Complementary Experimental Methods to Obtain Thermodynamic Parameters of Protein Ligand Systems

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Introduction

Motion of solutes in a solvent induced by a thermal gradient is termed as thermophoresis. Ratio of the established concentration gradient to the temperature gradient is quantified using Soret coefficient S_T (Köhler *et al.*, 2016). Since this phenomenon is very sensitive to the nature of solute-solvent interactions, it is used as a tool for quantifying biomolecular interactions, especially protein-ligand interactions (Niether *et al*. 2020). Although, a change in the thermophoretic behaviour of protein once the ligand binds is often attributed to the change in the hydration layer, underlying microscopic physical effect is not understood. To gain deeper insight into the interactions involved, we investigate whether the non-equilibrium coefficient measured can be related to equilibrium properties. The same, thermophoretic data measured using thermal diffusion forced Rayleigh scattering (TDFRS) (which is a non-equilibrium process) are compared with the thermodynamic data obtained by isothermal titration calorimetry (ITC) (which is an equilibrium method). To connect these parameters, we start from an early work by Eastman (Eastman 1928) which connects S_T to Gibb's free energy ΔG as follows

$$S_T = \frac{1}{k_B T}\frac{dG}{dT} \qquad (1)$$

Integrating Eq. 1 with temperature gives us access to a relation between S_T and ΔG for the individual compounds of the system (free protein, free ligand, and complex). These individual contributions calculated are then used to establish a relation between ITC and TDFRS measurements, as shown in Fig. 1. We hypothesize that $\Delta G_{T_{high}}$ can be calculated from the free energy change at a low temperature $\Delta G_{T_{low}}$ measured by ITC and the differences in $\Delta\Delta G$ corresponding to two temperatures for the individual components probed by TDFRS using the following equation

$$\Delta G_{T_{high}} = \Delta G_{T_{low}} + \Delta\Delta G_{AB} - \Delta\Delta G_A - \Delta\Delta G_B \qquad (2)$$

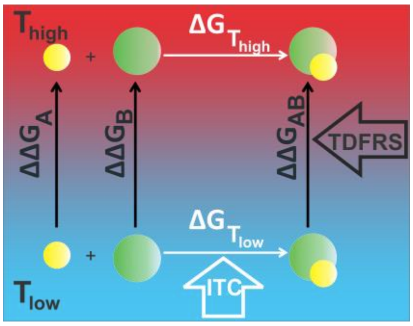

Figure 1: Schematic illustration of the calculation of ΔG and ΔΔG from ITC and TDFRS, respectively.

Choice of systems

To test our hypothesis, EDTA-CaCl$_2$ reaction in MES buffer is used as reference system. This is a well-known chelation reaction which is used as a validation standard for ITC measurements (Ràfols *et al*. 2016). Once the validation is done, Eq. 2 is tested for protein Bovine Carbonic Anhydrase I (BCA I) with two different ligands which belong to the group of arylsulfonamides. In our study, we used 4 fluorobenzenesulfonamide (4FBS) and pentafluorobenzenesulfonamide (PFBS). We carry out TDFRS and ITC measurements for these systems over a wide temperature range between 20°C to 45°C.

Results

1. TDFRS measurements

1.1 EDTA-CaCl$_2$

S_T of EDTA shows an abrupt drop between 25°C and 30°C. Although, there are several literature reports, which report a change in properties of several systems in the presence of EDTA in a similar temperature range, there exists no clear explanation for this behavior (Sugiyama *et al*. 2014, Minkevich *et al*. 2006, Yilmaz *et al*. 2011). Also, the temperature sensitive polymer poly(N-isoproplacrylamide) (PNiPAM) in water (*Kita* et al. 2005), which is influenced by the hydrogen bonds, shows an abrupt variation in S_T with temperature. Distinctive thermodiffusive properties exhibited by EDTA and the complex might have the same origin as in case of PNiPAM as it happens in similar temperature range. This also supports the hypothesis that a change in hydration has an effect on thermophoretic behavior.

1.2 Protein-ligand system

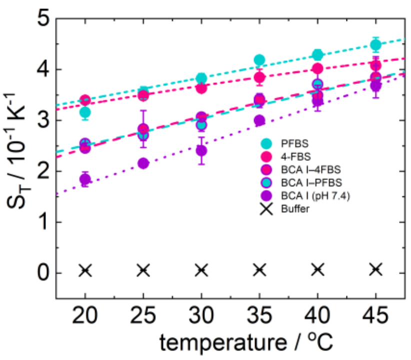

Figure 2: Temperature dependence of S_T for BCA I, 4FBS, PFBS, buffer and complexes formed.

Temperature dependence of S_T for protein-ligand systems is shown in Fig. 2. From TDFRS measurements, it can be concluded that the hydration shells of both complexes formed are very similar, but different from free BCA I. For both protein–ligand systems the difference between S_T of free protein and complex decreases with temperature, so that it is almost negligible at high temperatures. This is an indication that the binding of both ligands should become weaker with increasing temperature. A larger change in S_T with temperature for free BCA I indicate a higher hydrophilicity for the free protein in comparison to the protein-ligand complex formed. It has been reported that arylsulfonamides bind to the active site of different variants of carbonic anhydrase protein by replacing a water molecule from the active site (Krishnamurthy *et al.* 2007, Olander *et al.* 1973, Abbate *et al.* 2002). This implies that the complex is less hydrophilic than the free protein which is confirmed by TDFRS measurements.

2. Validation of relation between S_T and ΔG

The calculated values listed in Table 1 correspond to T_{high} = 30°C and T_{low} = 20°C. Mathematical expression is first applied to the EDTA-$CaCl_2$ system. With the S_T values of EDTA, CaCl2 and the complex measured at T_{high} and T_{low}, we have access to the change in Gibb’s energy ($\Delta\Delta G$) of the individual components. Later, the same procedure is used for protein-ligand systems.

Table 1: Comparison of ΔG that has been calculated and that has been measured with ITC

System	$\Delta G_{calculated}$ (kJ/mol)	ΔG_{ITC} (kJ/mol)
EDTA-$CaCl_2$	-36.5±1.2	-36.4±0.8
BCA I-4FBS	-40.5±1.1	-40.4±1.3
BCA I-PFBS	-39.9±3.9	-38.2±1.5

For all the three systems that have been studied, ΔG that has been calculated agrees with the measured values within the error limits.

Conclusions

One of the main goals of this work was to investigate whether it is possible to connect thermodynamic parameter obtained with ITC to thermodiffusion parameter measured with TDFRS. A mathematical relation connecting S_T and ΔG was derived which is tested for three different systems; low molecular weight reference system, EDTA–$CaCl_2$ and the protein BCA I with two ligands 4FBS and PFBS. For all the temperature pairs that have been studied for aforementioned systems, ΔG values calculated agreed with the measured values within the error limits. This implies that S_T measured at different temperatures can be used to predict the ΔG value at particular temperature. This newly developed connection can be utilized to open promising gates in the accurate acquisition of the underlying binding and molecular dissociation mechanisms from the studied systems.

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