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Thermodynamics and solvent linkage of macromolecule–ligand interactions ☆

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ABSTRACT

Binding involves two steps, desolvation and association. While water is ubiquitous and occurs at high concentration, it is typically ignored. *In vitro* experiments typically use infinite dilution conditions, while *in vivo*, the concentration of water is decreased due to the presence of high concentrations of molecules in the cellular milieu. This review discusses isothermal titration calorimetry approaches that address the role of water in binding. For example, use of D_2O allows the contribution of solvent reorganization to the enthalpy component to be assessed. Further, the addition of osmolytes will decrease the water activity of a solution and allow effects on Ka to be determined. In most cases, binding becomes tighter in the presence of osmolytes as the desolvation penalty associated with binding is minimized. In other cases, the osmolytes prefer to interact with the ligand or protein, and if their removal is more difficult than shedding water, then binding can be weakened. These complicating layers can be discerned by different slopes in ln(Ka) vs osmolality plots and by differential scanning calorimetry in the presence of the osmolyte.

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1. Introduction

A grand challenge in binding is the prediction of ligand affinity. This ability is desired in drug design, but is still lacking. One critical factor that is often overlooked is the role of solvent and/or solute in binding. *In vitro* assays typically involve high concentrations of water while *in vivo* assays involve high concentrations of macromolecules and metabolites that exist in the cell. How accurate/relevant is the binding measured in dilute conditions compared to the crowded cell? How do hydration and desolvation affect binding? Can solvation effects be deconvoluted from the observed enthalpy? Does the presence of other solutes affect affinity? Do osmolytes have different effects than crowders? This review considers some of the calorimetry studies that address these questions.

Ligand binding can be described by the following equation:

$$M + L \leftrightarrows ML \tag{1}$$

where M is the macromolecule and L is the ligand. Binding is described by the association constant, K_a, and the Gibbs free energy, ΔG, which equals $-RT \ln K_a$. Another common equation is $\Delta G = \Delta H - T\Delta S$ where ΔH is the enthalpy (a measure of the energy of interaction between the binding partners), T is the temperature and ΔS is the entropy (a measure of the order of the system). Isothermal titration calorimetry (ITC) allows direct measurement of the enthalpy as well as the stoichiometry and K_a associated with binding. Since molecules are hydrated in solution, the observed ΔH is composed of two parts, the intrinsic enthalpy associated with binding and the effect due to solvation. This relationship is described in Eq. (2) below as well as the Born–Haber cycle (Fig. 1).

$$\Delta H_{\text{observed}} = \Delta H_{\text{intrinsic}} + \Delta H_{\text{solvation}} \tag{2}$$

From the Born–Haber cycle, desolvation plays a role in binding. Water can also play an important role in binding and specificity by filling structural voids and also serving as a bridge between surfaces.

Abbreviations: ADP, adenosine diphosphate; APH, aminoglycoside phosphotransferase (3′)-IIIa; BSA, bovine serum albumin; Cc, iso-1-ferricytochrome c; CcP, ferricytochrome c peroxidase; CD, circular dichroism; ConA, concanavalin A; ΔC_p, change in heat capacity; ΔG, Gibbs free energy; ΔH, enthalpy; Δn_w, number of waters released or taken up upon binding; ΔS, entropy; D_2O, deuterium oxide; DHF, dihydrofolate; DHFR, dihydrofolate reductase; DMSO, dimethylsulfoxide; DSC, differential scanning calorimetry; EcDHFR, chromosomal *E. coli* dihydrofolate reductase; GK, guanylate kinase; GMP, guanosine monophosphate; HCA, human carbonic anhydrase; ITC, isothermal titration calorimetry; K_a, association constant; K_d, dissociation constant which equals $1/K_a$; MTX, methotrexate; MuP, major urinary protein; NADP($^+$/H), nicotinamide adenine dinucleotide phosphate (oxidized/reduced); NMR, nuclear magnetic resonance; PEG, polyethylene glycol; SANS, small angle neutron scattering; T, temperature; TBP, TATA-box binding protein; TEG, triethylene glycol.

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2. Solvent effects near hydrophobic surfaces

In a classical description of the hydrophobic effect, solvation of a hydrophobic surface involves ordering of water. Clathrate or iceberg structures have been observed for water near a hydrophobic surface. This behavior predicts that association of two hydrophobic surfaces will be entropy driven as the released water molecules will be more disordered as they enter the bulk solvent. Examples of entropy driven binding include early inhibitors of the AIDs protease [1] and a variant of subtilisin BPN' binding to GroEL [2]. Both systems show endothermic peaks in ITC titrations. Many other examples exist.

More recently however, Chandler has suggested water near an extended hydrophobic surface has properties similar to gas phase water [3,4]. In this case, water acts as if it is at a liquid–vapor interface and the water density has a larger variation as compared to bulk water. This model allows biomolecules to lose waters more readily (dewetting) to assemble via hydrophobic interactions. Melittin and 2,3-dihydroxybiphenyl dioxygenase were predicted to be near a dewetting–wetting transition and mutations were proposed to shift the equilibria towards one side of the transition or the other [4]. One could predict that using different solutes would also alter the ability of these assemblies to form.

