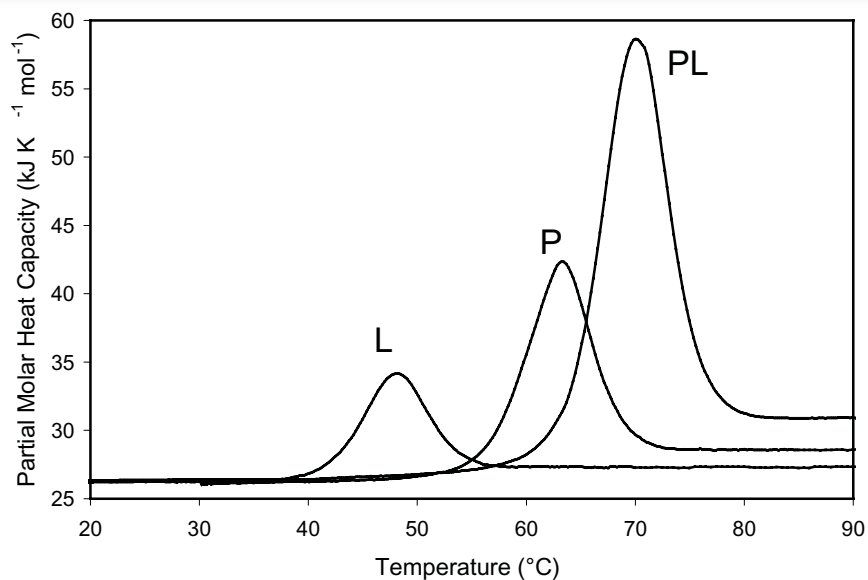


Characterizing Protein/Ligand Binding by DSC



DSC quickly establishes if ligand (L) binding to a protein (P) gives a stabilized complex (PL).

Like ITC, DSC is a valuable approach for studying binding between a biological macromolecule and a ligand such as another biopolymer or a drug. Unlike ITC, DSC allows the thermodynamics that drive binding to be correlated, at least to a degree, with conformational changes in the macromolecule caused by the binding reaction. DSC is particularly useful in characterizing very tight binding interactions.

Document No. 20111020706
February 2006

All proteins are capable of recognizing and binding specific molecules such as other proteins, cofactors, prosthetic groups or drugs. Efforts to understand the mechanisms controlling selective binding were initially prompted by the realization that recognition and binding are universal features of all biochemical processes. These efforts have intensified with the awareness that knowledge-based drug design requires not only high-quality structural data on both the protein and the drug candidate, but also a quantitative understanding of the thermodynamics driving binding. A ligand will bind to a protein (or other macromolecule) only if the resulting complex is more stable than the original, non-liganded protein. Binding can occur to the native, folded protein (stabilizing the native state), or it can bind preferentially to the denatured protein, in which case the ligand will destabilize the native protein. In either case, binding triggers changes in intramolecular and intermolecular interactions, and in the dynamics of both the protein and the ligand. Since the degree of stabilization or destabilization of the native protein depends on the magnitude of the binding energy, comparison of the stability of the complex with the stability of the ligand-free protein allows the binding energy to be estimated.

Differential scanning calorimetry (DSC) is particularly suited to studying the thermodynamics controlling conformational transitions in macromolecules such as proteins. As explained in CSC's overview note entitled 'Life Science Applications of DSC', DSC is generally used to measure the partial molar heat capacity of a protein over a temperature range of approximately 80 °C. If a ligand binds preferentially to the native state of the protein, the temperature at which the protein-ligand complex denatures will be higher compared to the temperature at which the free protein unfolds. DSC thus provides a direct measure of whether ligand binding to a protein is stabilizing or destabilizing, and so can complement studies of binding equilibria obtained by isothermal titration calorimetry (ITC). For a discussion of the utility of ITC in binding studies, please see CSC's application note entitled 'Characterizing protein/ligand binding by ITC'.

