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GENERALIZED BINDING PHENOMENA IN AN ALLOSTERIC MACROMOLECULE

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A general macromolecular partition function is developed in terms of chemical ligand activity, temperature and pressure for systems described by an array of species which are characterized by their state of allosteric conformation and ligand stoichiometry. The effects of chemical ligand binding, enthalpy change, and volume change are treated in a parallel manner. From a broad viewpoint all of these effects can be regarded as specific cases of generalized binding phenomena. This approach provides a general method for analyzing calorimetric and ligand binding experiments. Several applications are given: (1) Thermal scanning data for $tRNA^{phe}$ (P.L. Privalov and V.V. Filimonov, J. Mol. Biol. 122 (1978) 447) are shown to fit a general model with six conformational states. By application of linkage theory it is shown that sodium chloride is expelled as the molecule denatures. (2) The results of calorimetric titrations on the arabinose binding protein (H. Fukada, J.M. Sturtevant and F.A. Quiocho, J. Mol. Biol. 258 (1983) 13193) are shown to fit a simple two-state allosteric model. (3) A thermal binding curve is simulated for an unusual respiratory protein, trout I hemoglobin (B.G. Barisas and S.J. Gill, Biophys. Chem. 9 (1979) 235), in order to illustrate both the similarities and differences between enthalpy and chemical ligand binding processes.

1. Introduction

The analysis of both ligand binding and thermal transition phenomena has an extensive literature. In general, each area has been treated separately and the features common to both types of analysis have not been recognized. The intent of this study is to develop a theoretical framework which treats such phenomena from a unified viewpoint. In an earlier paper [1] we considered the effect of temperature upon the melting behavior of a nondissociating allosteric macromolecule. It was noted that a strong parallel exists between the description of transitions induced by heat and those induced by ligand binding. We wish to develop this parallelism further in this paper.

One finds the origin of this similarity in the common thermodynamic basis of these phenomena. In particular, the chemical potential of a macromolecule, μ_m, is dependent upon the physical variables, temperature (T) and pressure (p), and the chemical potentials of various components (μ_x, μ_y, etc.) by the Gibbs-Duhem equation *:

$$d\mu_m = -\bar{S}dT + \bar{V}dp + \bar{X}d\mu_x + \ldots \quad (1)$$

The entropy ($\bar{S}$), volume ($\bar{V}$), and amount of chemical ligand ($\bar{X}$) have been normalized to the moles of macromolecule as denoted by the superscript bar. The parallel between thermal effects and chemical ligand binding can be seen from eq. 1. Ligand binding is described in terms of $\bar{X}$; the exact parallel in thermal phenomena is $\bar{S}$, and the analog for pressure effects is $\bar{V}$. Entropy is a special quantity because its presence is known to us only indirectly through heat measurements, in accordance with the relation $dS = dQ/T$, where Q

* A more extensive description of the macromolecular chemical potential is contained within the concept of the binding potential [2,3].

is the heat absorbed under reversible conditions. Because of the thermodynamic parallelism between $\bar{S}$, $\bar{V}$, and $\bar{X}$ it is useful to think in terms of a generalized concept of binding. Formally this sets the stage for exploring the common features of thermal and ligand binding phenomena.

We usually describe binding in terms of definite interactions or changes that take place with the macromolecule. In particular we are interested in the amount of ligand bound to the macromolecule, and we disregard the amount of ligand which is free in solution. This applies most obviously to the determination of chemical ligand binding. The concept also applies with equal validity to binding of heat and volume, and we shall consider these terms to be physical binding parameters. The term 'binding' is used here in the operational sense and refers to the heat released or volume changes that are associated with particular reaction processes involving the macromolecule. In particular, one needs to delineate the amounts of these quantities which are specifically attributed to the presence of the macromolecule [2]. The determination of specific binding parameters then requires an operational procedure for excluding nonspecific effects. Dialysis and spectroscopic procedures are useful in determining specific chemical ligand binding. Calorimetry and densitometry serve to determine the specific thermal and volume effects due to the presence of the macromolecule.

The analysis of experiments, which consider thermal effects and chemical binding separately, has been developed for thermally induced transitions [4–6] and the temperature dependence of heats of reaction of specific ligand binding [7]. A thermodynamic development of linked binding effects was used to describe the effect of pH and NaCl concentration on the melting of DNA double helix [8] and to validate the concept of macromolecular state functions for lysozyme thermal transitions and guanidine hydrochloride binding reactions [9–11].

By recognizing the common thermodynamic features of physical and chemical binding it is possible to bring these phenomena within the same theoretical framework. We wish to show how this is done for the general situation of a macromolecule which is capable of undergoing a number of allosteric transitions to different states, each of which can specifically bind chemical ligands. The most useful representation of the system is given in terms of a partition function [6,12–15], from which the specific effects of physical and chemical binding can be derived.

