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Weakly hydrated surfaces and the binding interactions of small biological solutes

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Abstract Extended planar hydrophobic surfaces, such as are found in the side chains of the amino acids histidine, phenylalanine, tyrosine, and tryptophan, exhibit an affinity for the weakly hydrated faces of glucopyranose. In addition, molecular species such as these, including indole, caffeine, and imidazole, exhibit a weak tendency to pair together by hydrophobic stacking in aqueous solution. These interactions can be partially understood in terms of recent models for the hydration of extended hydrophobic faces and should provide insight into the architecture of sugar-binding sites in proteins.

Keywords Hydrophobic hydration · Molecular dynamics · Molecular aggregation · Serotonin

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Introduction

Water is the most abundant molecule on the surface of the earth and is ubiquitous in biological systems (Franks and Mathias 1982). As an integral part of living organisms, its presence is responsible, due to its unique hydrogen-bonding capacity, for much of the characteristic structure and chemistry of biomolecules. Water is required for most globular proteins to fold into their native conformations and is responsible for the formation of lipid bilayers, micelles, and vesicles. Water regulation is important not only in living organisms, but also in many practical applications, ranging from cosmetics to pharmaceuticals to foods. In order to control moisture, it is necessary to understand the ways in which water interacts with the many types of biological molecules and how these interactions may in turn govern the behavior of those molecules.

It is well-known that the special and unusual properties of water play an essential and controlling role in determining the behavior of biological systems. The special properties of water are primarily due to its unique hydrogen-bonding capacity. Its parity between hydrogen bond donors (protons) and acceptors (lone pairs) allows each water molecule to make up to four hydrogen bonds that do not interfere with one another, leading to the development of an extensive space-filling hydrogen bond network in condensed phases such as ice and liquid water. The great strength of these hydrogen bonds, on the order of 10 times k_BT at room temperature, means that they are not easily broken, although they can exchange with lifetimes in the picosecond range, and become less important as the temperature is increased toward the boiling point.

Complex biological solutes can significantly affect the structuring of the adjacent water molecules in aqueous

solutions, with important consequences for both the bulk properties of the solution and the conformation of the solute. When a solute molecule is in an aqueous environment, its functional groups must interact with the inherent hydrogen-bonding requirements of the surrounding water, and its presence can impose a structuring pattern on the adjacent solvent molecules, which differs from that of pure bulk water (Geiger et al. 1984; Israelachvili and Wennerstrom 1996; Stillinger 1980). The organization of solvent water molecules around a particular solute will in general involve both positional and orientational correlations with the specific chemical architecture of the solute, and thus will vary in its details from one molecule to the next. For many biological solutes, this structuring can be quite complex because of the interplay of chemical functionalities found in typical biopolymers, which frequently have polar or hydrogen-bonding functional groups juxtaposed in close proximity to non-polar groups (Liu and Brady 1996; Lounnas and Pettitt 1994; Mezei and Beveridge 1984; Resat and Mezei 1996; Schmidt et al. 1996).

The average structure of simple liquids is traditionally discussed in terms of spherically averaged radial distribution functions *g(r)*, defined as:

$$g(r) = \frac{1}{4\pi\rho r^2}\frac{dN(r)}{dr} \quad (1)$$

where ρ is the bulk density and $g(r)$ represents the probability of finding another atom as a function of the distance r away from a particular atom (Allen and Tildesley 1987; Hansen and McDonald 1976). The radial distribution function is fundamental to the discussion of the properties of simple liquids because it can be used to calculate the thermodynamic functions of the liquid such as the free energy and because $g(r)$ can be determined from diffraction experiments. However, the problem is more difficult for a polyatomic liquid, such as water. Furthermore, for polyatomic solutes, and particularly biological solutes, the solvent structures differently around each solute atom, and each has its own distribution function (Rossky and Karplus 1979). However, the close proximity of these atoms in the solute molecule means that the structuring around one atom contributes to the structuring around adjacent atoms as well, and that these functions are not isotropic, but rather are typically asymmetric. Such structuring can be very complex, so that averaging over all angles at each radial distance as in Eq. 1 would obscure this anisotropic character (Schmidt et al. 1996).

Computer simulations of aqueous solutions are capable of capturing the anisotropic character of this solvent structuring around large polyatomic solutes in complete detail. Various simulations have examined fully spatially resolved structuring in liquids (Kusalik and Svishchev 1994; Svishchev and Kusalik 1993; Svishchev and Kusalik 1994) and biological solutions (Liu and Brady 1996; Lounnas and Pettitt 1994; Schmidt et al. 1996), providing a direct picture of the non-uniform distribution of molecules in three dimensions, as well as the locations of preferred positions for "first shell" solvent molecules. Measuring such structuring experimentally, however, has proven more difficult. Neutron diffraction experiments have been one of the most useful methods of probing such structuring experimentally. For even moderately polyatomic solute molecules, including sugars and amino acids, however, such experiments are much more problematic. Recent advances in analysis of diffraction data in conjunction with computer simulations suggest the promise that such experiments might provide specific hydration information in the future (Mason et al. 2010). Whether or not the information comes from simulation or experiment, knowledge of how a solute structures solvent water could potentially be exploited to understand much about the properties of that solute in solution, such as its hydrodynamics, its solubility, and its interactions with co-solutes. This review will focus on a particularly significant example of solvent-mediated interactions leading to binding that involves the interactions of glucose with proteins, starting from concepts of simple hydrophobic hydration and leading up to representative molecules exhibiting amphiphilic character.

