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Heat capacity effects in protein folding and ligand binding: a re-evaluation of the role of water in biomolecular thermodynamics

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Abstract

Large "anomalous" heat capacity (ΔC_p) effects are a common feature of the thermodynamics of biomolecular interactions in aqueous solution and, as a result of the improved facility for direct calorimetric measurements, there is a growing body of experimental data for such effects in protein folding, protein–protein and protein–ligand interactions. Conventionally such heat capacity effects have been ascribed to hydrophobic interactions, and there are some remarkably convincing demonstrations of the usefulness of this concept. Nonetheless, there is also increasing evidence that hydrophobic interactions are not the only possible source of such effects. Here we re-evaluate the possible contributions of other interactions to the heat capacity changes to be expected for cooperative biomolecular folding and binding processes, with particular reference to the role of hydrogen bonding and solvent water interactions. Simple models based on the hydrogen-bonding propensity of water as a function of temperature give quantitative estimates of ΔC_p that compare well with experimental observations for both protein folding and ligand binding. The thermodynamic contribution of bound waters in protein complexes is also estimated. The prediction from simple lattice models is that trapping of water in a complex should give more exothermic binding ($\Delta\Delta H$ −6 to −12 kJ mol^{-1}) with lower entropy ($\Delta\Delta S^0 \approx -11$ J mol^{-1} K^{-1}) and more negative ΔC_p (by about −75 J mol^{-1} K^{-1}) per water molecule. More generally, it is clear that significant ΔC_p effects are to be expected for any macromolecular process involving a multiplicity of cooperative weak interactions of whatever kind.

Keywords: Protein folding; Protein–ligand; Protein–protein interaction; Hydrogen bond; Hydrophobic interaction

1. Introduction

The thermodynamics of biomolecular processes can now be measured routinely and accurately using direct calorimetric methods and, with the growing accumulation of reliable data on a wide range of systems, the time is ripe for a re-evaluation of what the data might have in common and what this tells us about the underlying forces responsible for controlling the stability and interaction of biological (macro)molecules. Here I wish to summarize briefly the current situation regarding heat capacity changes and related thermodynamic quantities in relation to the conventional interpretations based on hydrophobic interaction. I will show that, despite the enormous success of the hydrophobic concept, so-called "anomalous" heat capacity effects are in reality much more ubiquitous than has previously been assumed, and are not restricted solely to the interactions between non-polar groups in water. Indeed, both experimental data and theoretical considerations show that changes in heat capacity quantitatively similar to those seen in biomolecular processes are an inevitable consequence in any system involving cooperative transitions of a multiplicity of weak interactions—regardless of the nature of the interaction.

1.1. Protein folding

Differential scanning calorimetry (DSC) measurements on the thermal unfolding of simple globular proteins in solution typify the general observation that disruption of

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biomolecular assemblies results in an increase in heat capacity (positive ΔC_p). This is illustrated in Fig. 1, which shows how the heat capacity of unfolded polypeptide is generally higher than the compact native state from which it is derived. As a natural thermodynamic consequence, the higher the temperature of the unfolding transition, the more heat energy is required to unfold the protein. This is also shown by the data in Fig. 1, where the T_m for the unfolding transition has been varied by adjusting the pH of the sample. Not only does the baseline shift during the transition, but the magnitude of the transition endotherm increases with T_m. Such observations, coupled with structural information about the disposition of non-polar amino acid sidechains in globular protein structures, were among the first to support the hypothesis that protein folding is driven by hydrophobic interactions involving the burial of hydrophobic groups [1–3].

Detailed analysis of such experimental data gives the temperature dependence of the thermodynamic parameters that control folding stability. Such data (illustrated in Fig. 2) show how the very strong temperature dependencies of both ΔH_{unf} and $T \cdot \Delta S_{unf}$ compensate to give relatively much smaller changes in ΔG_{unf}. This is just one example of the widely reported phenomenon of entropy–enthalpy compensation, a natural consequence of weakly interacting systems itself [4,5].