Assessing ligand binding by DSC

Proteins are large and flexible, and constantly sample the continuum of conformational states from

partially folded to fully native. The native state itself is not one conformation, but a rapidly-changing ensemble of closely related structures (Ringe and Petsko, 1985; Palmer 1997). The binding between a flexible protein and a small ligand therefore produces a complex energy profile involving many favorable and unfavorable contributions. The thermodynamic assessment of a binding event is further complicated by the fact that ligands such as drug molecules often contain hydrophobic moieties which interact with hydrophobic patches on the surface (or in the binding crevice) of the protein. Water is more ordered adjacent to hydrophobic surfaces (Shinoda, 1977); when the protein and ligand bind and hydrophobic surfaces interact, bound water at these surfaces is transferred into the bulk solvent. Since the free energy of the entire system must decrease if binding is to occur, these solvent effects must be accounted for in addition to the energy changes resulting from direct noncovalent interactions between the protein and ligand (Jelesarov and Bosshard, 1999).

ITC is the most direct approach for assessing binding interactions: several rapid ITC experiments can provide a direct determination of the binding constant, the stoichiometry of binding, the enthalpy and entropy of the reaction and the change in heat capacity due to binding. However, as noted in the CSC ITC application note on binding studies, the most accurate binding constant measurements are obtained for reactions with medium to tight binding. Manipulation of the experimental conditions allows very weak and very tight association constants to be determined by ITC, but an independent assessment of K_a is advantageous in these cases. Since DSC compares the extent to which the ligand/protein complex is stabilized towards thermal denaturation compared to the free protein and ligand, DSC allows determination of binding energies for very tightly associated complexes, or complexes which equilibrate very slowly (minutes to hours) and thus are not compatible with the ITC timeframe (seconds) (Holdgate and Ward, 2005). DSC also allows the number of intermediate states in the binding pathway to be determined, whereas ITC ideally requires that the binding process be two-state. In addition, by conducting several DSC binding experiments at various concentrations of reactants, the population of free and bound species can be calculated as a function of temperature and concentration (Holdgate and Ward, 2002).

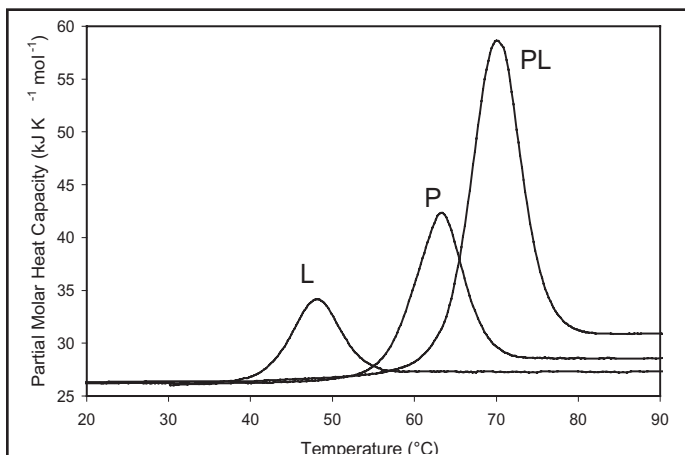


Fig. 1. Simulated DSC data showing the molar heat capacity of free ligand (L), free protein (P) and the protein-ligand complex (PL), where the complex is a tightly-associating system.

If a ligand (L) binds to the native conformation of a protein (P) with high affinity, the resulting protein/ligand complex (PL) will have a higher thermal stability than either of the two free components (Jelesarov and Bosshard, 1999). Figure 1 shows an idealized example where the stabilized complex has a significantly higher melting temperature than the protein or ligand alone. The symmetry of this PL peak suggests that complex dissociation and thermal denaturation are tightly coupled. In practice, the shape of the protein/ligand endotherm can be quite complex due to the presence of intermediates and/or denatured protein, the sensitivity of the complex to pH and ionic strength, or to the ligand preferentially binding to and stabilizing only part of the protein (*e.g.*, one domain) (Brandts and Lin, 1990; Jelesarov and Bosshard, 1999; Luque *et al.*, 2002). For many applications, only an approximation of the T_m of the complex (the temperature at which half the protein/ligand complex molecules are folded and half are unfolded) vs. the T_m of the free protein is required; this information can generally be obtained by visual inspection of the endotherms. However, since the free energy of complex formation results from a delicate balance between large favorable and unfavorable entropic and enthalpic contributions, precautions must be taken not to over-interpret the data. For example, a large observed T_m shift is not necessarily an indication of high affinity binding, since a range of different affinities, with different entropic and enthalpic contributions, could result in the same T_m .

