Paper 3: Thermodynamics of Living Systems and Bioenergetics

Module 28: Differential Scanning Calorimetry and its applications

Introduction :

In the previous module, you have learnt about ITC. Differential scanning calorimetry (DSC), is another calorimetric technique used in studying physicochemical parameters of polymers and biopolymers.If both are a kind of similar tehniques , the question then immediately comes to mind is

Why DSC ?

And when and where one can choose ITC or DSC ?Although, both are synonimous in calorimetry , sometimes both Itc and DSC are done to get different kinds of information.Theses are explained in this module. Generally DCS is used during protein engineering for comparison of thermostability between the parent and engineered antibodies.

DSC finds its applications in assessing the protein's thermal and conformational stability.

A DSC profile provides the melting temperature of proteins or individual domains.

- It is possible to determine the thermodynamic parameters of the unfolding in the case of a reversible reaction.
- If the transition temperature (T_m) of a protein is higher, then the protein exhibits better thermostability.
- Higher T_m or better thermostability is correlated with the reduced aggregation based on the comparison of thermostability data against the data of bioanalytical techniques such as size exclusion chromatography (SEC).
- Better long-term stability can be expected from antibodies having a higher T_m with reduced tendency towards aggregation, making them a potential candidate to produce better biotherapeutics

Why DSC is Unique ??

- What makes DSC unique is its specificity for the subset of small molecule ligands that do thermally stabilize a protein.
- Whereas the other techniques will only tell whether a small molecule binds, and can NOT tell whether it stabilizes.
- DSC is very easy to do the experiment, requiring almost no assay development work, but is relatively less amount of protein (200 μg per well).
- DSC-- used to measure a number of characteristic properties of a sample.

- DSC can be used to observe fusion and crystallization events as well as glass transition temperatures Tg.
- DSC can also be used to study oxidation, as well as other chemical reactions

Objectives

- **Differential Scanning Calorimetry-Introduction**
- **Technical and instrumentation details of DSC**
- **Informational content of DCS data**
- **Types of biomlecular experiments that can be addressed by DSC**

1.0. Differential Scanning calorimetry(DSC)

The technique was developed by E.S. Watson and M.J. O'Neill in 1962, and introduced commercially at the 1963 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. Compared to ITC,DSC provide a more comprehensive description of energetic of conformational or phase transition as a function of temperature of biological macromolecule.By measuring the difference in heat flow between sample cell and reference cell as a function of temerature, DSC gives immediate access to the thermodynamic mechanism that governs a conformational equilibrium. The reference cell in DSC contain an empty inert aluminum pan. At constant pressure the enthalpy and entropy changes are measured by increasimg the temperature of both sample and reference cell at constant rate. DdH/dt so obtained is the heat capacity of the reaction. During an endothermic reaction like DNA melting, protein denaturation , reduction process, decomposition reaction etc DdH/dt is positive. However during an exothermic reaction like some cross-linking processes ,oxidation reactions, crystallization etc DdH/dt is negative. This can be explained by the following reaction:at constant pressure heat flow dH/dt (mcal sec-1) is equal to enthalpy changes:

$$\left(\frac{dq}{dt}\right)_P = \frac{dH}{dt} \quad \ldots\ldots\ldots\ldots\ldots 1$$

$$\Delta\frac{dH}{dt} = \left(\frac{dH}{dt}\right)_{sample} - \left(\frac{dH}{dt}\right)_{reference} \quad \ldots\ldots\ldots\ldots\ldots 2$$