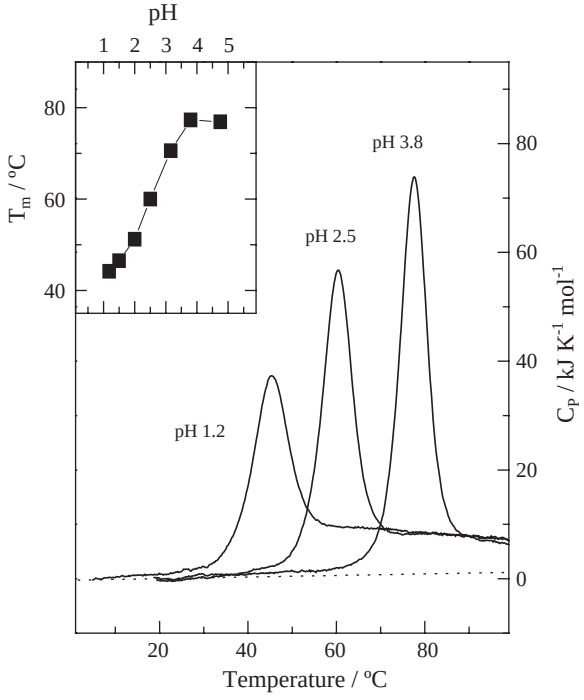


Fig. 1. Typical DSC data for the unfolding of a small globular protein (lysozyme) in solution at various pH values. The insert shows the variation in mid-point unfolding temperature (T_m) as a function of pH. The increase in area under each endotherm with higher T_m, and the higher heat capacity baselines after the unfolding transitions, are both indications of the significant positive ΔC_p commonly associated with such processes. (Adapted from [5].)

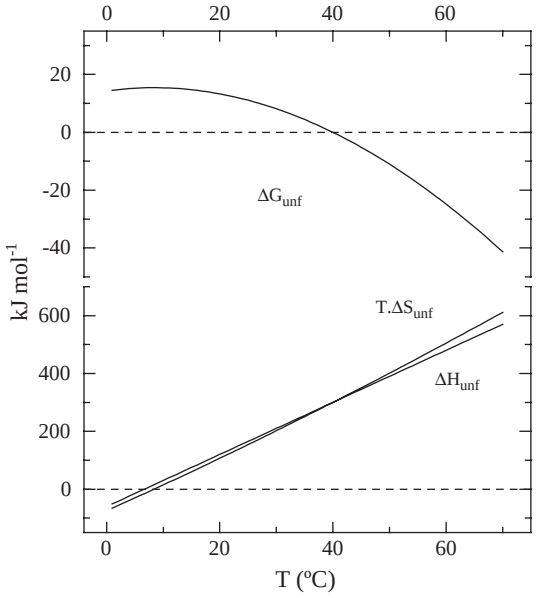


Fig. 2. Typical thermodynamic data for reversible two-state unfolding of a globular protein, such as derived from analysis of DSC data of the kind illustrated in Fig. 1.

1.2. Protein aggregation

Nor are such heat capacity changes just a property of native protein (un)folding, since they are also seen in much less specific condensation processes such as protein aggregation. For example, Fig. 3 shows DSC data for the thermal aggregation of insulin in solution under conditions

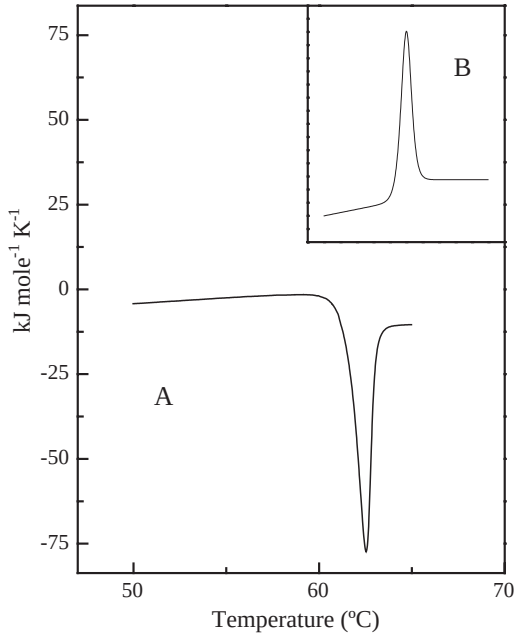


Fig. 3. DSC thermogram of the thermal aggregation of insulin in solution (A). Under these conditions (5 mg ml^{-1} insulin, in 0.1 M KCl/HCl pH 2.0; DSC scan rate=5 °C h^{-1}), the unfolded protein undergoes a kinetically limited exothermic nucleation-growth transition to a condensed protein aggregate similar to amyloid fibrils [6,7]. Inset (B) shows the more typical thermogram seen for protein unfolding (without aggregation).

where it forms amyloid-like fibrils upon thermal denaturation. In this case the DSC thermogram shows a *negative* ΔC_p effect, similar in magnitude but opposite in sign to the positive ΔC_p seen for unfolding without aggregation. This indicates that heat capacity changes reflect general changes in polypeptide environment, and that condensed/closely packed polypeptides – either as a result of specific folding or less-specific aggregation – have a lower heat capacity than the unraveled chain exposed to water.

