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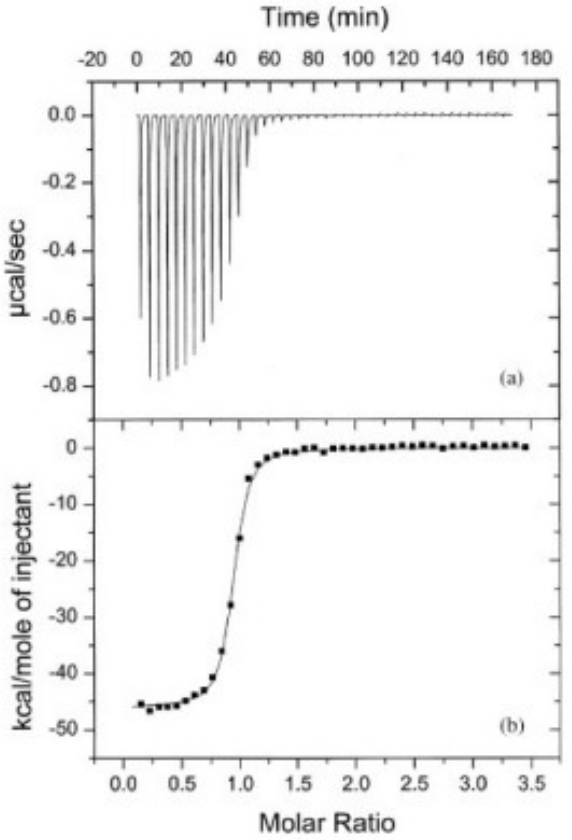
Calorimetry

Principles of Physical Biochemistry

Van Holde, Johnson & Ho

Pages 93-103



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Calorimetry

Two common calorimetric methods in Biochemistry:

1) Differential Scanning Calorimetry (DSC)

Measure changes in heat capacity as a function of temperature

Useful for identifying phase transitions (ie. conformational changes)

2) Isothermal Titration Calorimetry (ITC)

Measure changes in enthalpy as a function of protein or ligand concentration

Preferred method for quantifying binding interactions

Note: 'bomb' calorimetry is a constant volume measurement $q_v = \Delta E$

DSC and ITC are constant pressure measurements $q_p = \Delta H$

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Experimental Calorimetry

The K_{eq} of a conformational change or binding interactions can be measured by identifying the concentrations of all components at equilibrium

From the K_{eq}, it is straightforward to calculate $\Delta G°$

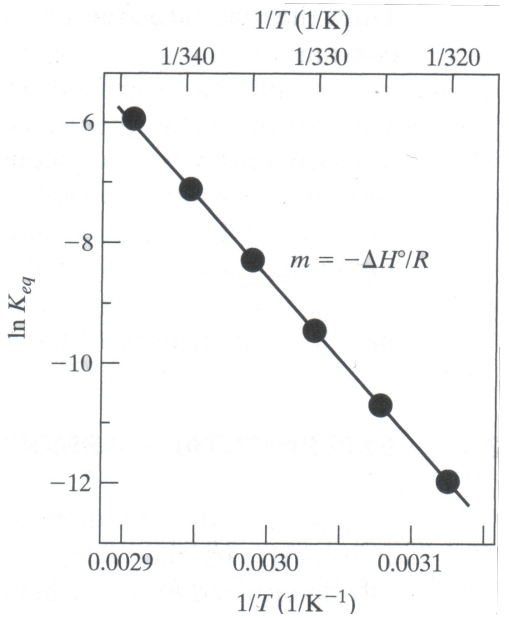
$$\Delta G° = -RT \ln K_{eq}$$

How do we measure the $\Delta H°$ and $\Delta S°$ components?

van't Hoff Relationship

$\Delta G° = - RT \ln K_{eq} = \Delta H° – T\Delta S°$, rearranging yields

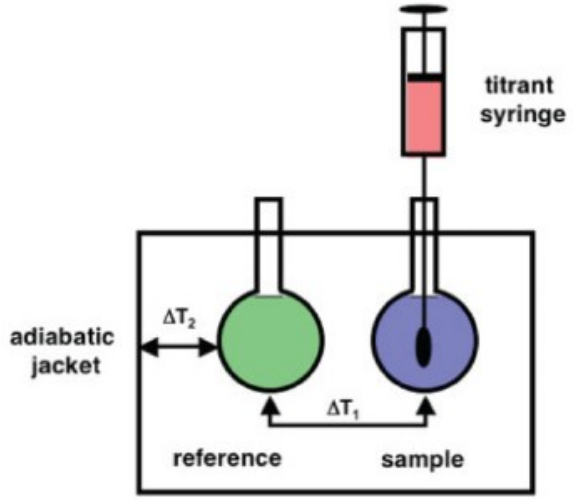
$\ln K_{eq} = -(\Delta H° – T\Delta S°)/RT$ or $\ln K_{eq} = (1/R)(\Delta S° - \Delta H°/T)$