If determination of the free energy of complex formation is required and the endotherm has a simple shape (indicative of a two-state process), the free energy change due to binding is calculated by subtracting the free energy change of unfolding of the complex (ΔG_{comp}) from the free energy change of unfolding of the protein (ΔG_{prot}) (*i.e.*, $\Delta G = \Delta G_{\text{prot}} - \Delta G_{\text{comp}}$). If the endotherm is complex, indicative of multiple folding states, the endotherm must be deconvoluted to extract the free energy contributions of each state. DSC and ITC thus allow a binding reaction to be studied from two different angles. The power of ITC is that it gives accurate values of ΔH , and allows the determination of ΔC_p if heat capacity is independent of temperature and the interacting molecules form a two-state complex. The versatility of DSC means that although measurements may produce a complex endotherm, following deconvolution the data allow the enthalpy and heat capacity measured by ITC to be correlated, at least to a degree, to conformational changes occurring upon binding (Jelesarov and Bosshard, 1999). The deconvolution process and its application to the analysis of complex binding systems are described in Jelesarov and Bosshard (1999), Cooper *et al.* (2001), and references therein.

Summary

When combined with structural and ITC data, DSC provides an independent approach to elucidating the free energy changes accompanying the formation of protein/ligand complexes, which in turn can shed light on the mechanism of the association reaction. Used together, ITC and DSC complement and augment each other and provide the most direct, comprehensive approach for monitoring and interpreting binding interactions involving biological macromolecules.

References:

(Preference has been given to current references. Citation does not imply that a paper is necessarily the original reference to a study.)

- Brandts, J. F. and L-N Lin. (1990) Study of strong to ultratight protein interactions using differential scanning calorimetry. *Biochemistry* **29**, 6927-6940.
- Cooper, A., M. A. Nutley and A. Wadood. (2001) Differential scanning calorimetry. p. 287-318. In S. E. Harding and B. Z. Chowdry (Eds.) *Protein-Ligand Interactions: hydrodynamics and calorimetry*. Oxford University Press, Oxford.
- Holdgate, G. A. and W. H. J. Ward. (2005) Measurements of binding thermodynamics in drug discovery. *Drug Discov. Today* **10**, 1543-1550.
- Jelesarov, I. and H. R. Bosshard. (1999) Isothermal titration calorimetry and differential scanning calorimetry as complementary tools to investigate the energetics of biomolecular recognition. *J. Mol. Recognit.* **12**, 3-18.
- Luque, I., S. A. Leavitt and E. Freire. (2002) The linkage between protein folding and functional cooperativity: two sides of the same coin? *Annu. Rev. Biophys. Biomol. Struct.* **31**, 235-256.
- Palmer, A. G. (1997) Probing molecular motion by NMR. *Curr. Opin. Struct. Biol.* **7**, 732-737.
- Ringe, D. and G. A. Petsko. (1985) Mapping protein dynamics by X-ray diffraction. *Prog. Biophys. Mol. Biol.* **45**, 197-235.
- Shinoda, K. (1977) 'Iceberg' formation and solubility. *J. Phys. Chem.* **81**, 8069-8072.

CALORIMETRY

SCIENCES CORPORATION

890 West 410 North, Suite A
Lindon, Utah 84042
Phone: 801.763.1500 Fax: 801.763.1414
Web: www.calorimetrysciences.com
E-mail: info@calorimetrysciences.com