1.3. Protein–protein interactions

Large ΔC_p effects are also seen in many protein–protein interactions. In some cases, such as illustrated in Fig. 4 for the binding of a small protein domain at the dimer interface of the E3 subunit of pyruvate dehydrogenase from *B. stearothermophilus* [8–10], the temperature dependence of the observed heat of binding is so great that it can even change sign over a relatively small temperature range. Again, the entropy component ($T \cdot \Delta S^\circ$) of this interaction shows a similar compensating dependence, such that the overall Gibbs free energy of the interaction (ΔG°) scarcely changes. (We have termed this phenomenon "thermodynamic homeostasis" [5] since, whatever its origin, it might have evolutionary significance for organisms during adaptation to hostile environments.)

ΔC_p for the E3/di-domain interaction depends somewhat on ionic strength [8], but is around -1.9 kJ mol^{-1} K^{-1} in 50 mM phosphate buffer, pH 7.4 (Fig. 4). Prevailing wisdom is that ΔC_p effects may be correlated with changes in polar and non-polar exposed surface areas (ΔASA). The crystal structure of this complex [11] gives a calculated total surface area buried at the protein–protein interface ΔASA_{tot}=1125 Å^2, with a polar/non-polar ratio, $\Delta ASA_p/\Delta ASA_{np}$=0.67. Using published algorithms [12–14] that relate ΔC_p to changes in accessible surface areas (ΔASA), we would predict ΔC_p=−640 to −780 J mol^{-1} K^{-1} (different algorithms have different coefficients), compared to the measured ΔC_p=−1900 J mol^{-1} K^{-1}. Such discrepancy is disturbing, and suggests that ΔC_p/ΔASA correlations should be treated with caution when applied out with the limited data sets from which they were derived. Significantly, such a large ΔC_p as observed here would normally be taken to indicate a dominant role for hydrophobic interactions, yet the crystal structure, supported by mutagenesis studies [9,10], shows that the key interactions are predominantly electrostatic in this instance. Similar discrepancies between measured ΔC_p and predicted surface area burial have been reported recently for protein–DNA interactions [15] and for drug–protein and inhibitor binding [16,17].

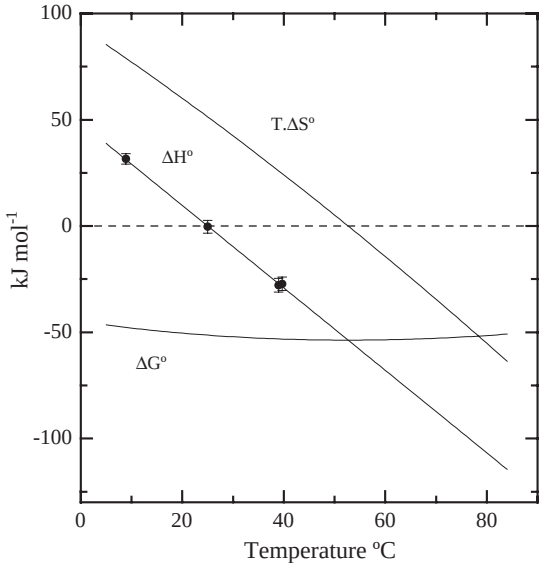


Fig. 4. Thermodynamic data for protein–protein interactions: in pyruvate dehydrogenase multi-enzyme complexes—E3/di-domain interaction [8]. These data were determined directly using isothermal titration calorimetry (ITC). The slope of ΔH vs. T gives $\Delta C_p \sim -1.9$ kJ mol^{-1} K^{-1} for this association process.

1.4. Protein–ligand interactions

The binding to proteins of seemingly very hydrophilic molecules also shows significant ΔC_p effects. This has been demonstrated in an ITC study of the binding of specifically engineered tri-saccharides to the lectin, concanavilin-A (ConA) summarized in Fig. 5 [18].

These two tri-saccharide ligands (Fig. 5) were specifically designed and constructed [18] to probe the effects of displacement of a single water molecule identified in structural studies of ConA–ligand complexes. The water molecule trapped in the ConA/ligand **1** complex is displaced from the active site by the additional hydroxyethyl group in ligand **2**. Two points to note here: (a) sugars are water-soluble molecules, not normally considered to have any substantial hydrophobic content, yet their heats of binding to ConA show significant temperature dependence/ΔC_p behaviour; (b) incorporation of the larger hydroxyethyl group and consequent water displacement with ligand **2** reduces both the heat and entropy loss on binding. It also reduces the magnitude of the (negative) ΔC_p effect. This is surprising since, all other things being equal, one might anticipate that the slight increase in hydrophobicity of ligand **2** compared to ligand **1** (from the –CH_2–CH_2– group) should have the opposite effect on ΔC_p.