This is an equation for a straight line with slope ($– \Delta H°/R$) and intercept ($\Delta S°/R$)

Thus, the enthalpy (and entropy) of a process can be determined by measuring K_{eq} at multiple temperatures

DSC



Experimental Setup

Energy is simultaneously added to two 'cells' while keeping T constant

A) Sample cell containing protein and buffer system

B) Reference cell containing buffer system

Energy is absorbed (or released) by the buffer system in the reference cell and by both the protein and buffer system in the sample cell

In order to keep T constant, additional energy in the form of electrical energy is added to one of the cells

The difference in input energy is proportional to the amount of excess heat absorbed (endothermic) or released (exothermic) by the protein sample

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DSC

Excess heat absorbed or released is proportional to the heat capacity C_p of the protein

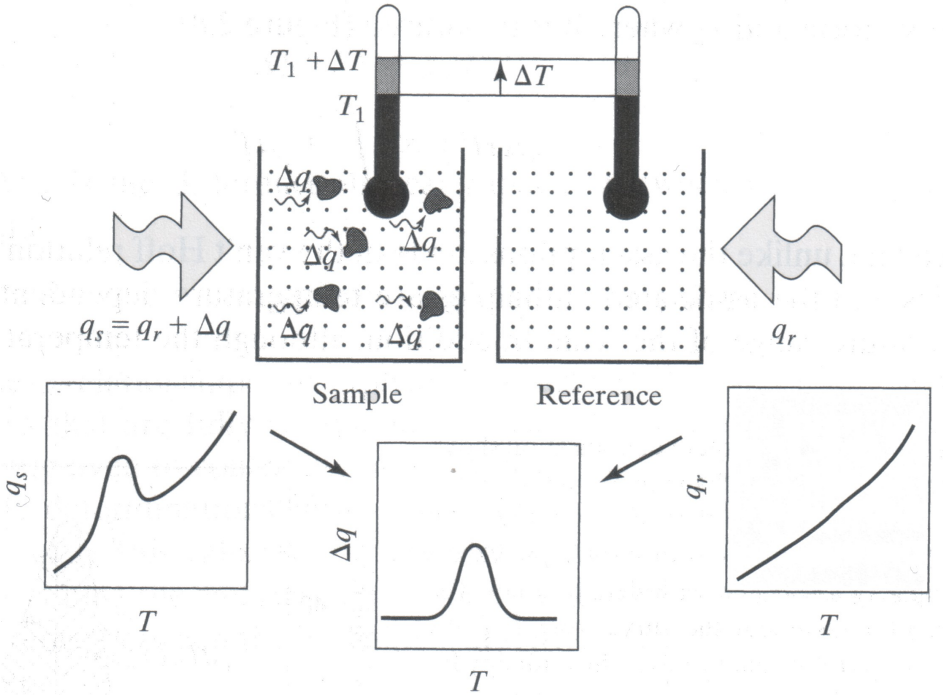
$C_p = dq / dt$ and at constant P $C_p = dH / dT$

Thermal denaturation

- **C_p for native and denatured state of a differ**
- **Average enthalpy of denaturation ($\Delta H°$) is determined by integrating the area of the resulting bell-shaped curve**

At T_m, $\Delta G°_m = 0$ and $\Delta S°_m = \Delta H°_m / T_m$

- **For simple two state models, the T dependence of $\Delta H°$ and $\Delta S°$ can be calculated**



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DSC

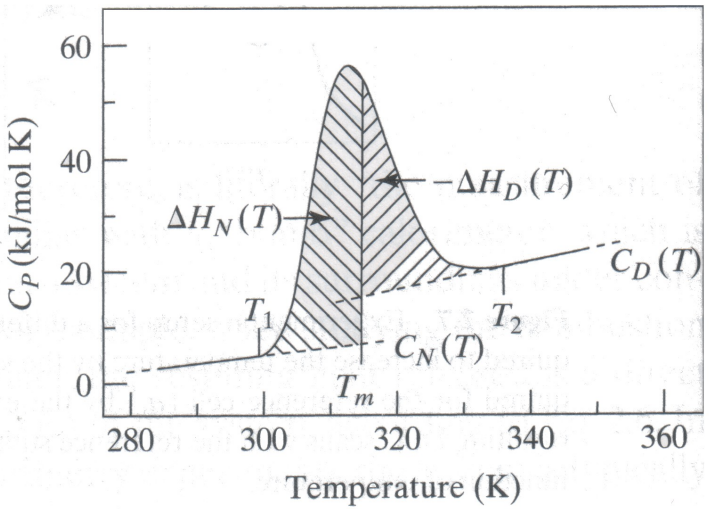
The ratio of native and denatured protein (at some T) is proportional to the ratios of their ΔH° (at the same T)