The practical application of this formalism will be illustrated for two types of thermal experiments, temperature scanning calorimetry and isothermal titration calorimetry. By considering the more general aspects of binding it is possible to analyze such experiments in terms of linkage between the physical quantities, heat and volume, and the chemical processes which are the underlying basis of such changes. These relations have largely been overlooked before.

2. Theory

In this section we wish to examine the effects of temperature, pressure, and chemical ligand activity upon the state of a macromolecular system. Consider the situation in which a macromolecule exists in several different conformations in equilibrium with one another. We assume that each of these allosteric forms has a number of binding sites for a set of different chemical ligands. * We shall consider the specific case where the macromolecule has $q + 1$ conformations, 0, 1, ...q, each of which has r binding sites for a ligand X. ** The macromolecular partition function is defined by the sum of the relative concentrations of all macromolecular species which correspond to the different conformational and ligation states. Fig. 1 shows a representation of the macromolecule in its different states which are specified by conformational form and number of X ligands bound.

* The familiar two-state MWC allosteric model has only two conformational forms, each of which can bind a number of ligands independently [16]. The general form of this model has been especially useful in describing cooperative effects found in the binding of chemical ligands, but it also proves useful in describing thermal effects caused by temperature changes or chemical ligation.

** In the general case each allosteric form may have a different number of binding sites and can be treated by suitable modification of the derivation presented here.

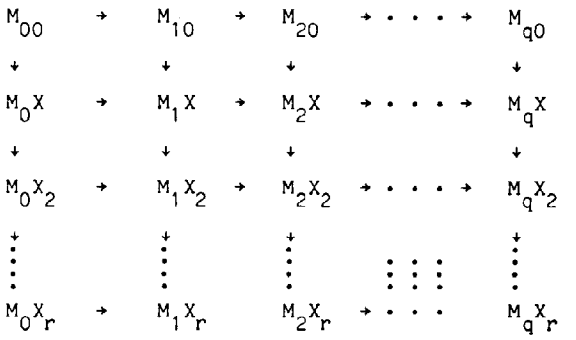

Fig. 1. Array of conformational and chemically ligated species. Columns represent different conformational forms and rows denote different amount of ligand X bound.

Chemical ligation and conformational change can be represented by the following reaction:

$$M_{00} + jX \rightarrow M_iX_j \tag{2}$$

where M_{00} is the unligated macromolecule in the zeroth conformational form, j the number of ligands which are involved in the specific binding reaction, and M_iX_j the macromolecule in the i-th conformational form with j ligands of X bound. The reaction is characterized by changes in enthalpy ($\Delta\bar{H}_{ij}$), the amount of X ligand bound ($\Delta\bar{X}_{ij}$) and volume ($\Delta\bar{V}_{ij}$). Generally the reference state is unligated and thus $\Delta\bar{X}_{ij} = j$.

The concentration of the M_iX_j species is proportional to the concentration of M_{00}:

$$[M_iX_j] = [M_{00}]\beta_{ij} \tag{3}$$

Here we have defined β_{ij} to be a general equilibrium constant for reaction 2 which depends upon ligand activity, a_x, as well as temperature and pressure. The binding partition function Q is the sum of all species concentrations taken relative to the reference species M_{00}:

$$Q = \sum_{i=0}^{q}\sum_{j=0}^{r}\frac{[M_iX_j]}{[M_{00}]} = \sum_{i=0}^{q}\sum_{j=0}^{r}\beta_{ij} \tag{4}$$

The partition function * of eq. 4 represents the rectangular array of species shown in fig. 1. This array can be reduced in two ways: (1) collecting all the various states of ligation according to their specific allosteric forms or (2) collecting the allosteric forms according to their ligand stoichiometry. In the first case we initially sum the elements of the array over the degree of ligation for each allosteric form i. This sum defines an equilibrium constant L_i which represents the concentration of all chemically ligated forms of the allosteric state i relative to the reference species M_{00}:

$$L_i = \sum_{j=0}^{r}\beta_{ij} \tag{5}$$

In terms of allosteric forms, Q is then given by

$$Q = \sum_{i=0}^{q} L_i \tag{6}$$

Alternatively, we may initially sum over the allosteric forms, collecting the specific terms with j ligands bound. This yields another set of equilibrium constants, defined as β_j, which represent the total concentrations of all species with j ligands bound, relative to the concentration of the M_{00} references species. This is expressed as follows:

$$\beta_j = \sum_{i=0}^{q}\beta_{ij} \tag{7}$$

* Eq. 4 may also be obtained from the traditional partition function given by the sum of all states for a system:

$$Q = \Sigma g_n e^{-\epsilon_n/kT}$$

where g is the degeneracy of energy state ϵ_n. The number of molecules in state n, N_n, is then given as:

$$N_n = N g_n e^{-\epsilon_n/kT}/Q$$

where N is the total number of molecules. In terms of the ground state ($n = 0$)

$$N_n = N_0(g_n/g_0)e^{-(\epsilon_n-\epsilon_0)/kT}$$

which then allows Q to be expressed as

$$Q = g_0 e^{-\epsilon_n/kT}\Sigma(N_n/N_0)$$

Defining the free energy as $G_n = \epsilon_n - kT\ln g_n$ and setting $G_0 = 0$ yields:

$$Q = \Sigma(N_n/N_0)$$

This expression is equivalent to eq. 4 provided one specifies the state of a molecule in terms of the degree of ligation and allosteric form.