Glucose-protein interactions

The prototypical hexose sugar glucose is one of the more important and interesting examples of a complex biological solute. Molecules such as sugars will have a very complicated pattern of interactions with solvent water. Like other sugars, with their many hydrogen-bonding hydroxyl groups, glucose is an osmolyte in a traditional Hofmeister sense. With its high solubility, glucose is drawn into the bulk of a solution, away from surfaces, including the surfaces of proteins, thus raising the surface tension of the solution and stabilizing globular proteins towards denaturation. By raising the chemical potential, however, glucose also lowers protein solubility and favors precipitation. In general, then, glucose is preferentially excluded from the surfaces of proteins. However, there are a number of proteins that have been designed by evolution to specifically bind to carbohydrates (Sharon and Lis 1990), including, for example, lectins (Delbaere et al. 1989; Einspahr et al. 1986; Frankel et al. 1996), sugar-binding proteins (Quiocho and Vyas 1984), and enzymes that have carbohydrate substrates, such as glycosidases (Henrissat 1994; McCarter and Withers 1994; Vasella et al. 2002; Wilson 1988) and sugar isomerases (Blow et al. 1992; Lavie et al. 1994). In these proteins, the binding sites are specifically designed so as to be complementary to the functional characteristics of

the target sugars. Naturally, this complementarity requires hydrogen bond partners for the sugar hydroxyl groups. In addition, however, it often also involves pairing the hydrophobic portions of the sugar molecules with hydrophobic side chains in the protein.

While it is highly soluble in water, glucose does indeed have some hydrophobic character as well. In particular, glucose has apolar C–H groups, which are all in axial positions in the lowest-energy $^4C_1$ conformation for the β anomer. With all of its hydrogen-bonding hydroxyl groups in equatorial positions, it resembles a puckered disk with a hydrophobic top and bottom but with a polar periphery (see Fig. 1; the slightly less favorable α anomer has an axial C1 hydroxyl group, which somewhat disrupts this segregation). In general, glucose molecules exhibit little tendency to aggregate. To the extent that they are forced to interact by close proximity at high concentrations, they do so by hydrogen bonding (Mason et al. 2005).

Surveys of the known structures of proteins that bind to glucose have revealed a frequent tendency for the binding sites to have tryptophan, phenylalanine, tyrosine, and histidine side chains in those sites, often with their planar faces in van der Waals contact with the hydrophobic "tops" and "bottoms" of the bound glucose ligands. An example of this can be seen in Fig. 2, which displays the interactions between a cellulose chain in the active site tunnel of the cellulase enzyme Cel48F from *Clostridium cellulolyticum* and the side chains of two tryptophan residues (Parsiegla et al. 2008). Notice that the planar indole groups of these side chains are paired against the H1–H3–H5 triads of two glucose residues in the cellulose chain [the β-linkage of cellulose puts all three of these protons in the axial configuration, and the two-fold screw character of cellulose has these protons pointing "up" (see Fig. 2), in the opposite direction, for the intervening residue].

The frequency of occurrence of glucose/planar ring stacking of this type in protein structures suggests that there is a particular affinity of glucose for such species. It has been shown that the π systems of aromatic rings can function as weak hydrogen bond acceptors for water (Atwood et al. 1991; Linse 1990; Suzuki et al. 1992), and such an interaction between the aliphatic protons of glucose and aromatic rings may partially explain the observed stacking, but not necessarily why glucose should out-compete water in this role. The results of a number of recent molecular mechanics simulations suggests that there is a significant contribution to such binding from hydrophobic hydration of extended surfaces (Mason et al. 2011; Wohlert et al. 2010).

Hydrophobic and amphiphilic hydration

Weak associations like those described above can potentially be understood in terms of recent theories of the way hydration of hydrophobic surfaces changes as the spatial extent of the surfaces increases (Ashbaugh and Paulaitis 2001; Chandler 2005; Huang and Chandler 2002; Zangi and Berne 2008). For small hydrophobic solutes with a high curvature, such as methane or argon, an adjacent water molecule can straddle the solute to make hydrogen

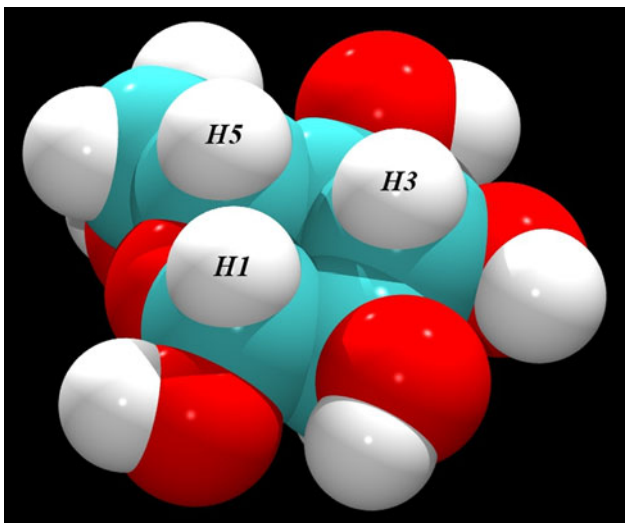

Fig. 1 A van der Waals representation of the β-D-glucopyranose molecule, demonstrating how the axial H1, H3, and H5 aliphatic protons in all lie on the same side of the ring and constitute a triangular hydrophobic surface on the "*top*" of the molecule, with H2 and H4 constituting a smaller region of hydrophobicity on the "*bottom*." All the polar hydroxyl groups are thus in equatorial positions around the periphery