These are just a few examples of the ubiquitous nature of ΔC_p effects. The classic explanation, stemming from the influential paper by Kauzmann [1], is that such effects are a consequence of the unusual properties of solvent water and the way in which water molecules – having a greater affinity for each other in the liquid phase – tend to repel non-polar molecules and groups: the hydrophobic effect. Simple empirical observations on model compounds in water (e.g. [19]) suggest that the thermodynamics of such interactions should show complicated temperature dependence. For example, the solubility of non-polar compounds in water – contrary to expectation – initially *decreases* with increase in

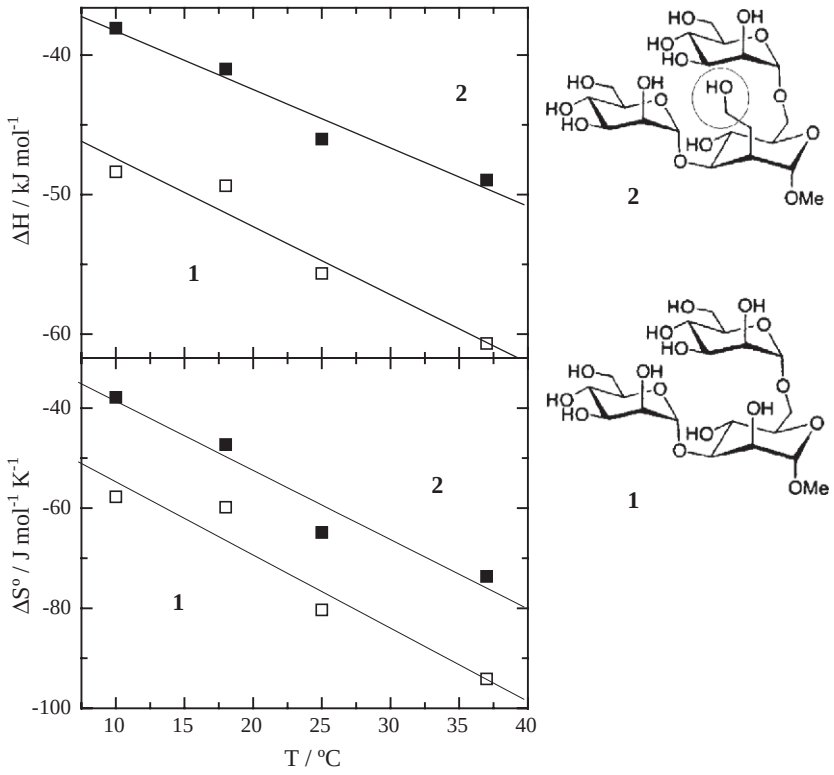


Fig. 5. Enthalpy (ΔH) and entropy (ΔS^0) data for the binding of tri-saccharides **1** (open symbols) and **2** (closed symbols) to ConA over a range of temperatures. Linear regression of the ΔH data gives: $\Delta C_p(1)=-460$ (±20) J mol^{-1} K^{-1} and $\Delta C_p(2)=-385$ (±55) J mol^{-1} K^{-1}.

temperature (hydrophobic association endothermic at low T) before behaving more rationally at higher temperatures (hydrophobic association exothermic at higher T). Consequently, the association of hydrophobic groups in water should have a negative ΔC_p. Such observations have been variously described in terms of the structural re-organization ("ice-bergs", "clusters", "clathrates", etc.) of solvent water molecules around hydrophobic groups but, regardless of the detailed explanation, hydrophobic interactions are expected to show this characteristic ΔC_p "signature". But is it unique? Can other interactions show similar effects? We have seen in some of the examples described above how sometimes it is difficult to reconcile observed ΔC_p effects with hydrophobicity alone.

It is worth noting that Kauzmann [1] even in his seminal 1959 review recognized that the thermodynamic signature of hydrophobic interactions was not unique, in particular that both hydrophobic and electrostatic interactions ("salt linkages") in water are expected to be endothermic and entropy-driven, at least at low temperatures. This has not always been appreciated in subsequent literature.