Treating the ratio at an equilibrium constant, we can use the van't Hoff relationship to derive the following expressions

$$\Delta H^\circ_{vH}(T) = -RT^2 \left[\left(\Delta C_n(T)/\Delta H^\circ_n(T)\right) - \left(\Delta C_d(T)/\Delta H^\circ_d(T)\right) \right]$$

Simple the diff

Thus the heat capacity, enthalpy and fraction bound determine the overall enthalpy



A plot of heat capacity vs T derived from a DSC power vs T scan. The T_m is the midpoint of the integrated area calculation

Isothermal Titration Cal.

Preferred method for study of the energetics of binding events

- **Measures amount of electrical energy required to kee sample and reference cell at constant T (just like DSC)**

Dependent upon enthalpy at a particular temperature and the moles of complex formed

$$q = \Delta H°(T)\, n_{PL} = \Delta H°(T)\, V\, [PL]$$

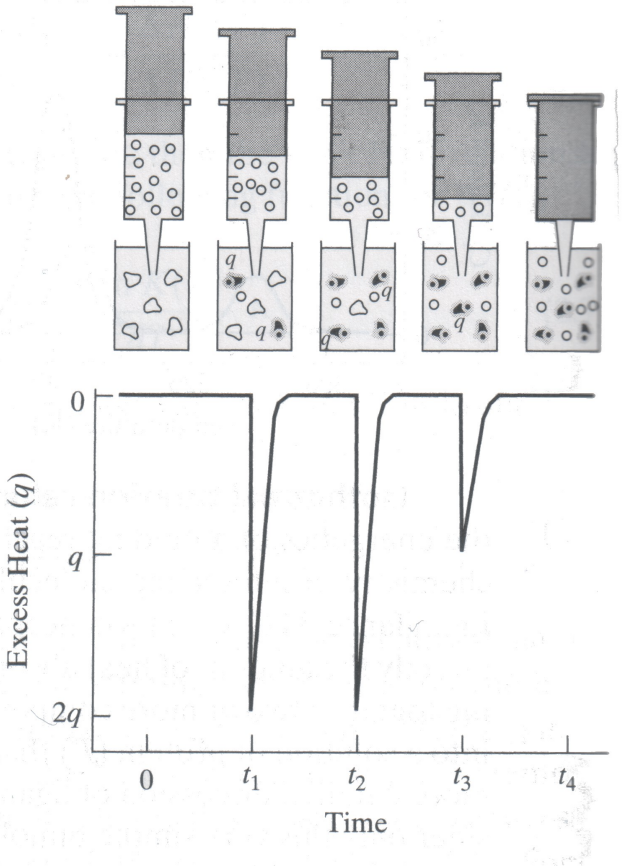
Note: For the equilibrium $P + L \leftrightarrow PL$

$$[PL] = [P_T]\,(K_a[L] / (1 + K_a[L]))$$

(Equilibrium and Conservation equations)

Thus the integrated area of a peak is

$$q = \Delta H°(T)\, V\, [P_T]\, \{(K_a[L] / (1 + K_a[L])_i - (K_a[L] / (1 + K_a[L])_{i+1}\}$$



ITC

Typically, we do not know the K_a and therefore cannot know [L] (free form)

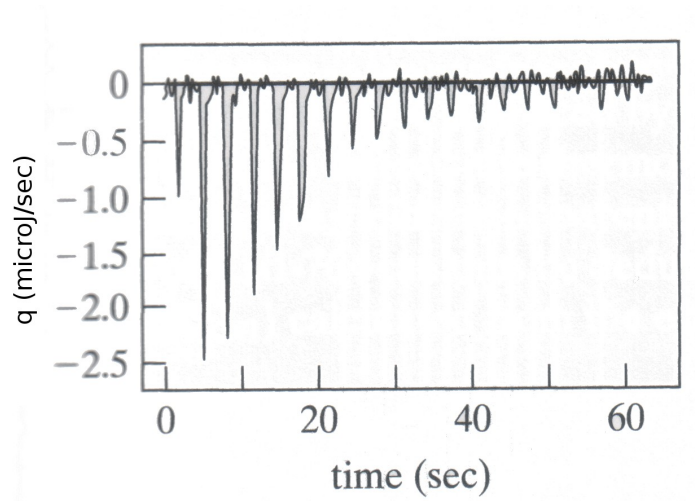
We only know the q and R (ratio of total L and P)