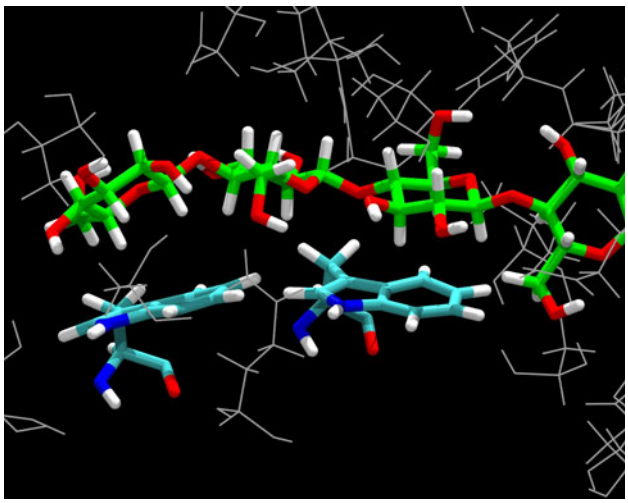

Fig. 2 An illustration of the relationship between the indole side chain groups of two tryptophan residues, shown in atomic detail, at the entrance of the active site tunnel of the cellulase Cel48F from *Clostridium cellulolyticum* (Parsiegla et al. 2008) and a cellulose chain bound into this tunnel. Other nearby protein residues are shown as *light grey lines*

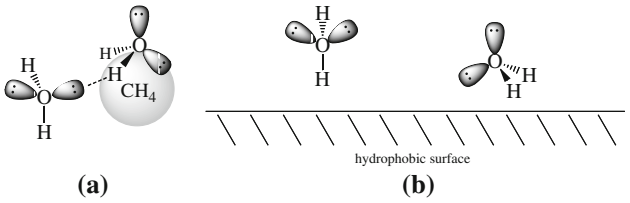

Fig. 3 **a** A schematic representation of a water molecule hydrating a methane molecule by "straddling" it and making a hydrogen bond to other water molecules off to the side. In this geometry, none of the protons or lone pairs of the water molecule are pointing directly at the carbon atom of the methane, which would involve the loss of a hydrogen bond. **b** A schematic representation of water molecules near an extended hydrophobic surface. If the water molecules adopt an orientation similar to the hydration of methane (as on the *right*), they lose three hydrogen bonds to water, but if they point a hydrogen atom or lone pair directly at the surface, as on the *left*, only one hydrogen bond is lost

bonds to other water molecules, with neither of its protons or lone pairs directly pointing at the solute, which would involve the loss of a hydrogen bond (see Fig. 3) (Stillinger 1980). However, this type of hydration restricts the rotational freedom of these water molecules, because if they rotate, they will sacrifice a hydrogen bond, and thus their entropy is lowered and the heat capacity raised by solvation. If two such solutes aggregate, their intervening water molecules are liberated and recover their rotational freedom and associated entropy, so that the spontaneous hydrophobic aggregation of such species is entropy driven. However, for an extended hydrophobic surface, it becomes impossible for water molecules to straddle the surface and still make hydrogen bonds to other water molecules off to its sides (Zangi and Berne 2008). Under these conditions, the water molecules point one hydrogen atom or lone pair directly at the non-hydrogen-bonding surface (Fig. 3), because the resulting loss of one hydrogen bond is nevertheless energetically better than the loss of the three hydrogen bonds that would result if it adopted the orientation of waters adjacent to a methane molecule (Chandler 2005; Lee et al. 1984; Lee and Rossky 1994). The aggregation of such extended surfaces in aqueous solution would then be enthalpically driven, since the pairing of two such surfaces would allow the liberated water molecules to regain their lost hydrogen bonds. It has been demonstrated that there is a gradual transition from entropic domination of the solvation-free energy to enthalpic domination as a function of size for the case of a featureless spherical cavity, with a characteristic length scale for the transition of $\sim$1 nm (Chandler 2005; Huang and Chandler 2002). This type of hydration also results in a depletion of water molecule density close to the hydrophobic surface, so that the hydration of these surfaces is characterized by a de-wetting, with an extended zone from which water is excluded (Chandler 2005).

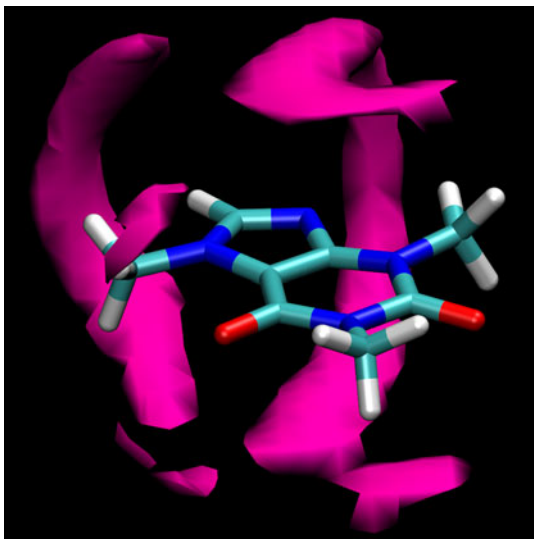

Fig. 4 Contours of solvent water density calculated with respect to a reference frame fixed on the center of mass of caffeine, as calculated from a molecular dynamics simulation. The *purple* surface encloses those regions around the caffeine molecule that on average have a water density 1.3 times higher that the average bulk density (Tavagnacco et al. 2011b). The H8 proton, bound to C8, is the one at the 10 o'clock position, the only proton bound to a ring atom