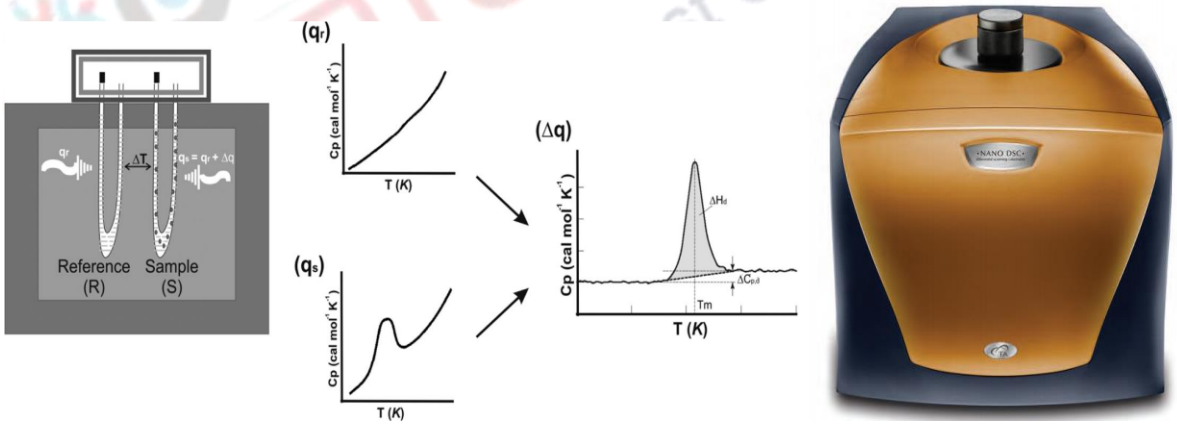
The difference in heat flow between the sample and the reference can be positive or negative.

For a meaningful comparison of the thermal transition curves of different substances it is important to compare the calorimetric enthalpy (Hcal) with van't Hoff enthalpy H_{VH}. Calorimetric enthalpy (Hcal) is the total integrated zone below the thermogram peak, which indicates total heat energy uptake by the sample after suitable baseline correction affecting the transition.While the van't Hoff enthalpy is determined by shape analysis of experimental graph and is measurement of the transitional enthalpy .If $H_{VH} = H_{cal}$, the transition occurs in a two-state mode.When $H_{VH} > H_{cal}$, the intermolecular cooperation has

occurred. Comparison between HVH and Hcal also indicates the cooperative nature of the transition. Moreover the ratio of H_{VH}/H_{cal} gives the fraction of the structure, which is melted as a thermodynamical.

The Nano DSC differential scanning calorimeter(**Figure 2**) is designed to measure the amount of heat absorbed or released by dilute in-solution bio-molecules as they are heated or cooled. Macromolecules such as proteins respond to heating or cooling by unfolding at a characteristic temperature. The more intrinsically stable the biopolymer, the higher the midpoint temperature of the unfolding transition. As these processes often exchange microjoule levels of heat, the sensitivity of the Nano DSC is critical for successful investigation of the reaction. The Nano DSC obtains data with less sample than competitive designs and produces unmatched short term noise (±15 nanowatts) and baseline reproducibility (±28 nanowatts). Solid-state thermoelectric elements are used to precisely control temperature and a built-in precision linear actuator maintains constant or controlled variable pressure in the cell. Increased sample throughput is realized by adding on the Nano DSC Autosampler. It provides true walk-away capability for up to 96 samples. With convenient USB connectivity, built-in pressure perturbation capability and capillary cell design, the Nano DSC provides maximum flexibility with a cell design that minimizes sample aggregation and precipitation, resulting in high quality data.