2. Positive ΔC_p is normal for cooperative order–disorder transitions, especially in H-bonded lattices

A clue to the ubiquitous nature of ΔC_p effects comes from examination of the heat capacity changes that take place upon melting of pure crystalline solids, particularly those involving weaker non-covalent lattice forces [20]. This is summarized in Fig. 6, which shows heat capacity

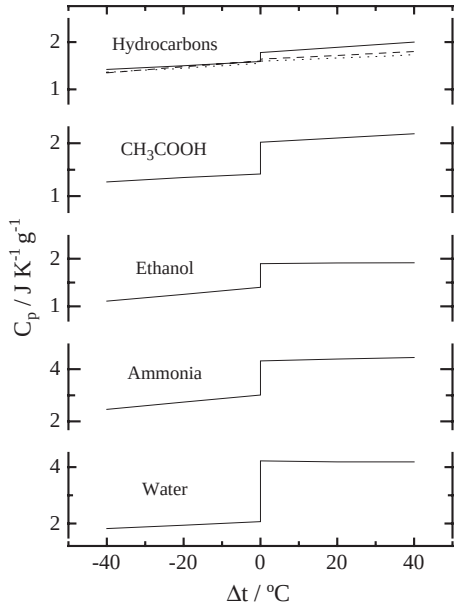


Fig. 6. (Adapted from [20]) Examples of absolute heat capacities for pure solid and liquid compounds as a function of temperature, plotted with respect to the normal melting point ($\Delta t=T-T_m$). Empirical data are taken from [21]. For simplicity, the heat capacity increments at the melting points ($\Delta t=0$) omit the large C_p discontinuity associated with heat of melting at this point.

data taken from the older literature [21] for simple compounds above and below their melting points.

This increase in heat capacity during order–disorder (solid–liquid) transitions is to be expected. The heat capacity of any substance reflects its ability to absorb heat energy without change in temperature. For a (crystalline) solid, heat energy goes mainly into lattice vibrations, but in the more disordered (liquid) state, there are additional translational and rotational degrees of freedom that can soak up thermal energy. In addition, for those liquids – like water and other polar compounds – which retain some degree of hydrogen-bonded structure, the breakdown of this structure with increase in temperature requires additional heat energy, and consequently increases the heat capacity even further.

So what have these classical, macroscopic phase transitions got to do with processes on a much smaller scale, as in protein unfolding, for example? We know that globular proteins are quite compact structures in their native folded state, comprising an ordered structure in which most (if not all) hydrogen-bonded interactions are satisfied. But they can also undergo a cooperative thermal (or chemical/denaturant) unfolding process to a much more disordered state in which the flexible polypeptide chain has many more degrees of freedom. In some respects therefore, apart from differences in size, we might view the process of protein unfolding as akin to the melting of an ordered polymeric substance. Also, the effect of small size should only be reflected in the size of the cooperative unit and the shape (breadth) of the thermal "melting" transition.

Consider the thermodynamics of melting of a snowflake. A small ice crystal about the size of a small globular protein, 30 Å diameter, would contain around 500 water molecules. Ignoring surface effects for simplicity, and using standard parameters for the melting of ice under normal conditions, cooperative melting of such a crystallite would have a transition enthalpy (at 0 °C) corresponding to ~+3000 kJ mol^{-1} and a heat capacity increment, ΔC_p (ice→water), of about +20 kJ mol^{-1} K^{-1} (where the cluster is taken as the cooperative unit). The calculated DSC thermogram for such a transition is shown in Fig. 7. Although it is technically difficult to do the actual experiment with ice microcrystals of this size, it is clear that the DSC profile would have features in common with those seen for the thermal unfolding of proteins. In particular, although the actual transition for an ice crystal is somewhat sharper than for protein (because of the relatively larger heat of melting of ice compared to protein), the heat capacity increment is similar if not larger (cf. Fig. 1 for protein data). The melting of ice involves the cooperative breakdown of the tetrahedral hydrogen-bonded lattice and, naturally, involves no trace of hydrophobic interaction. Yet it shows the same thermodynamic signature.

Such considerations lead us to the conclusion that, in the case of protein unfolding at least, the observed increase in heat capacity is a natural consequence of the general properties of matter, and not necessarily diagnostic of hydrophobic exposure alone. More detailed theoretical calculations based on the statistical mechanics of cooperative lattice models have been presented elsewhere [20] and support this both qualitatively and quantitatively.

