Protein Binding

DOI: 10.1002/ange.200602227

Contribution of Ligand Desolvation to Binding Thermodynamics in a Ligand–Protein Interaction**

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Despite enormous advances in the structure determination of protein complexes, our ability to predict binding affinity from structure remains severely limited. Affinities are governed by both structure and dynamics, including solvent rearrangement. Although a number of studies have examined the contribution of water molecules in the protein binding pocket,[1–11] ligand solvation has received little attention. Herein, we examine the latter contribution in the major urinary protein (MUP), an abundant pheromone-binding protein for which the subtle recognition of a series of related compounds is essential to its biological function.[12–14] Our approach involves the experimental determination of solvation thermodynamics of relevant ligands by measuring air/water partition coefficients. These measurements are interpreted in the context of thermodynamic binding data[15] and allow the interaction thermodynamics to be resolved to a level of detail unreported for any system.

We determined experimental standard free energies of solvation for a pyrazine-derived ligand of MUP, namely 2-methoxy-3-isopropylpyrazine (IPMP). We also determined solvation thermodynamics for methylpyrazine (MP), for which data have been reported previously, to assess the validity of our approach (see the Experimental Section). In each case we found that the temperature dependence of the standard free energy of solvation is approximately linear and the value for MP at 25 °C (-24.4 ± 0.1 kJ mol^{-1}, Table 1) is very close to that obtained previously by Buttery et al. (-23.1 kJ mol^{-1}).[16] Standard enthalpy and entropy values derived from the temperature dependence of the standard free energy are given in Table 1.

Table 1: Solvation thermodynamic parameters (in kJ mol^{-1}) of pyrazine derivatives at 298 K.

Ligand	ΔG^0_{solv} [a]	ΔH^0_{solv}	$T\Delta S^0_{solv}$
MP	-24.4 ± 0.1	-50.5 ± 0.96	-26.1 ± 0.99
IPMP	-17.0 ± 0.04	-43.8 ± 8.2	-26.7 ± 8.4

[a] Errors are reported for duplicate experiments.

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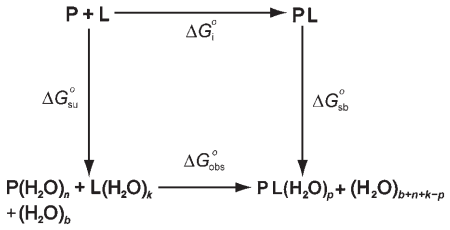
[**] This work was supported by the BBSRC (grant numbers 24/B19388 and BB/C500679/1) and by The Wellcome Trust (grant numbers 062164 and 072568).

It is convenient to represent the binding process in terms of a conventional Born–Haber cycle (Scheme 1).[17] Since G is a state function, the observed standard free energy of binding



Scheme 1. Born–Haber cycle for ligand L binding to protein P, showing the relationship between the observed free energy of binding ΔG^0_{obs}, the "intrinsic" (solute–solute) term ΔG^0_i, and the solvation free energies of unbound (ΔG^0_{su}) and bound (ΔG^0_{sb}) species.

is given by Equation (1), in which ΔG^0_i represents the "intrinsic" solute–solute contribution, and the quantity in braces contains solvation processes, that is, the standard free energies of solvation of the ligand (ΔG^0_{solL}) and the protein (ΔG^0_{solP}), which together comprise ΔG^0_{su}, and the standard free energy of solvation of the complex ΔG^0_{solPL}, which is equivalent to ΔG^0_{sb}.

$$\Delta G^0_{obs} = \Delta G^0_i + \{\Delta G^0_{sb} - \Delta G^0_{su}\} \quad (1)$$

Thus Equation (1) can be rewritten as Equation (2). A similar equation can be written for the standard enthalpy and entropy of binding.

$$\Delta G^0_{obs} = \Delta G^0_i + \{\Delta G^0_{solPL} - (\Delta G^0_{solP} + \Delta G^0_{solL})\} \quad (2)$$

We consider first ΔS^0_i. This term comprises changes in the dynamics of the protein and the ligand following association. The entropic contribution from protein degrees of freedom to the binding of IPMP to MUP determined from NMR relaxation measurements is zero within experimental error.[15] Furthermore, we have estimated the entropic contribution from the loss of translational and rotational degrees of freedom of a ligand in earlier work on the binding of an oligosaccharide to cholera toxin B-subunit.[18] Since this entropic component depends on the logarithm of the molecular mass, this loss of degrees of freedom represents an unfavorable contribution of approximately -25 kJ mol^{-1}. If the internal degrees of freedom of the ligand are assumed to be essentially "frozen" on binding, the corresponding unfavorable contribution from the two internal degrees of freedom of IPMP amount to approximately -12 kJ mol^{-1}.[19] Herein, we ignore degrees of freedom about the symmetry axis of methyl groups since rotation about the latter will not be frozen on binding. The crystallographic B factors for the ligand, which are lower than those for the protein backbone, show that the ligand degrees of freedom are indeed frozen on binding.[15] From these data together with the standard entropy of solvation of IPMP determined in the present

study, the breakdown of the standard entropy of binding is shown in Table 2.

The term $T\Delta S^0_{\text{solvPL}} - T\Delta S^0_{\text{solvP}}$ is inaccessible experimentally, but can be inferred by comparison of the sum of the above contributions with $T\Delta S^0_{\text{obs}}$. Remarkably, these data suggest that the entropic contribution from desolvation of the protein is small or zero. It has been surmised that the release of ordered water molecules from the binding pocket of a protein following ligand binding gives rise to a substantial favorable entropic contribution to binding. However, the binding pocket of MUP is suboptimally hydrated, which results in a dearth of well-ordered water molecules.[11,20] Thus, the characteristic entropy-driven thermodynamic signature of "hydrophobic binding" is not realized, since the favorable entropic contribution arising from ligand desolvation is insufficient to overcome the unfavorable contribution from "freezing" the protein and ligand degrees of freedom on binding.