2. Experimental Setup of Differential Scanning Calorimeter



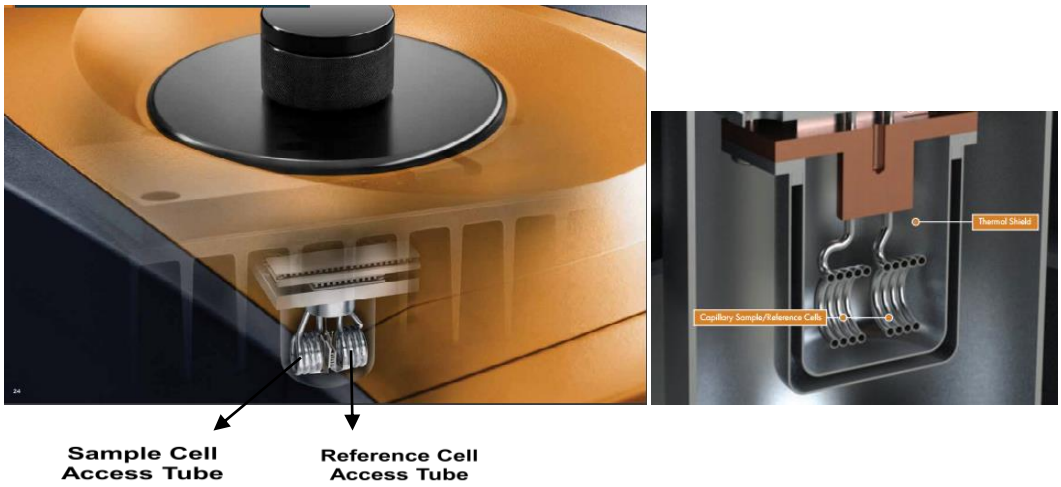


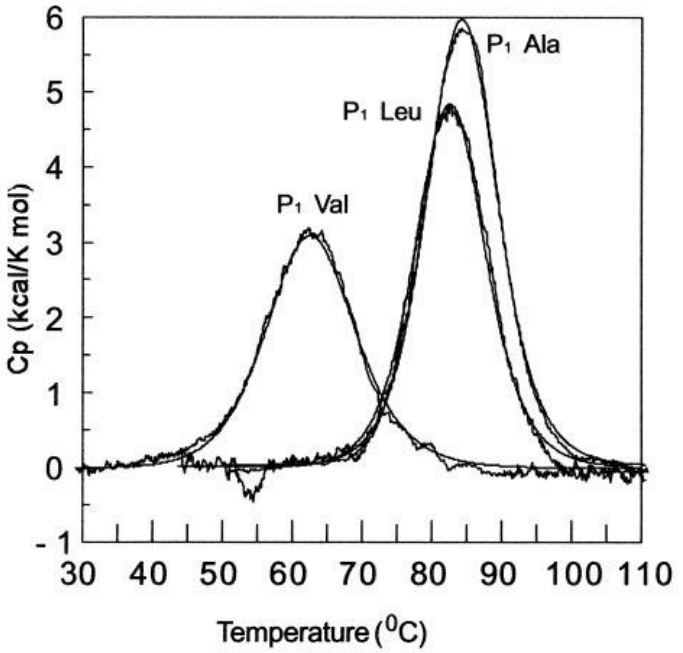
Figure 2: Representative diagram of a Differential Scanning Calorimeter experiment. The basic feature of Nano DSC instrument is reference and sample cell made to allow high temperature operation. Power is supplier to both the cell containing a resistance heater and temperature sensor to increase the temperature at constant rate. The difference between the power of the two holders is used to calculate DdH/dt

Figure 3: Below shown is another model of DSC

In a basic DSC experiment, the calorimeter contain a sample holder and a reference holder made up of platinum to allow high temperature operation **Figure 2**. The sample is sealed into a small aluminum pan that hold about 10mg of material and the reference is usually an empty pan. Both the holder are connected to temperature sensor and resistance heater . Energy is applied to both the heater to simultaneously increase temperature at the selected rate. The difference in the power input required to maintain the temperature of sample holders to that of reference holder at the same temperature, is used to calculate excess heat absorbed or released by the molecule in the sample (during an endothermic or exothermic process, respectively) DdH/dt . During the experiment the sample holder is supplied with constant flow of nitrogen gas to create a dry and reproducible atmosphere. At high temperature the dry atmosphere prevent the oxidation of air in the samples.