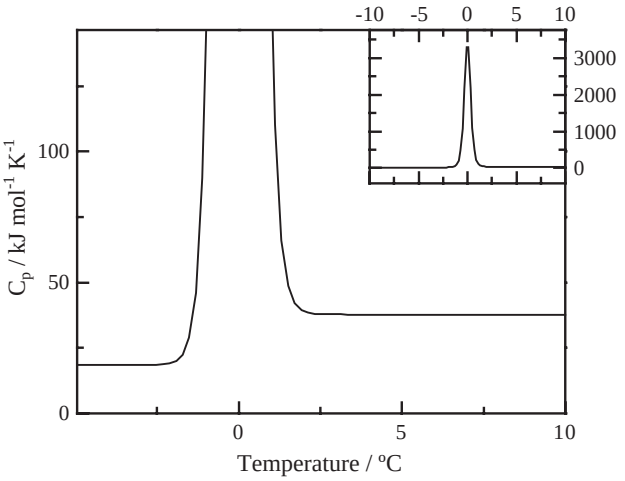


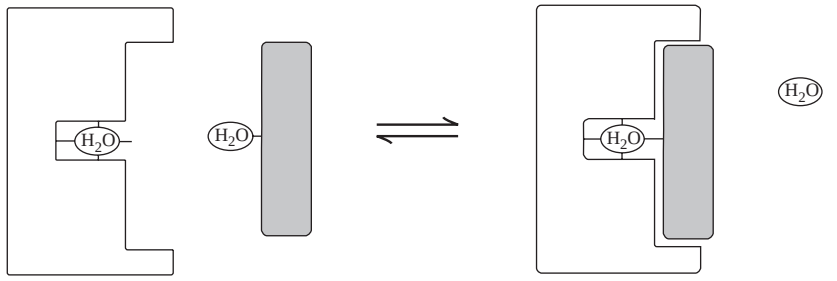
Fig. 7. Simulated DSC thermogram for melting of a microscopic ice crystal of size similar to a globular protein. Calculated assuming a cluster of 500 water molecules, using the following standard thermodynamic parameters: ΔH (ice-water, 0 °C)=334 J g^{-1} (80 cal g^{-1})≡6.01 kJ mol^{-1} (1.44 kcal mol^{-1}); ΔS (ice-water, 0 °C)=22.0 J mol^{-1} K^{-1} (5.26 cal mol^{-1} K^{-1}); C_p (ice, 0 °C)=2.05 J g^{-1} K≡36.9 J mol^{-1} K^{-1}; C_p (water, 0 °C)=4.2 J g^{-1} K≡75.6 J mol^{-1} K^{-1}; ΔC_p (ice-water, 0 °C)=38.7 J mol^{-1} K^{-1} (9.25 cal mol^{-1} K^{-1}).

3. Protein–carbohydrate binding: the thermodynamics of trapped waters

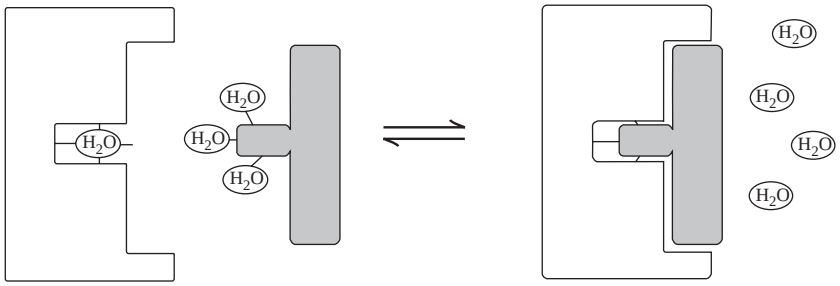
We can extend these arguments to explore protein–ligand interactions, and in this section we will exemplify this by an analysis of the protein–carbohydrate interaction described above.

Determining the role of solvation/hydration changes in the thermodynamics of protein–ligand interactions is complicated because of the need to take account of *all* the waters in the system, not just those that might be most apparent in well-resolved crystal structures. A generic approach based on the thermodynamics of hydrogen-bonded lattices has recently been successful in reproducing, both qualitatively and quantitatively, the enthalpy and heat capacity changes for unfolding of globular proteins in water [20], and such an approach might be instructive here.

We start with the premise that every one of the (N_w) water molecules in the system may, at least in principle, form four hydrogen bonds with neighbouring donor/acceptor groups, based on the tetrahedral ice/water lattice. In bulk water, each of these four bonds is shared between two molecules, so each bond counts a maximum of ½ in the overall H-bond count (i.e. 2 per bulk water molecule). In practice, of course, because of thermal fluctuations and the nature of the liquid state, not all bonds will be made at any one time, and we must take account of the fractional occupancy (f) of H-bonds. Water molecules bound to protein sites may make any



Scheme 1



Scheme 2

Fig. 8. Sketch showing the way in which two different ligands (shaded) may bind in a protein active site with entrapment (Scheme (1)) or displacement (Scheme (2)) of a water molecule.

number (x=0,1,2,3,4) of bonds to the protein, with any remaining donor/acceptor sites taken up by (fractional) occupancy by surrounding waters. These x bonds to water will be replaced by bonds to donor/acceptor sites on the ligand molecule in the complex. These ligand sites will themselves be (fractionally) solvated in the free state.