If this type of hydration is involved in the self-association of small planar molecules like imidazole, purines, and benzene derivatives, then it should be possible to detect certain signatures of this behavior in MD simulations of the solvation of such molecules. Figure 4 displays the density of water molecule oxygen atoms around the purine caffeine in an aqueous solution (Tavagnacco et al. 2011b). This purine, which closely resembles the indole group of tryptophan, is a good subject for such studies because it is somewhat soluble in water, and as a result, experimental thermodynamic data for its solvation are available (Cesàro et al. 1976; Stoesser and Gill 1967). As can be seen from Fig. 4, the distribution of water molecules around caffeine is highly anisotropic and quite complex. This reflects the complicated arrangement of hydrogen-bonding functional groups, such as the ring nitrogen atom and the carbonyl oxygen atoms adjacent to non-hydrogen-bonding methyl groups and with extended non-hydrogen-bonding planar surfaces. Because of the significant molecular dipole oriented approximately toward the C–H group at C8 (see Fig. 4), this proton also makes strong interactions with water molecules that are in some ways similar to hydrogen bonds (Tavagnacco et al. 2011b), as has been seen in other purines (Teng et al. 1988). Note how a band of water density wraps around the ring nitrogen atom, merging at the top and bottom with a small, localized cloud of density directly over the center of the molecule. The banana-shaped part of this cloud is due to water molecules hydrogen bonding to the N9 atom, but the caps over the top

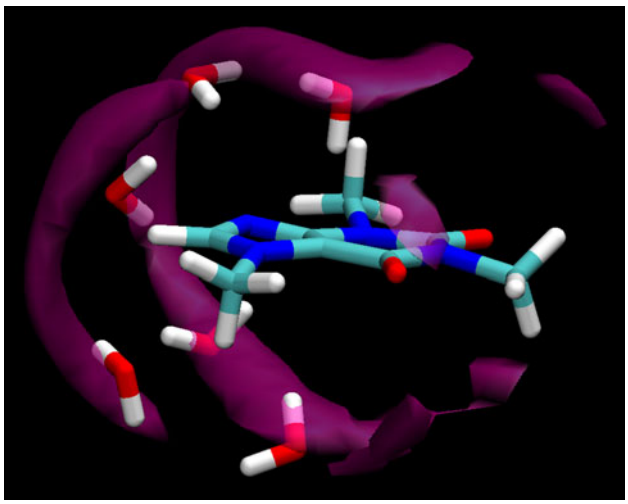

Fig. 5 The areas of high water density are shown contoured in *purple*, as in Fig. 4, along with the instantaneous positions of six water molecules selected because they were close to the atoms of the five-atom ring. Note that the two water molecules directly over the planar faces of the caffeine, one above and another below, are pointing their protons directly at the C4–C5 bond regions in the center of the caffeine molecule, at the cost of a hydrogen bond, because there is no hydrogen bond partner for them in that region (Tavagnacco et al. 2011b)

and bottom represent water molecules that are hydrophobically hydrating the planar faces. Figure 5 displays the instantaneous position of a few selected water molecules from a single frame of the simulation, superimposed on the density clouds averaged over the entire simulation (Tavagnacco et al. 2011b). As can be seen, the water molecules directly over the caffeine faces are oriented so as to point one of their protons directly at the non-hydrogen-bonding faces, in principle sacrificing a hydrogen bond to water in the process. However, this arrangement is preferable to the alternate possibility, in which three lone pair and proton positions are directed toward the surface while one proton points directly away. In that arrangement, the water molecule sacrifices three hydrogen bonds. Thus, the simulations appear to agree with the predictions from the theories about the hydration of extended surfaces. However, the positions of these water molecules are no further from the hydrophobic faces than water molecules would be from the central carbon atom of methane, so no significant de-wetting is seen. This is not necessarily surprising, however, because the caffeine molecule is considerably shorter than the 1-nm length predicted by Chandler for the transition to de-wetting hydration behavior. In addition, the caffeine face is not a uniform hydrophobic surface, but instead, as noted, has hydrogen-bonding functionalities all around its periphery, and the water molecules hydrogen bonded to these groups also impose structuring requirements on the water molecules directly above the plane of the rings as well as around the periphery.

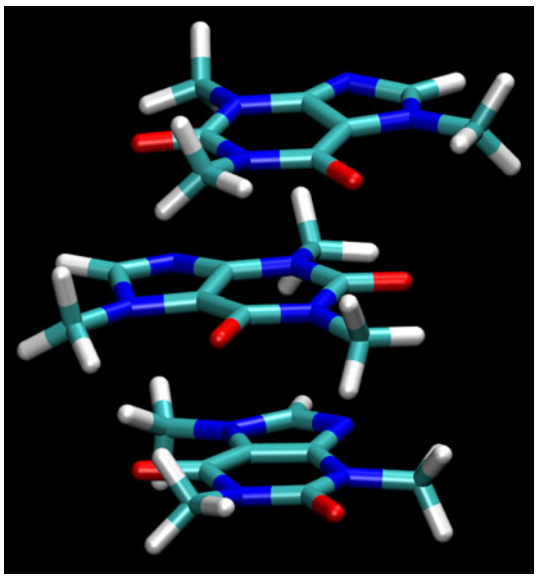

Fig. 6 A typical "snapshot" from a simulation of caffeine in water, in which extensive face-to-face stacking aggregation was observed, as seen in this typical trimer

If two such faces pair up, these hydrophobically structured water molecules are liberated and recover their full complement of hydrogen bonds to water. Such pairing would thus be expected to be enthalpically driven. It is known from experimental studies that there is extensive aggregation in caffeine solutions and that this aggregation is enthalpically driven (Cesàro et al. 1976; Stoesser and Gill 1967). Such an association is indeed found in MD simulations of caffeine solutions (Tavagnacco et al. 2011b), as can be seen in a typical "snapshot" of a caffeine cluster from these simulations (Fig. 6). The removal of water molecules structured as seen in Fig. 5 would explain the enthalpy dominance of the free energy change upon such clustering, although the simulations conducted to date did not calculate the free energy change or its components (Tavagnacco et al. 2011b).