Table 2: Breakdown of the thermodynamics of binding of IPMP to MUP at 298 K.

Description	Value [kJ mol^{-1}]	Description	Value [kJ mol^{-1}]
$T\Delta S^0_i$		ΔH^0_i	
protein degrees of freedom	-0.8 ± 3.8[a]	new solute–solute interactions	ca. -76
ligand degrees of freedom $-T\Delta S^0_{\text{solvL}}$	ca. -37	changes in ligand/protein structure $-\Delta H^0_{\text{solvL}}$	ca. 0
ligand desolvation $T\Delta S^0_{\text{solvPL}} - T\Delta S^0_{\text{solvP}}$	$+26.7 \pm 8.4$	ligand desolvation $\Delta H^0_{\text{solvPL}} - \Delta H^0_{\text{solvP}}$	$+43.8 \pm 8.2$
desolvation of protein/complex $T\Delta S^0_{\text{obs}}$	$+0.4 \pm 9.2$	desolvation of protein/complex ΔH^0_{obs}	-12.3 ± 8.4
observed entropy	-10.7 ± 0.5[a]	observed enthalpy	-44.5 ± 0.4[a]

[a] From reference [15].

The term ΔH^0_i comprises changes in the structure of the protein and the ligand, together with the formation of new solute–solute nonbonded interactions following association. There are no significant changes in the structure of MUP on binding either pyrazine-derived or alternative surrogate ligands.[11,20] Moreover, quantum chemical calculations at the 6-31G* level indicate that IPMP is bound in a low-energy conformation that is energetically indistinguishable from the global minimum-energy conformation. Thus ΔH^0_i can be equated with the formation of new solute–solute interactions. In a recent study on the binding of aliphatic primary alcohols to MUP, we concluded that binding was driven by favorable solute–solute interactions.[20] The linear relationship between ΔH^0_i and the length of the carbon chain enabled an estimate of the contribution from a methylene group (-8.4 ± 0.2 kJ mol^{-1}). Assuming this contribution is proportional to the van der Waals surface area of the ligand, we estimate $\Delta H^0_i \approx -76$ kJ mol^{-1} for the association of IPMP with MUP. Given a desolvation enthalpy $-\Delta H^0_{\text{solvL}} = +43.8 \pm 8.2$ kJ mol^{-1} for IPMP as above together with $\Delta H^0_{\text{obs}} = -44.5 \pm 0.4$ kJ mol^{-1},[15] the contribution from desolvation of the protein on formation of the complex $\Delta H^0_{\text{solvPL}} - \Delta H^0_{\text{solvP}}$ can be estimated as approximately -12.3 ± 8.4 kJ mol^{-1} (Table 2).

In summary, the favorable entropic contribution arising from desolvation of IPMP on binding to MUP is insufficient to overcome the strongly unfavorable component arising from "freezing" of ligand degrees of freedom. These data thus explain the unfavorable global entropy of binding $T\Delta S^0_{\text{obs}}$, which is paradoxical in view of the hydrophobic nature of the interaction. The enthalpic contribution to binding is more difficult to deconvolute with accuracy, but the data above indicate that the unfavorable enthalpic contribution arising from desolvation of the ligand is offset both by favorable solute–solute dispersive interactions,[20] with a contribution from desolvation of the protein binding pocket.[11]

Experimental Section

A microfuge tube containing ligand solution (1.5 mL) of known concentration (0.4–4 mM) in water was placed in a closed glass bottle (25 L) at a constant temperature (6, 13, and 20 °C for IPMP and 13, 20, and 27 °C for MP). Trial experiments were used to find the appropriate equilibration period, which ranged from 7 to 14 days. The pressure was kept constant by means of a conventional 50-mL glass gas syringe connected through the screw-top lid. Initial and equilibrated ligand concentrations were determined from optical-density measurements at 220 nm ($\varepsilon_{220} = 144\,\text{M}^{-1}\text{cm}^{-1}$ for MP and $4590\,\text{M}^{-1}\text{cm}^{-1}$ for IPMP). The mass of each microfuge tube was recorded before and after the equilibration period to account for evaporation. The equilibrium constant K for $L_{air} \rightleftharpoons L_{sol}$ (L = ligand) in this experiment is given by Equation (3).

$$K = \frac{[L]_{sol}}{[L]_{gas}} = \frac{[L]_{sol}}{[L]_0\, m_0 - [L]_{sol}\, m_{sol}} V_{bottle}\, \rho_{water} \quad (3)$$

In Equation (3), $[L]_{sol}$ and $[L]_{gas}$ are the ligand concentrations in solution and gas phase, respectively, after equilibration, $[L]_0$ is the initial ligand concentration, m_0 and m_{sol} are the masses of the initial and equilibrated solutions, V_{bottle} is the volume of the bottle with syringe attachment, and ρ_{water} is the density of water at the relevant temperature.

Standard free energies of solvation of each ligand were calculated according to Equation (4).

$$\Delta G^0_{solv} = -R\,T \ln K \quad (4)$$

Standard enthalpies and entropies of solvation were determined from plots of the standard free energy versus temperature in a conventional van't Hoff analysis.

Global binding thermodynamics data were obtained from a previous report.[15]

Received: June 3, 2006
Published online: August 14, 2006

Keywords: noncovalent interactions · solvation · thermodynamics

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