Consider what might happen with water molecules in the very schematic view of ligand binding possibilities shown in Fig. 8 (Schemes 1 and 2), taking into account the fluid dynamic nature of the system in which water molecules will interact (at least partially) with both themselves and with –OH and other H-bonding groups on both ligand and protein. In Schemes 1 (corresponding roughly to what might be occurring with the natural ligand **1**), binding of (partially) solvated ligand to (partially) solvated protein active site results in displacement of one of the solvated waters, but specific entrapment of the other. By contrast, in Scheme 2 (corresponding to ligand **2** in Fig. 5), the bulky polar side chain, engineered to fit the water cavity, results in displacement of both waters in the binding process. Ignoring all other interactions, these two schemes differ in the extent of hydrogen bond changes during the binding process that might be reflected in the observed thermodynamics.

In order to quantify the possible effects, define the following ("p"=protein, "l"=ligand, "w"=water):

N_w the total number of water molecules (per protein) in the system
x the number of protein–water H-bonds made in the specific water site (x=0–4)
h_{pw} enthalpy of formation of a protein–water hydrogen bond in the active site cavity
h_{lw} enthalpy of formation of a ligand–water hydrogen bond
h_{ww} enthalpy of formation of a water–water hydrogen bond
h_{pl} enthalpy of formation of a protein–ligand hydrogen bond (in Scheme 2)

Except in special circumstances, the hydrogen-bonding network involving solvent water, protein and ligand will be quite dynamic at normal temperatures, and we must define terms that describe the (temperature dependent) partial occupancy of hydration sites:

f_{pw} fractional occupancy of the protein–water site in the free enzyme
f_{lw} fractional occupancy of the ligand–water site in the free ligand
f_{ww} fraction of water–water bonds in bulk solvent
f_{pl} fractional occupancy of "trapped" water site in the protein–ligand complex (assume f_{pl}=1 in the following model)

Considering separately the total numbers of H-bonds in the bound/free states of Schemes (1) and (2) leads to estimates of the hydrogen bond contributions (including water) to the enthalpies of complex formation in each case:

$$\Delta H(1) = \left(4 - x f_{pw}\right) h_{pw} - (4 - x) f_{lw} h_{lw} \qquad \text{(Scheme1)}$$

$$\Delta H(2) = x.\left(h_{pl} + f_{ww} h_{ww} - f_{pw} h_{pw} - f_{lw} h_{lw}\right) \qquad \text{(Scheme2)}$$

and the difference in binding enthalpies between the two models is:

$$\Delta\Delta H = \Delta H(2) - \Delta H(1)$$
$$= x\left(h_{\mathrm{pl}} + f_{\mathrm{ww}} h_{\mathrm{ww}}\right) + (4-2x) f_{\mathrm{lw}} h_{\mathrm{lw}} - 4h_{\mathrm{pw}}$$

In order to proceed to numerical estimates, we must make some further approximations. For simplicity, and in the absence of detailed information, let us assume that all H-bonds have equal heats of formation, h. Let us further assume that, with the exception of the trapped water molecule in Scheme (1), the fractional water occupancies also have similar values, f.

With these approximations (which may be relaxed when more detailed information is available), we get for the enthalpy changes associated with the two modes of binding:

$$\Delta H(1) \approx 4h(1-f)$$

$$\Delta H(2) \approx xh(1-f) \text{ and}$$

$$\Delta\Delta H = \Delta H(2) - \Delta H(1) \approx -h(4-x)(1-f)$$

$$\approx -h(1-f) \qquad \text{if } x = 3, \text{for example.}$$

Bearing in mind that h is defined as an exothermic heat of formation, with negative sign, and that f is a fraction somewhere between zero and one, this indicates that this contribution to ligand binding is exothermic (ΔH negative) in both cases. Furthermore, this would predict that binding of ligand **1** (with entrapment of water) should be more exothermic than ligand **2**, as indeed we observe (Fig. 5), although the absolute values would depend on choice of x.

Actual numerical values are more difficult to obtain and, though they have been considered in more detail elsewhere [20], only rough estimates are possible. Typical values of h will likely fall in the range −12.5 to −25 kJ mol^{-1}, and f is likely to be of order 50% at normal temperatures. Consequently, a binding enthalpy difference of 6 to 12 kJ mol^{-1} between compounds **1** and **2** might not be unreasonable.

