein Sci.* **1999**, *8*, 1075−1086.