Interactions of glucose with planar hydrophobic ring biomolecules

This same type of hydrophobic face pairing drives glucose to aggregate with caffeine in aqueous solution, as can be seen from Fig. 7 (Tavagnacco et al. 2011c). In this type of pairing the three aliphatic protons H1, H3, and H5, which constitute a small non-polar planar surface, are more likely to point toward the surface of a caffeine molecule than the other way around. However, there is still significant probability for the other face, which consists of only two such protons, H2 and H4, to pair with caffeine, as can be seen from the two layers of density in Fig. 7.

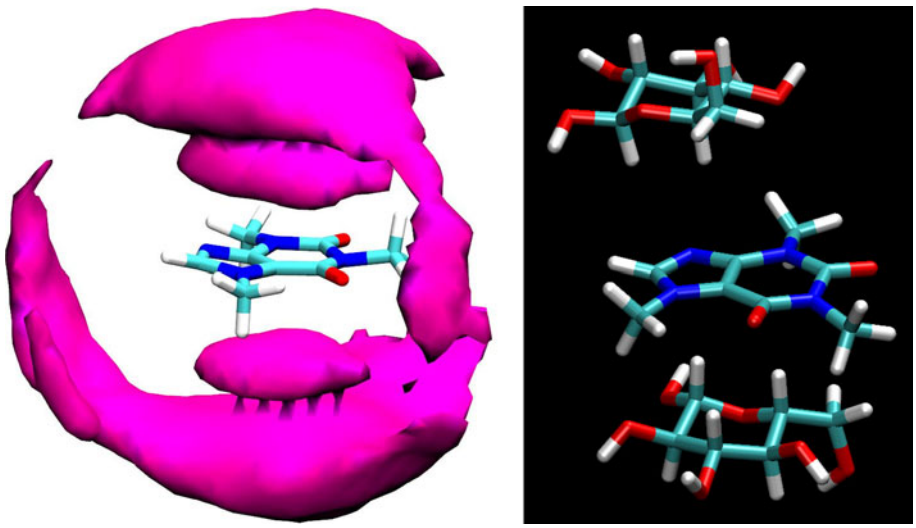

Fig. 7 *Left* the density of H2 and H4 atoms from glucose at 2× bulk density, as calculated from an MD simulation of a mixed aqueous solution of β-D-glucopyranose and caffeine, each 3 molal in concentration; *right* an example configuration from the simulation illustrating two β-D-glucopyranose molecules stacking their H1–H3–H4 faces against the caffeine faces

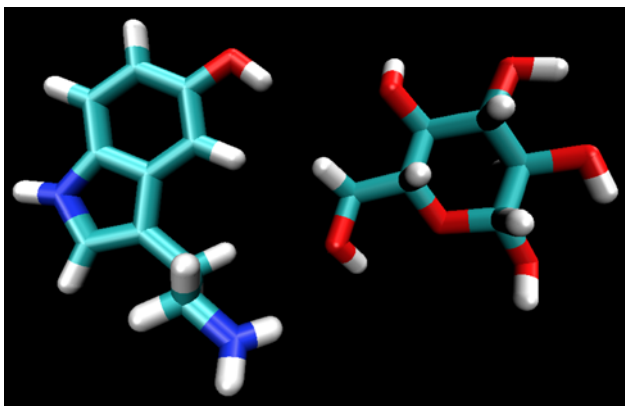

Fig. 8 The covalent structure of serotonin, compared with that of β-D-glucopyranose

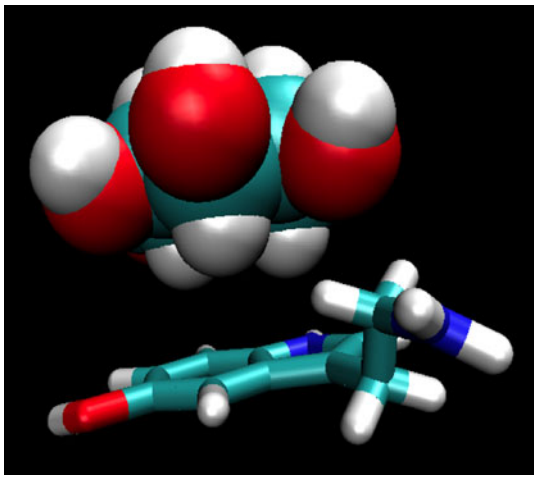

Fig. 9 An example configuration of a glucose molecule interacting with a serotonin molecule, stacking its H1–H3–H5 atom face against the flat surface of the serotonin

Similar structuring is also seen for the interaction of glucose with the indole-containing neurotransmitter serotonin (Fig. 8). MD simulations of serotonin again found pairing of the glucose hydrophobic triad of β-D-glucopyranose with the planar faces of serotonin, as shown in Fig. 9. Figure 10 displays the trajectory-averaged density of glucose ring atoms relative to a reference frame fixed on the serotonin solute. As can be seen, this density is only slightly perturbed by the $–CH_2–CH_2–NH_2$ oriented on one side rather than the other. Using the calculated density probabilities, it was possible to estimate the glucose-serotonin binding affinity as around −1.4 kcal/mol. This value is approximately the same as previously found for the interaction of glucose binding to Trp in a model protein (Mason et al. 2011; note that in this previous study, equilibrium constants were incorrectly calculated using $\log_{10}$; actual energies were thus 2.3 times larger than reported), and for the interaction of glucose with indole as found from free energy calculations, when corrected for concentration (Wohlert et al. 2010), or approximately $2k_BT$.

