

Microcalorimetric Methods for Peptide Studies

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Microcalorimetry is a widely used technique for the study of biological systems with over 7,000 published references relating to binding, enzyme kinetics, characterization of macromolecular structure and stability, amongst others [1].

Isothermal Titration Calorimetry (ITC) measures the heat liberated or absorbed during a chemical process such as binding or catalysis. In a single experiment, ITC provides a complete profile of a molecular interaction including Binding Constant (K_B), Enthalpy (ΔH), Entropy (ΔS) and Stoichiometry (n) of the interaction [2]. The thermodynamic quantities, ΔH and ΔS provide valuable insight into the mechanism of binding not obtainable from a Binding Constant alone. With ITC, molecules can be studied in their native state, as no modification or immobilization is required.

In biological systems enthalpically driven reactions (heat generated) are typically associated with the formation of hydrogen bonds and electrostatic interactions. Entropic driven processes in biological systems are usually due to the displacement of ordered water into solution. This later process is associated with hydrophobic surfaces coming into contact with surfaces having bound or ordered water which displaces the ordered water into solution, a more disordered state. Due to the critical distances required for the formation of hydrogen and ionic interactions, enthalpically driven systems tend to be highly specific binding. Conversely, hydrophobic interactions tend to be non-specific binding. Thus, with the data from an ITC experiment, in addition to determining the binding constant and stoichiometry, the thermodynamic quantities of ΔH and ΔS provide valuable information relating to the mechanisms of binding and relative binding specificity.

Differential Scanning Calorimetry (DSC) measures the heat liberated or absorbed by a solute while temperature is increasing for the entire system. Thus, the conformational energy of solutes, including macromolecules, membranes, etc., may be determined. In a single DSC experiment T_m , ΔH and ΔC_p are measured [3]. T_m is the temperature at which 50% of the transition or unfolding has occurred, ΔH is the enthalpy, which corresponds to the total conformational energy of the structure being investigated and ΔC_p the change in heat capacity comparing the unfolded to native states of the macromolecule or structure. This information can be utilized to better understand the effect various molecules and conditions have on membrane structures or prediction of stability of macromolecules in solution⁴, amongst other applications. Remele et al. [4] have demonstrated a direct correspondence between increasing T_m and the stability of proteins in solution. This information has been used extensively to define the optimal conditions for solution stability of protein pharmaceuticals as well as other applications.

Jing et al. [5] employed microcalorimetry to better understand the mechanism of action of a 13-residue

antimicrobial peptide including how this peptide affects membrane structure thereby inducing membrane leakage. Their studies found that when PuroA, a 13 peptide residue, binds to dipalmitoylphosphatidylglycerol (DPPG) there is a resultant dose-response decrease in the T_m of DPPG due to disruption of the liquid crystalline phase. When the effect of PuroA was studied with dipalmitoylphosphatidylcholine (DPPC), little effect on the phase behavior was observed. They further studied the effect of different polar head-groups in membrane structures and how these different structures affect the binding of this peptide. Studies using ITC compared the binding characteristics of PuroA to 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and POPC, the choline variant of the above lipid. Binding to the choline variant was almost 10-fold tighter as compared to the glycerol variant due to ionic interactions with the charged groups in the choline moiety. This data was consistent with data from DSC.

Brandenburg et al. [6] used both ITC and DSC to explain the temperature dependence for binding of endotoxins to Polymyxin B and its nonapeptide. At temperatures below 31° C (gel phase) binding is endothermic (absorbs heat) and is driven by an entropically driven process due to the disruption of the ordered water structure and cation assembly of the lipid and adjacent molecules. Above 35°C (liquid crystalline phase), binding is exothermic (releases heat), which is mainly due to electrostatic interactions between the Polymyxins and the negative charges of the endotoxins. Furthermore, the stoichiometry of Polymyxin binding corresponds to pure charge neutralization with short sugar chains. From this and other calorimetric data the authors concluded that the binding of Polymyxin B and its nonapeptide is phase specific, is driven by electrostatic interactions and influenced by the small acyl chain in PMB, thus further elucidating the mechanism of binding.

References

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