Examples of Applications of DSC

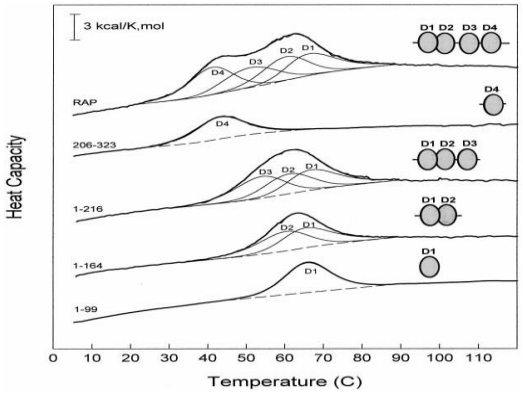
1. Protein stability



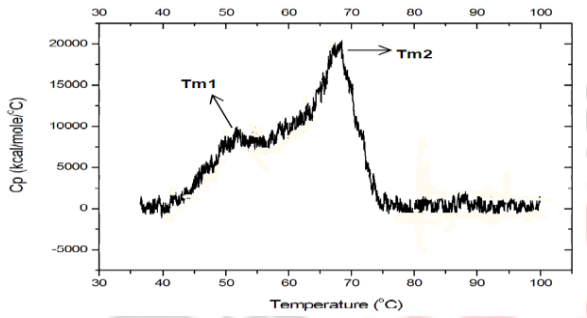
Example: Mutate proteins to make them more stable, more specific, faster, have new properties, etc.Useful to predict outcome of a mutation, so need a database of thermodynamically characterized proteins. Complicated network of interactions. Also, enthalpic-entropic compensation. Enthalpic changes (changes in hydrophobic interactions, hydrogen bonding, electrostatic interactions) compensated by entropic changes (changes in solvation, conformational freedom)..

3 Protein-Protein subunit interaction

- Unfolding of domains and subunits with different thermal stabilities produce asymmetric thermograms.
- Deconvolution of the unfolding thermogram provides the number of domains or subunits.
- A small change in sequence, or other alteration, can affect the stability of the whole protein, or the stability of one domain or subunit.
- DSC quickly reveals these stability changes, sheds light on how sequence and thermodynamics interact.
- Practical implications: how the altered protein might cause a disease, how it could be targeted by a drug, etc.



4. Nucleic Acids



This is an example of DNA triplex.The DSC result demonstrates biphasic melting transition one with Tm1 value of 56.28±0.15ºC which corresponds to the denaturation of DNA triplex and another of Tm2 value of 67.67±0.028oC which is related to melting of DNA duplex. For the triplex melting the vant hoff enthalpy, ΔHv = 1.8×102±0.7 kcal M-1 and the Calorimetric enthalpy, Δ*Hc = 0.44×102 ±3.2kcal M-1. On the other hand, the ΔHv* =1.23×102±0.5kcal M-1 and ΔHc = 1.22×102±2.5kcal M-1 were observed for the second transition. The ratio of calorimetric to Van't Hoff enthalpy for the DNA duplex melting is unity indicating two state transitions, i.e, the transition occur in all or none fashion, without any intermediate state.

Determining the thermodynamic parameters of a protein by differential scanning calorimetry (DSC) using the Nano DSC requires about the same amount of protein as surface plasmon resonance or fluorescence studies. Because of the Nano DSC's extreme sensitivity and baseline reproducibility, and the sample cell's small volume (300 µL), a complete, interpretable, accurate scan can be obtained on essentially any protein of interest.

5. Limitations of DSC

- **However, DSC is relatively slow, requiring over an hour per cone sample.**
- **DSC can miss weak binders (K_d > 500 µM).**
- **SPR or ITC are preferred for K_d determination, in such cases.**

works Only if ligand binding is entropically driven

- **DSC is sometimes used in dose-response to determine K_d, but this only works if the binding is mostly entropically driven**

Summary

- Differential scanning calorimetry or DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature.
- The result of a DSC experiment is a curve of heat flux versus temperature or versus time that can be used to calculate enthalpies of transitions.
- Differential scanning calorimetry can be used to measure a number of characteristic
- properties of a sample. Using this technique it is possible to observe fusion and crystallization events as well as glass transition temperatures Tg. DSC can also be used to study oxidation, as well as other chemical reactions.
- It is most often used to **study the binding of small molecules (such as medicinal compounds) to larger macromolecules (proteins, DNA , lipids, membrane etc.).**
- **Applications to proteins, nucleic acids etc are discussed in detail in this module**