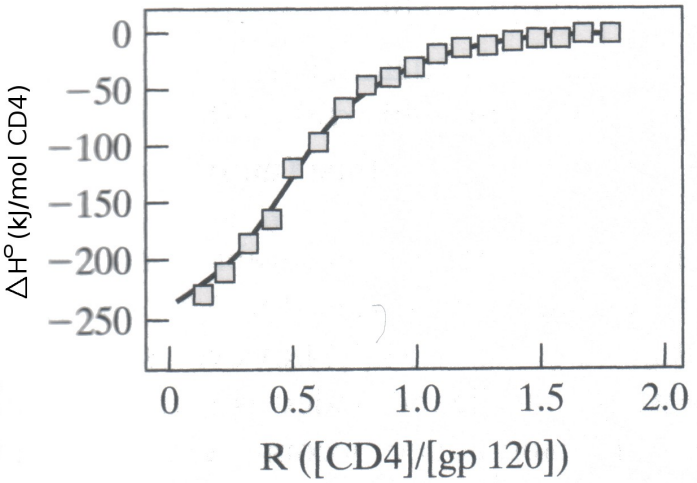
Curve fitting experimental q vs R data yields values for K_a and $\Delta H°(T)$

Note: Curve fitting requires expression for L in terms of L_T, P_T and K_a

$$[L]_i = (1/2)([L]_T - [P]_T - 1/K_a \pm ([L]_T - [P]_T - 1/K_a)^2 - 4[L]_T)^{1/2}$$

ie. Solving a quadratic equation in terms of L





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ITC Limits

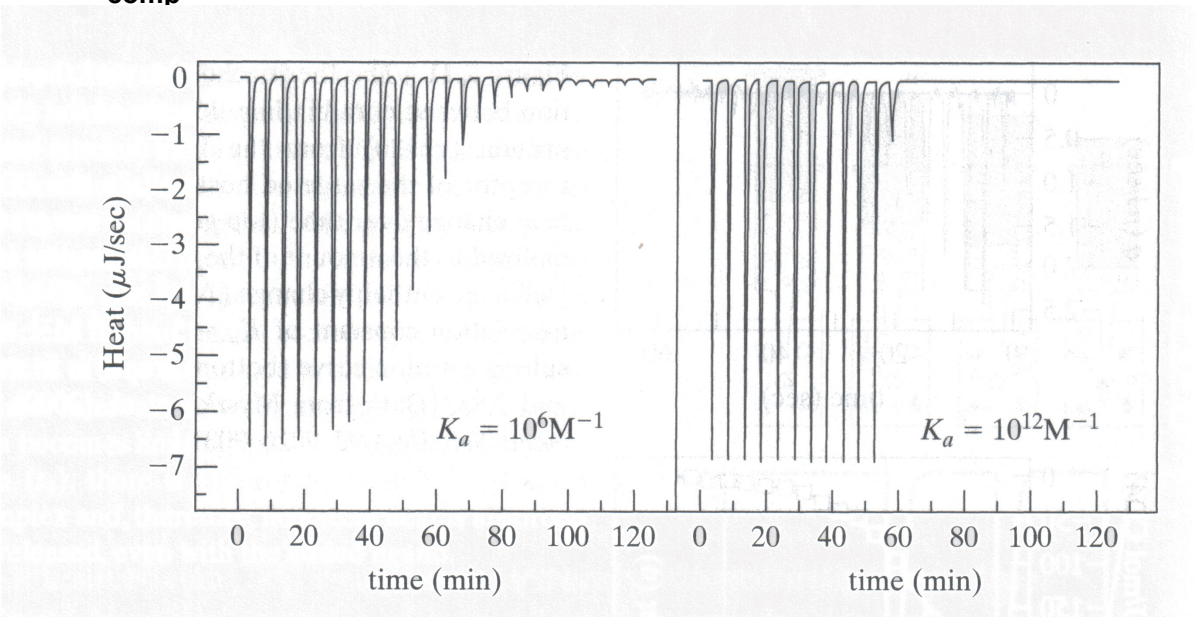
ITC cannot directly measure K_a values below nM

- **The q vs R transition is too sharp to produce points for curve fitting**
  - **ie. When [L]T > [P]T, [P] ~ 0**

Only option is to utilize a competition experiment

- **Requires characterization of binding of competitor followed by**

$K_{app}=K_a/(1+K_{comp}[Comp])$



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ITC 'c value'

ITC uses relatively large volumes of sample (~1.4 mL)

The 'c-value' is Ka · [sample] and defines the shape of the resulting ITC curve

10 < c < 100 produces optimal data

c < 10 produces a too shallow curve and the curve is not completed in a single experiment

c > 100 produces a too sharp curve that cannot be fit accurately