and

$$Q = \sum_{j=0}^{r} \beta_j \tag{8}$$

In studies of chemical ligand binding one usually chooses the reference state to include all the unligated allosteric forms. This is accomplished by normalizing the partition function of eq. 8 by dividing by β_0. This gives Q in the form in which the various coefficients serve to define the Adair parameters. This form is also termed the binding polynomial once it is recognized that each β_j term contains the ligand activity to the j-th power.

The thermodynamic properties due to the binding reactions may be evaluated by partial differentiation of $\ln \beta_{ij}$ with respect to the appropriate independent variables of the system:

$$\frac{\partial \ln \beta_{ij}}{\partial \ln a_x} = \Delta \bar{X}_{ij} \tag{9}$$

$$\frac{\partial \ln \beta_{ij}}{\partial \tau} = \frac{-\Delta \bar{H}_{ij}}{R}, \tau = 1/T \tag{10}$$

$$\frac{\partial \ln \beta_{ij}}{\partial p} = \frac{-\Delta \bar{V}_{ij}}{RT} \tag{11}$$

In order to obtain the total amount of ligand, enthalpy, or volume change which occurs upon taking the macromolecular system from its reference state to any general state of the system, one differentiates $\ln Q$ with respect to the appropriate independent variable. For the case of chemical ligation this gives:

$$\frac{\partial \ln Q}{\partial \ln a_x} = \frac{1}{Q} \sum_i \sum_j \frac{\partial \ln \beta_{ij}}{\partial \ln a_x} \beta_{ij}$$

$$= \frac{1}{Q} \sum_i \sum_j \left(\Delta \bar{X}_{ij}\right) \beta_{ij} = \bar{X} - \bar{X}_{00} \tag{12}$$

The term $\bar{X}_{00}$ represents the amount of X ligand bound to the reference state and has been retained here to account for the general situation where the amount of X ligand bound to this state may not be zero. The fraction of macromolecules of the type represented by M_iX_j is given by β_{ij}/Q. This expression allows one to see that the last term of eq. 12 is obtained by taking the weighted sum of the $\Delta \bar{X}_{ij}$ values over all species.

Similarly, the amount of enthalpy and volume change involved in going from the reference state to a general state of the macromolecule, $\bar{H} - \bar{H}_{00}$ and $\bar{V} - \bar{V}_{00}$, are obtained by differentiation in $\ln Q$ with respect to τ and p. The inclusion of $\bar{H}_{00}$ and $\bar{V}_{00}$ is necessary because the enthalpy and volume for the M_{00} reference state are not zero. In summary we write:

$$\bar{X} - \bar{X}_{00} = \frac{\partial \ln Q}{\partial \ln a_x} \tag{13}$$

$$\bar{H} - \bar{H}_{00} = -R \frac{\partial \ln Q}{\partial \tau} \tag{14}$$

$$\bar{V} - \bar{V}_{00} = -RT \frac{\partial \ln Q}{\partial p} \tag{15}$$

The parallel form of these expressions serves to emphasize how chemical and physical binding phenomena can be considered in the same general context.

The preceding development stresses the general relation between the partition function and $\bar{X}$, $\bar{H}$ and $\bar{V}$. A more detailed view of the factors affecting these quantities is obtained by using a representation formulated in terms of the fraction of the macromolecules in the i-th form with j ligands bound, α_{ij}:

$$\alpha_{ij} = \frac{1}{Q} \beta_{ij} \tag{16}$$

In these terms, the average amount of chemical ligand bound per mole of macromolecule is given by the sum of the fraction of molecules in a given state times the amount of ligand bound to that state. The average enthalpy and volume are expressed in a similar manner. The resulting equations are given as follows:

$$\bar{X} - \bar{X}_{00} = \sum_i \sum_j \Delta \bar{X}_{ij} \alpha_{ij} \tag{17}$$

$$\bar{H} - \bar{H}_{00} = \sum_i \sum_j \Delta \bar{H}_{ij} \alpha_{ij} \tag{18}$$

$$\bar{V} - \bar{V}_{00} = \sum_i \sum_j \Delta \bar{V}_{ij} \alpha_{ij} \tag{19}$$