Similar simulations have also been conducted for glucose and imidazole as co-solutes in water, with the concentration of each at 3 *m* (Chen et al. 2011). As with the caffeine simulations, imidazole self-aggregation was observed, with the molecules associating less by face-to-face stacking than by T-type edge-to-face interactions. In addition, significant stacking of the β-D-glucopyranose molecules against the imidazole faces was seen, as shown in Fig. 11. As in the caffeine case, there was a preference for the H1–H3–H5 faces to pair with the imidazole face, but again there was also significant pairing with the opposite face as well. However, in the imidazole case, there was little tendency for water molecules to structure as in Fig. 5, with a proton pointing directly at the center of the ring. This is presumably due to the much smaller size of the imidazole face and thus the more dominating orienting effect of the ring nitrogen atoms, as well as the possibility of straddling

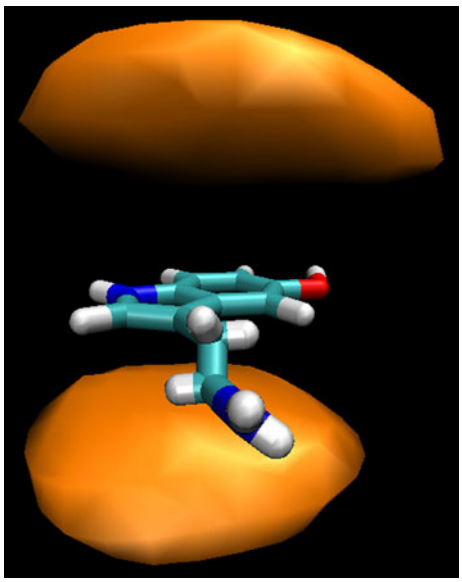

Fig. 10 Contours of glucose ring atom density relative to serotonin, enclosing the volume with an average density two times higher than the bulk density for these atoms

this solute as in the methane case. In spite of this difference, the binding Gibbs free energy for β-D-glucopyranose to imidazole was estimated from the molecular densities to be approximately -0.5 kcal/mol (again, it was not possible to break this down into entropy and enthalpy contributions).

Although the free energies observed for glucose pairing with these planar molecules are small, they could help to explain the prevalence of such groups in the binding sites for glucose and other sugars in proteins. In particular, in binding sites such as illustrated in Fig. 2, where there is more than one such residue to interact with the carbohydrate ligand, the cumulative contribution could be much more significant. In a related simulation of a small globular binding domain portion of a cellulase in a concentrated aqueous solution of β-D-glucopyranose (Tavagnacco et al. 2011a), the sugars were found to have a marked tendency to bind to three surface tyrosine residues that are known to be important for the binding of this protein to cellulose. The glucose molecules paired up with the flat faces of these tyrosine side chains in exactly the same fashion as seen above. These residues are spaced along the surface of the binding module at intervals that match up well with every other residue of cellulose, similar to the pattern seen in Fig. 2. The accumulation of small energy contributions from a large number of such interactions may even help explain the insolubility of cellulose. Potential of mean force calculations of the free energy between chains of cellulose in water (Bergenstråhle et al. 2010) found that there is a significant pairing energy due to solvation that grows with the length of the chains. However, this cooperativity leads to a much larger interaction energy than would come from just adding up the small contributions from each individual glucose pairing.

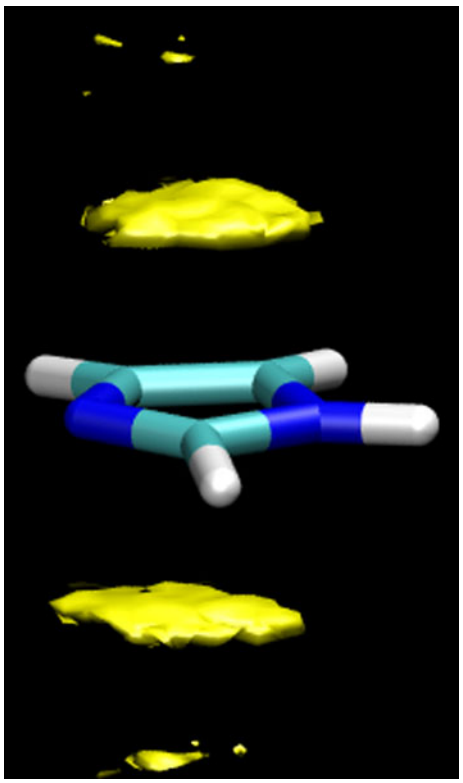

Fig. 11 Contours of the density of H1, H3, and H5 atoms from β-D-glucopyranose relative to imidazole as calculated from an MD simulation of imidazole and glucose in water. As before, note the higher probability for these atoms pairing with the planar co-solute face than orienting the opposite way, with the H2 and H4 protons in van der Waals contact with this surface

It should be noted that the affinities discussed here were all calculated using conventional molecular mechanics simulations (Brooks et al. 2009). However, it has been suggested that binding of the sort described is also favored by interactions with the π systems of aromatic ring molecules like benzene, indole, serotonin, and caffeine, and that the π system of a molecule like benzene can serve as a weak hydrogen bond acceptor (Atwood et al. 1991; Linse 1990; Ramírez-Gualito et al. 2009; Suzuki et al. 1992). Such interactions are not directly present in molecular mechanics simulations, of course, but the force fields employed are parameterized to the results of quantum mechanical calculations, including interactions with water, so that this type of interaction is generally properly mimicked (MacKerell et al. 1998).