3. Enthalpy driven hydrophobic effects?

To study the role of water on hydrophobic effects, ITC is typically coupled with additional structural information derived from crystallography or nuclear magnetic resonance (NMR) experiments. For example, the Klebe lab has studied water effects in thermolysin using ITC and high resolution crystal structures [5–7]. Using ligands with either a terminal methyl or a terminal carboxylate or both moieties that bind in the S2′ site, they find that the energetics are dependent on the route taken to the doubly substituted ligand [5]. In other words, a $-CH_3$ group has a different effect when added to the initial molecule or when added to the derivative possessing the carboxylate group. As the ligands show almost identical binding modes, their non-predictable behavior was proposed to be associated with differences in the first hydration shell surrounding the ligands. The Klebe lab found that the larger and more cohesive the water network formed on the surface of the bound ligand, the greater the enthalpy associated with binding. In this "non-classical hydrophobic effect," a more negative enthalpy was observed when rigid water networks formed on the surface of a hydrophobic ligand in the bound complex. In other studies by this group [6,7], a series of 12 different ligands was examined where the substituent binding to the S2′ site in thermolysin ranged from hydrogen to longer alkyl chains to a benzene moiety. While the ΔG varied from −7.52 to −9.73 kcal/mol ($\Delta\Delta G$ = 2.21 kcal/mol), the associated enthalpy varied from −2.50 to −8.03 kcal/mol ($\Delta\Delta H$ = 5.53 kcal/mol), a much larger range. All these data sets led the authors to conclude that desolvation is not the sole driving force in binding as addition of more methyl groups does not linearly affect the ΔG and ΔH values. Rather the authors posit that water which bridges from the bound ligand surface to bulk water strongly impacts the thermodynamics of the interaction. If a strong network of H-bonds is formed in the first hydration shell of the complex, this contributes to an enthalpic signature associated with binding.

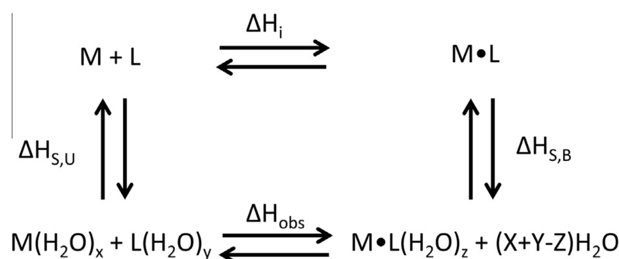

Fig. 1. The Born–Haber cycle. A ligand (L) and macromolecule (M) bind in the gas phase, giving the enthalpy intrinsic to the binding contacts made upon complex formation (ΔH_i top line). The solvation enthalpy of the unbound species ($\Delta H_{S,U}$), where S stands for solvation and U for unbound species, is shown on the left, while that of the bound complex is shown on the right ($\Delta H_{S,B}$), where B means the bound species. In an actual experiment, the species are already solvated (bottom row) and the observed binding enthalpy (ΔH_{obs}) includes the change in solvation between the free and bound species in addition to the ΔH_i.

Another window on the hydrophobic effect is provided by studies of human carbonic anhydrase II (HCA) by the Whitesides group [8–10]. Most recently, they have monitored the thermodynamics of binding by benzothiazole sulfonamide that has been substituted with one to four fluorines [8]. While substitution of fluoride for hydrogen does not greatly alter the ligand size, fluorination does alter the dipole moment of the ligand, which can in turn affect the active site water network. Binding of eight fluorinated ligands to HCA was examined by ITC and no substantial change in the ΔG associated with binding was found. However the ΔH values for two members of the series were found to vary by up to 5 kcal/mol. Crystallography studies found no changes in structure for either the protein or these two bound ligands. Computational WaterMap predictions of the associated entropy suggest a linkage of the entropy changes with ligand desolvation. They conclude desolvation and positioning of the active site water network both contribute to the observed enthalpy change.

An alternate solution to how proteins may affect solvent and thus the entropy associated with binding has been uncovered by the Homans group [11–15]. They study pheromone binding in mouse major urinary protein (MUP), a lipocalin. The pheromones are hydrophobic yet binding is enthalpy driven. This group has deconvoluted the various enthalpy and entropy terms associated with the Born–Haber cycle (see Table 2 in Homans Ref. [11]). As the pheromone is volatile, they can measure its partitioning between water and the gas phase to describe ligand desolvation [12]. To measure changes in the degrees of freedom associated for the protein in the bound vs the unbound state, they use NMR. Surprisingly, while residues in the active site of MUP tighten upon ligand binding, the rest of the molecule becomes more dynamic [14]. Further, the active site is not well solvated. The active site of MUP is occluded and the sock shape of the binding pocket precludes contact of any bound water in the apo enzyme with bulk water [16]. Since there is little water to remove from the active site, the desolvation penalty is minimal. (Other groups have found dry active sites, supporting this condition [16,17].) Changes in the degrees of freedom for the ligand upon binding were calculated [14,18]. The Homans group concluded that the dry active site does not contribute to the observed entropy, allowing the enthalpic signal to predominate.

One signature of changes in water structure appears to be enthalpy-entropy compensation. Results from both the Klebe and Whitesides groups support this notion, previously proposed by Ben-Naim [19], Yu and Karplus [20] and Frisch et al. [21].

To summarize, formation of water networks near hydrophobic surfaces can contribute to the observed enthalpy. In addition, dewetting of protein active sites can provide an alternate route to allowing enthalpy driven hydrophobic binding.

4. Heat capacity changes

Heat capacity describes the ability of a substance to absorb heat without a change in temperature. Previously a change in heat capacity (ΔC_p) has been correlated with changes in the accessible

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