Life Science Applications of ITC
Overview Note

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Proteomics research is uncovering a vast array of new proteins, including enzymes. It is now clear that the old assumption that one gene encodes one protein is incorrect. One gene can encode for several proteins, and many proteins have multiple functions: which function is displayed is controlled by the protein's molecular environment. The structure and function of a protein are controlled by interactions with small molecule ligands and cofactors, and by interactions with macromolecules such as other proteins, nucleic acids and lipids. Studying a protein in isolation (for example, obtaining the crystal or NMR structure of a purified protein) is critical to establishing a structural basis for the protein's function(s). However, true functional characterization of the protein generally requires manipulation of multi-component systems under stringently-controlled conditions, and evaluating the roles of the various components in regulating and tuning the physical characteristics of the protein. Proteomics has highlighted the need for a technique that can quantify the interactions between molecules under physiologically-relevant conditions.

Isothermal titration calorimetry (ITC) is a universally-applicable technique for determining the thermal effects arising from molecular interactions. For life scientists, ITC is a particularly powerful approach for quantifying molecular interactions between two or more proteins or other macromolecules, or a protein and small molecule ligands such as drugs or enzyme inhibitors. Since nearly every reaction is accompanied by the absorption or evolution of heat, ***most reactions, often requiring only nanomoles of material, can be studied using calorimetry***. There is no requirement for chromophores, fluorophores, optical transparency, chemical modification or immobilization, and since the measurement of heat is independent of the complexity of the sample, impurities in the sample are tolerated. This introductory note provides an overview of the types of biophysical questions that can be addressed using ITC, and briefly describes how a typical experiment is performed and the data interpreted.

Applications of ITC:

Life science applications of ITC can be broadly characterized as either binding interactions, or enzyme-substrate interactions. ***All the examples outlined below can be investigated using the same ITC instrument.***

Binding interactions:

This is perhaps the most frequent use of ITC, where the affinity of one molecule to bind to another at physiologically-relevant temperatures is characterized. In a single titration experiment, ***ITC can measure the binding affinity, the binding enthalpy, and the stoichiometry*** with which two compounds interact with each other. Common systems studied by ITC include:

- Protein/Ligand Binding
- Protein/Protein Interactions
- Biopolymer/Drug Binding
- Lipid Vesicle and Liposome Interactions

Calorimetry does not distinguish the size or function of molecules, so the assignment of "ligand", "macromolecule", "drug", "receptor" or other nomenclature is purely arbitrary, and is solely for

the convenience of the experimenter. Thus, all the interactions listed above would be studied using similar approaches. To determine the affinity with which, for example, a protein binds a small ligand (*e.g.,* a drug candidate), a solution of the protein (1 mL of an approx. 10 μM solution) is loaded into the calorimeter sample cell, and the ligand solution (approx. 100 μL) is loaded into an injector syringe connected to the sample cell. The ligand is titrated incrementally into the protein sample, and heat is generated and registered as a deflection peak by the instrument. Integration of the area under each deflection peak allows the amount of heat produced by each addition of ligand to be calculated, until the protein is saturated with ligand and no further binding is detected. A typical, fully-automated ITC experiment takes about one hour to run. Using the 'Bindworks' software supplied by CSC, a global fit of the data from a single titration experiment is often sufficient to allow the association constant (K_a), the binding enthalpy (ΔH) and the stoichiometry (n) of the reaction to be calculated.

Drug binding to DNA, RNA and polysaccharides can all be studied in an analogous manner, as can protein/protein interactions (with the second protein taking the place of the small molecule ligand in the syringe). Importantly, for any of these applications, conducting titration experiments with two different pH buffers (having the same pH but different enthalpies of protonation) allows the number of protons exchanged between the ligand and the binding site to be determined. This provides information on which functional groups are involved in the binding reaction.

Enzyme/substrate interactions:

Enzymes drive every biochemical pathway and can exhibit different catalytic functions depending on the physiological state of the organism. For this reason, enzymes are implicated in practically every disease and are therefore increasingly important targets for drugs. ***ITC can rapidly analyze essentially any enzymatic reaction, using a wide range of physiological or synthetic substrates and inhibitors***, without the need for chromogenic derivatives or coupling enzymes. Additionally, since the observed thermal signal is directly proportional to the rate of the reaction, data analysis is straightforward.

To determine the rate of catalysis, enzyme is loaded into the calorimeter's sample cell, and the substrate, dissolved in the same buffer as the enzyme, is loaded into the injector syringe. Appropriate concentrations of substrate and enzyme will vary depending on the exact reaction being studied, but generally concentrations are in the order of 1-20 nM enzyme and 10 -100 μM substrate. Approximately 25μL of substrate is injected, and the rate of heat production is monitored continuously until the substrate is depleted. Using software supplied with the instrument, the data are plotted (rate *vs*. substrate concentration) and fit to yield the catalytic rate constant (k_{cat}) and the Michaelis constant (K_M). The experiment can be repeated by injecting a second aliquot of substrate. If there is no product inhibition, the same curve will be obtained; if there is product inhibition, its severity can be determined over the course of several injections. Using a variation of this approach, pseudo-first-order kinetics can be studied, using multiple small injections of substrate.

Summary:

In summary, ITC is a universally-applicable technique that has two major life science applications: first, for studying binding interactions between, for example, proteins and drugs, or ligands and receptors, and second, for quantifying rates of enzymatic catalysis and product or drug inhibition. Experiments are rapid (typically requiring an hour or so of unattended operation), sample derivatization is not required, impurities are tolerated, and often only nanomoles of the target macromolecule are required.

For further details regarding applications to life science research, please refer to CSC's application notes 'Characterizing binding reactions by ITC' and 'Characterizing enzyme kinetics by ITC'. Please contact us by phone at 801.763.1500, or by email at info@calscorp.com.