The temperature dependence of binding enthalpies ($\Delta C_{\mathrm{p}} = \partial\Delta H/\partial T$) in this model arises from the decrease in fractional occupancies, f, with increase in temperature. Consequently:

$$\Delta C_{\mathrm{p}}(1) \approx -4h \cdot \partial f/\partial T \text{ and}$$

$$\Delta C_{\mathrm{p}}(2) \approx -xh \cdot \partial f/\partial T$$

Since both h and $\partial f/\partial T$ are negative quantities (f decreases with increase in temperature), ΔC_{p} is predicted to be also negative in both instances, with ΔH getting more exothermic with increased temperature—as we (and others) observe. For more quantitative comparisons, we need to make a reasonable estimate of the crucial quantity, $h \cdot \partial f/\partial T$. Assuming, as we have done here in the approximate versions of these expressions, that all water H-bonds behave similarly, it follows that the heat capacity of liquid water should itself be related to the temperature dependence of f, on the assumption that the major contribution to the anomalously high heat capacity of water comes from the energy required to break intermolecular H-bonds (reduce f) as the temperature is increased. Consequently [20], $h \cdot \partial f/\partial T \approx C_{\mathrm{p,water}}$, where $C_{\mathrm{p,water}}$ (=1 cal g^{-1} K^{-1}=4.184 J g^{-1} K^{-1}≡75 J K^{-1} mol^{-1}) is the standard isobaric heat capacity of liquid water. With $h \cdot \partial f/\partial T \approx 75$ J K^{-1} mol^{-1} as a reasonable order of magnitude estimate:

$$\Delta C_{\mathrm{p}}(1) \approx -300 \text{ J } \mathrm{K}^{-1} \text{ } \mathrm{mol}^{-1} \text{ and}$$

$$\Delta C_{\mathrm{p}}(2) \approx -75x \text{ J } \mathrm{K}^{-1} \text{ } \mathrm{mol}^{-1}$$

$$\approx -225 \text{ J } \mathrm{K}^{-1} \text{ } \mathrm{mol}^{-1} \text{ for } x = 3$$

Bear in mind that we have ignored all other interactions taking place between protein and ligand, so the absolute values of ΔH and ΔC_{p} will not be directly comparable to experiment. However, the model predicts that ΔC_{p} for binding of ligand **2** should have a less negative ΔC_{p} (by about 75 J K^{-1} mol^{-1}) than ligand **1**. This is identical to the experimentally observed difference in ΔC_{p} for the tri-saccharide ligands binding to ConA (Fig. 5, [18]) though, bearing in mind the rather large experimental uncertainties, such close agreement is probably fortuitous. Nonetheless, the observed trends are all in the predicted direction and the magnitudes are comparable.

Similar arguments may be applied to determine the entropic contributions coming from solvated/trapped waters. Without going into details, but following the procedures of Cooper [20], one obtains:

$$\Delta\Delta S = \Delta S(2) - \Delta S(1) \approx -s(4-x)(1-f)$$

where s is the entropy of formation of any hydrogen bond, expected to be negative due to the loss of degrees of freedom entailed. Consequently, ΔS for binding of **2** should be less negative than **1**, again as observed (Fig. 5). We can only speculate on possible numerical values for s, but an order-of-magnitude estimate might be the entropy of melting of ice (see Fig. 7) which gives $s \approx -22$ J mol^{-1} K^{-1}. For x=3, this would give $\Delta\Delta S \approx -11$ J mol^{-1} K^{-1} as the contribution form one trapped water molecule in the ConA/tri-saccharide complex, compatible with the observed data (Fig. 5). Similar estimates have been made elsewhere using different approaches on different systems [22,23].

Interestingly, this analysis of the role of water will apply to *any* water displacements that might take place during ligand binding, not just those involved in a specific cavity. Consequently, we might anticipate, for each water site that becomes unavailable in the complex, a ΔH

contribution of maybe −6 to −12 kJ mol^{-1} , $\Delta S^0 \approx -11$ J mol^{-1} K^{-1}, and a ΔC_p of around −75 J K^{-1} mol^{-1} or greater, depending on the number of hydrogen bonds that each water molecule makes with the protein. These numbers are not inconsistent with the gross thermodynamic values seen for mono- and tri-saccharide ligands [24–27], and with the range of values seen more generally in a variety of interactions [17].

4. Conclusions

- "Anomalous" heat capacity effects are ubiquitous, and are a natural property of any transition or binding process involving a multiplicity of weak interactions—not just hydrophobics.
- Simple estimates based on cooperative lattice models give thermodynamic parameters comparable to observation.
- This may require re-evaluation of the role of hydrophobic interactions.
- In general it can be shown that large ΔC_p effects are to be expected in any system comprising multiple, cooperative, weak (ca. kT) interactions.
- This does not imply that Kauzmann was "wrong"—simply that there may be other, equally valid contributions.
- In actual systems, all these effects may be involved at any one time, and the relative proportions of the different kinds of interaction might vary with experimental conditions.

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