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Growing Popularity of Ultrasensitive Microcalorimetry

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Abstract

Non covalent interactions in biological macromolecules mediate almost all the chemical processes in living systems. Developments in microcalorimetry has enabled a quantitative understanding of such interactions and contributed significantly to biotechnology, medicine, and novel drug design. This report addresses advancement and use of ultrasensitive microcalorimetry in connecting bioenergetics with possible applications in chemical and biological processes.

Keywords: Bioenergetics; Isothermal titration calorimetry; Differential scanning calorimetry; Novel drug design; Biothermodynamics; Protein folding and stability

Introduction

Most of the interactions which are essential for cellular organization or proper functioning of biological macromolecules are non-covalent in nature. A variety of ligands which can act as potential drugs, also interact via non-covalent interactions. Precise and accurate measurements of such weak interactions and correlation with structure and functional groups of reacting species can provide insights into functions and organization of such biologically important systems, and rational drug design. The conformational stability of a biological macromolecule is important for its function. The techniques which have rapidly grown in addressing these issues successfully are Isothermal Titration Calorimetry (ITC) and Differential Scanning Calorimetry (DSC).

Tronac titration calorimeter, prototype model of which was developed by Christensen and Izatt [1] in the year 1968 was mainly useful for the measurement of excess enthalpies or heats of dilutions. The sensitivity and amount of sample requirement did not allow this instrument to be a popular choice amongst scientists having biological interests. Efforts were continuously being put to develop calorimeters in which the reaction vessel was changed from an open to an adiabatic system with improved sensitivity. Such calorimeters either indigenous or commercially available were accurately able to determine the value of heat of neutralization of acid by alkali close to -13.3 kcal mol-1 which is amongst the best values known in literature [2]. However, the measurement of heats of interaction in biologically important reactions was not possible to high level of accuracy because of the above mentioned reasons. The differential scanning calorimeters started appearing in mid-sixties [3-5]. With the developments in technology, the sensitivities of ITC and DSC improved manifolds. Therefore, these techniques have become fundamental choice due to their inherent capability to determine thermodynamics of biologically important reactions irrespective of the limitations on molecular mass, intrinsic/extrinsic labeling, immobilization of species or stringent requirement on transparency of the solutions. Currently, the ultrasensitive isothermal titration calorimeters which have made an impact in biologically important systems are VP-ITC and ITC200 from Malvern (Microcal), and Nano ITC Standard Volume and Nano ITC Low Volume from TA Instruments. Further developments in terms of improved sensitivity and low volume requirements are constantly being tried by these manufacturers (for example, recently launched PEAQ ITC by Malvern, and Affinity ITC by TA Instruments).

Differential Scanning Calorimetry has enabled understanding the forces which are responsible for the folded conformation of proteins and understanding protein folding intermediates. Not only just for monitoring the thermal stability of biological macromolecules, DSC is also extremely useful in unraveling the mechanism of protein unfolding. Reversible thermal unfolding transitions of proteins allow measurements of both calorimetric and Van't Hoffenthalpies, comparison of which suggests absence or presence of intermediate states, or onset of association of protein molecules during the thermal unfolding process [6,7]. Appearance of reduced thermal transitions or complete disappearance of cooperative transitions have been reported by different research groups for thermal unfolding of proteins from molten globule to unfolded state [8,9]. Nevertheless, whether it is reduced thermal transition with ratio of Van't Hoff to calorimetric enthalpy less than unity or disappearance of thermal transition, either non two-state unfolding mechanism or denatured state of the protein is inferred which can further be confirmed by using circular dichroism or fluorescence spectroscopy [10,11]. The role of specific amino acid residues in maintaining the three dimensional native conformation of proteins has also been answered by DSC through mutant proteins [12-14].

Desired affinity, selectivity, and solubility have been major challenges in discovery of novel drugs. The significance of enthalpic contribution in affinity by choosing examples of HIV protease inhibitors and statins from the first in class to the best in class over a period of about 12 years have been elegantly described by Ernesto Friere [15]. Though unplanned, the emergence of new drugs with stronger exothermic association as a key parameter indicates undisputed and unquestioned role of direct isothermal titration calorimetry in deriving guidelines for target oriented synthesis and rational drug design. Such a correlation can be established only if the energetics of interaction can be correlated with the functional groups both on the drug and at the binding site, suitable alterations of which

leads to improved affinity and selectivity. Exploding synthetic approaches to obtain potential novel drugs can attain focus with the availability of such guidelines.

The role of calorimetry and thermodynamics in biological macromolecules and drug design has been acknowledged from time to time [16-19]. ITC is also being employed in understanding the interactions of nucleic acids with variety of molecules ranging from small synthetic compounds to large proteins [20-22]. The result of such studies have great potential on design and evaluation of DNA interaction of small molecules that can enter cells and modify function of targeted cells in a programmed manner. Application of microcalorimetry has led to discovery of several potential compounds targeted at inhibition of telomerase enzyme which is an excellent method to selectively attack cancer cells [23,24]. Efforts directed towards discovery and characterization of small molecules that target the RaS-like GTPase, pathway components of which are currently being studied as breakthroughs for potential cancer therapies [25]. Clinical applications of Plasma thermograms have also been discussed [26]. Recently the applications of ITC in Nanoscience and Nanotechnology ranging from understanding agglomeration or stabilization mechanism of nanoparticles [27] to biomolecular nanoparticle interactions [28] have been reported or reviewed. Some other recently reported applications of ITC include monitoring the growth of bacterial activity which can be beneficial in designing anti-microbial agents [29]. Calorimetry has also found its use in the investigation of the fate of food products in the gastrointestinal tract [30]. Interaction of proteins with essential 1st row transition metals has also recently been reported [31,32]. Isothermal titration calorimetry and differential scanning calorimetry have also found role in getting insights into inhibition of aggregation or fibrillation of proteins, which is essential in prevention of neurodegenerative diseases [33,34].

Subsequent to the optimum design of the experiments and obtaining high quality data, suitable data analysis is extremely important. Even though, the data analysis software provided by instrument manufacturers is sufficient most of the times, situations may arise where additional modeling is required. Brautigam et al. [35] have recently suggested methods for global analysis of ITC calorimetric data for interaction of macromolecules.

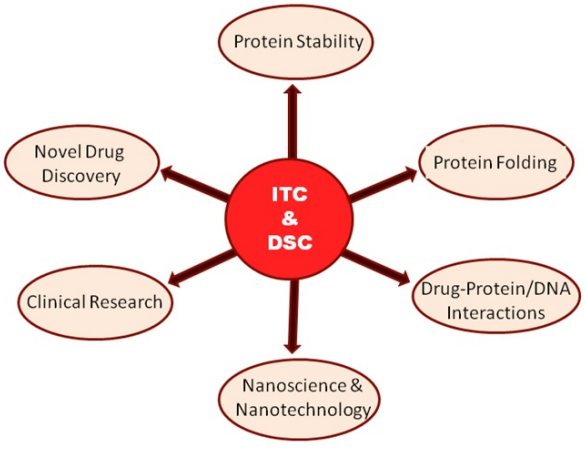

Figure 1: Potential applications of ITC and DSC.

Technical advances in isothermal titration calorimetry and differential scanning calorimetry will undoubtedly broaden the scope of their applications (Figure 1) in the years to come and make a huge impact in fundamental chemical and biological research, as well as in applied science and technology including those targeted at drug development, and finding answers to much awaited solution to protein folding problem.

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