It is also possible that the aggregation observed in these various studies may have been affected by the system size (Singh et al. 2008). However, in our previous simulations of guanidinium aggregation, which studied aggregation as a function of system size and concentration, no such effects were found (Mason et al. 2004). The quantitative

agreement with experimental thermodynamic measurements of osmotic data in the case of caffeine aggregation further demonstrates that the relatively small sizes of our systems are not introducing serious artifacts of this type (Tavagnacco et al. 2011b).

Conclusions

Although glucose is very hydrophilic, and behaves as an osmolyte, it does possess hydrophobic character in its nonpolar faces, particularly in the $^4C_1$ chair conformation of its beta pyranose form. In aqueous solution these hydrophobic faces can interact with the planar hydrophobic faces of amino acid side chains like tryptophan, tyrosine, phenylalanine, and histidine to give a weak binding affinity. While this binding energy is weak, in the range of 0.5–1.4 kcal/mol, it apparently plays a role in the specificity of a number of proteins that have sugar substrates or ligands, and may even help explain the insolubility of cellulose in water. These interactions have some of the elements expected from the hydration of extended hydrophobic surfaces, but because the sizes of both the glucose faces and the rings to which they bind are in the intermediate size range for the transition from so-called wetting to de-wetting behavior, these systems do not exhibit the de-wetting that would be seen for more extended hydrophobic surfaces (Chandler 2005). Nonetheless, they may well exhibit enthalpy-driven binding, as is observed from experiment for the binding of two caffeine molecules together (Cesàro et al. 1976). Such interactions should be considered when using bioengineering to design modified enzymes intended to bind to sugars like glucose.

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References

Allen MP, Tildesley DJ (1987) Computer simulation of liquids. Clarendon Press, Oxford

Ashbaugh HS, Paulaitis ME (2001) Effect of solute size and solute-water attractive interactions on hydration water structure around hydrophobic solutes. J Am Chem Soc 123:10721–10728

Atwood JL, Hamada F, Robinson KD, Orr GW, Vincent RL (1991) X-ray diffraction evidence for aromatic pi hydrogen-bonding to water. Nature 349:683–684

Bergenstråhle M, Wohlert J, Himmel ME, Brady JW (2010) Simulation studies of the insolubility of cellulose. Carbohydr Res 345:2060–2066

Blow DM, Collyer CA, Goldberg JD, Smart OS (1992) Structure and mechanism of D-xylose isomerase. Faraday Discuss 93:1–7

Brooks BR, Brooks CL, MacKerell AD, Nilsson J, Petrella L, Roux RJ, Won B, Archontis Y, Bartels G, Boresch CS et al (2009) CHARMM: the biomolecular simulation program. J Comput Chem 30:1545–1614

Cesàro A, Russo E, Crescenzi V (1976) Thermodynamics of caffeine aqueous solutions. J Phys Chem 80:335–339

Chandler D (2005) Interfaces and the driving force of hydrophobic assembly. Nature 437:640–647

Chen M, Bomble YJ, Himmel M E, Brady JW (2011) Molecular dynamics simulations of the interaction of glucose with imidazole in aqueous solution. Carbohydr Res (submitted)

Delbaere LTJ, Vandonselaar M, Prasad L, Quail JW, Nikrad PV, Pearlstone JR, Carpenter MR, Smillie LB, Spohr U, Lemieux RU (1989) Structures of *Griffonia Simplicifolia* Lectin IV and its complex with a Synthetic Lewis b Blood Group Determinant. Trans Am Crystallogr Assoc 25:65–76

Einspahr H, Parks EH, Suguna K, Subramanian E, Suddath FL (1986) The crystal structure of pea lectin at 3.0-Å resolution. J Biol Chem 261:16518–16527

Frankel AE, Burbage C, Fu T, Tagge E, Chandler J, Willingham MC (1996) Ricin toxin contains at least three galactose-binding sites located in B chain subdomains 1α, 1β, and 2γ. Biochemistry 35:14749–14756

Franks F, Mathias S (eds) (1982) Biophysics of water. Wiley, Chichester

Geiger A, Mausbach P, Schnitker J, Blumberg RL, Stanley HE (1984) Structure and dynamics of the hydrogen bond network in water by computer simulations. Journal de Physique 45:13–31

Hansen JP, McDonald JR (1976) Theory of simple liquids. Academic Press, London

Henrissat B (1994) Cellulases and their interaction with cellulose. Cellulose 1:169–196

Huang DM, Chandler D (2002) The hydrophobic effect and the influence of solute-solvent attractions. J Phys Chem B 106:2047–2053

Israelachvili J, Wennerstrom H (1996) Role of hydration and water structure in biological and colloidal interactions. Nature 379:219–225

Kusalik PG, Svishchev IM (1994) The spatial structure in liquid water. Science 265:1219–1221

Lavie A, Allen KN, Petsko GA, Ringe D (1994) X-ray crystallographic structures of D-xylose isomerase-substrate complexes position the substrate and provide evidence for metal movement during catalysis. Biochemistry 33:5469–5480

Lee SH, Rossky PJ (1994) A comparison of the structure and dynamics of liquid water at hydrophobic and hydrophilic surfaces—a molecular dynamics simulation study. J Chem Phys 100:3334–3345

Lee CY, McCammon JA, Rossky PJ (1984) The structure of liquid water at an extended hydrophobic surface. J Chem Phys 80:4448–4455

Linse P (1990) Molecular dynamics simulation of a dilute aqueous solution of benzene. J Am Chem Soc 112:1744–1750

Liu Q, Brady JW (1996) Anisotropic solvent structuring in aqueous sugar solutions. J Am Chem Soc 118:12276–12286

Lounnas V, Pettitt BM (1994) A connected-cluster of hydration around myoglobin: correlation between molecular dynamics simulations and experiment. Proteins Struct Funct Genet 18:133–147

MacKerell AD, Bashford D, Bellott M, Dunbrack RL, Evanseck JD, Field MJ, Fischer S, Gao J, Guo H, Ha S et al (1998) All-atom empirical potential for molecular modeling and dynamics studies of proteins. J Phys Chem B 102:3586–3616

Mason PE, Neilson GW, Enderby JE, Saboungi M-L, Dempsey CE, MacKerell AD, Brady JW (2004) The structure of aqueous guanidinium chloride solutions. J Am Chem Soc 126:11462–11470

Mason PE, Neilson GW, Enderby JE, Saboungi M-L, Brady JW (2005) The structure of aqueous glucose solutions as determined by neutron diffraction with isotopic substitution experiments and molecular dynamics calculations. J Phys Chem B 109:13104–13111

Mason PE, Neilson GW, Price DL, Saboungi M-L, Brady JW (2010) Observation of pyridine aggregation in aqueous solution using neutron scattering experiments and MD simulations. J Phys Chem B 114:5412–5419

Mason PE, Lerbret A, Saboungi M-L, Neilson GW, Dempsey CE, Brady JW (2011) The interactions of glucose with a model peptide. Proteins 79:2224–2232

McCarter JD, Withers SG (1994) Mechanisms of enzymatic glycoside hydrolysis. Curr Opin Struct Biol 4:885–892

Mezei M, Beveridge DL (1984) Generic solvent sites in a crystal. J Comput Chem 5:523–527

Parsiegla G, Reverbel C, Tardif C, Driguez H, Haser R (2008) Structures of mutants of cellulase Cel48F of Clostridium cellulolyticum in complex with long hemithiocellooligosaccharides give rise to a new view of the substrate pathway during processive action. J Microbiol 375:499–510

Quiocho FA, Vyas KK (1984) Novel stereospecificity of the L-arabinose-binding protein. Nature 310:381–386

Ramírez-Gualito K, Alonso-Ríos R, Quiroz-García B, Rojas-Aguilar A, Díaz D, Jiménez-Barbero J, Cuevas G (2009) Enthalpic nature of the CH/π interaction involved in the recognition of carbohydrates by aromatic compounds, confirmed by a novel interplay of NMR, calorimetry, and theoretical calculations. J Am Chem Soc 131:18129–18138

Resat H, Mezei M (1996) Grand canonical ensemble Monte Carlo simulation of the dCpG/proflavine crystal hydrate. Biophys J 71:1179–1190

Rossky PJ, Karplus M (1979) Solvation. A molecular dynamics study of a dipeptide in water. J Am Chem Soc 101:1913–1937

Schmidt RK, Karplus M, Brady JW (1996) The anomeric equilibrium in D-xylose: free energy and the role of solvent structuring. J Am Chem Soc 118:541–546

Sharon N, Lis H (1990) Carbohydrate-protein interactions. Chemistry in Britain, 679–682

Singh G, Brovchenko I, Oleinkova A, Winter R (2008) Peptide aggregation in finite systems. Biophys J 95:3208–3221

Stillinger FH (1980) Water revisited. Science 209:451–457

Stoesser PR, Gill SJ (1967) Calorimetric study of self-association of 6-methylpurine in water. J Phys Chem 71:564–567

Suzuki S, Green PG, Bumgarner RE, Dasgupta S, Goddard WA, Blake GA (1992) Benzene forms hydrogen bonds with water. Science 257:942–945

Svishchev IM, Kusalik PG (1993) Proton chemical shift of water in the liquid state: computer simulation results. J Am Chem Soc 115:8270–8274

Svishchev IM, Kusalik PG (1994) Structure in liquid methanol from spatial distribution functions. J Chem Phys 100:5165–5171

Tavagnacco L, Mason PE, Schnupf U, Pitici F, Zhong L, Himmel ME, Crowley M, Cesàro A, Brady JW (2011a) Sugar binding sites on the surface of the carbohydrate binding module of CBH I from *Trichoderma reesei*. Carbohydr Res 346:839–846

Tavagnacco L, Schnupf U, Mason PE, Saboungi M-L, Cesàro A, Brady JW (2011b) Molecular dynamics simulation studies of caffeine aggregation in aqueous solution. J Phys Chem B 115:10957–10966

Tavagnacco L, Schnupf U, Saboungi M-L, Cesàro A, Brady JW (2011c) Simulation studies of sugars interacting with caffeine in aqueous solution. J Phys Chem B (in preparation)

Teng M-K, Usman N, Frederick CA, Wang AH-J (1988) The molecular structure of the complex of Hoechst 33258 and the DNA dodecamer d(CGCGAATTCGCG). Nucleic Acid Res 16:2671–2690

Vasella A, Davies GJ, Böhm M (2002) Glycosidase mechanisms. Curr Opin Chem Biol 6:619–629

Wilson DB (1988) Cellulases of *Thermomonospora fusca*. Methods Enzymol 160:314–323

Wohlert J, Schnupf U, Brady JW (2010) Free energy surfaces for the interaction of glucose with planar aromatic groups in aqueous solution. J Chem Phys 133:155103

Zangi R, Berne BJ (2008) Temperature dependence of dimerization and dewetting of large-scale hydrophobes: a molecular dynamics study. J Phys Chem B 112:8634–8644