As shown by these equations, in order to evaluate $\bar{X}$, $\bar{H}$, or $\bar{V}$ in any specific problem one needs the

array of species parameters, $\Delta\overline{H}_{ij}$, $\Delta\overline{V}_{ij}$, and $\Delta\overline{X}_{ij}$, along with the values of the species fractions α_{ij}. In chemical ligand binding, $\Delta\overline{X}_{ij}$ ranges in integer values from 0 to r. In thermal binding the values of $\Delta\overline{H}_{ij}$ are not necessarily integer or positive. This is also true for the values of $\Delta\overline{V}_{ij}$. This means that the description of chemical ligand binding is inherently much simpler than that for heat and volume effects. In fig. 2 we contrast the integer 'staircase' situation of chemical ligand binding to the situation which results from an arbitrary set of $\Delta\overline{H}_{ij}$ values.

In order to describe the explicit dependence of Q upon the independent variables, $\ln a_x$, τ and p, we need to express β_{ij} in terms of these variables. This can be done by integrating eqs. 9–11 as follows:

$$\int_{\ln(\beta_{ij})_0}^{\ln\beta_{ij}} \mathrm{d}\ln\beta_{ij} = -\int_{\tau_0}^{\tau}\frac{\Delta\overline{H}_{ij}}{R}\mathrm{d}\tau - \int_{p_0}^{p}\frac{\Delta\overline{V}_{ij}}{RT}\mathrm{d}p + \int_{\ln a_{x0}}^{\ln a_x}\Delta\overline{X}_{ij}\,\mathrm{d}\ln a_x \qquad (20)$$

For simplicity let us assume that $\Delta\overline{H}_{ij}$ is independent of temperature (i.e., the heat capacity change $\Delta\overline{C}_{ij}$ is zero), $\Delta\overline{V}_{ij}$ is independent of pressure (i.e., the compressibility is the same for all species) and $\Delta\overline{X}_{ij} = j$ (i.e., the reference state M_{00} has no ligand bound). Integration from the reference condition of τ_0, p_0 and a_{x0} then gives the expression for the equilibrium constant between any form and the reference form M_{00} as:

$$\beta_{ij} = (\beta_{ij})_0 e^{-(\Delta\overline{H}_{ij}/R)(\tau-\tau_0)} e^{-(\Delta\overline{V}_{ij}/RT)(p-p_0)} \times e^{j(\ln a_x - \ln a_{x0})} \qquad (21)$$

Here $(\beta_{ij})_0$ is the equilibrium constant under the reference state conditions defined by τ_0, p_0 and a_{x0}. *

The partition function Q as found by substitution into eq. 4 is given as:

$$Q = \sum_{i=0}^{q}\sum_{j=0}^{r}(\beta_{ij})_0 e^{-(\Delta\overline{H}_{ij}/R)(\tau-\tau_0)} e^{-(\Delta\overline{V}_{ij}/RT)(p-p_0)} \times e^{j(\ln a_x - \ln a_{x0})} \qquad (22)$$

The first two exponentials in this expression account for the effect of the physical variables T and p while the third exponential accounts for the effect of the chemical ligand X. The presence of additional ligands will contribute exponential terms analogous to that of X with appropriate summations.

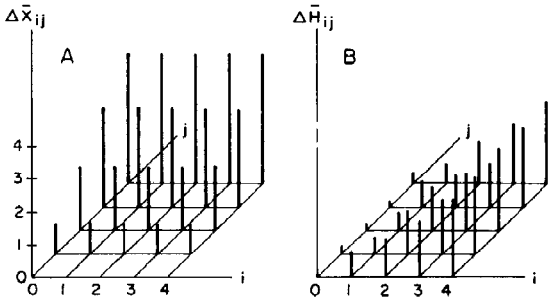

Fig. 2. (A) Representation of allowable values ($\Delta\overline{X}_{ij}$) for chemical ligand binding per mole of macromolecule versus the allosteric forms (i) and the number of ligands bound (j). Note the values of $\Delta\overline{X}_{ij}$ must be integers. (B) Representation of the enthalpy change values, $\Delta\overline{H}_{ij}$, per mole of macromolecule versus the allosteric forms (i) and the number of ligands bound (j). The values of $\Delta\overline{H}_{ij}$ are in general arbitrary and are here shown for the situation where the heats of ligation are small compared to the heats of transition between allosteric forms.

3. A simple case: Allosteric forms with equal heat capacities

The preceding development has been directed towards the general aspects of multi-state equilibria in order to show the features which are common to the description of thermal and ligand binding phenomena. For practical situations this general formulation has far too many parameters to evaluate and one must resort to simplifying assumptions. In this section we wish to develop a description for the total enthalpy and heat capacity of a system in which the difference between the heat capacities of allosteric forms is effectively zero. This case is useful for systems whose allo-

* In the case where the heat capacity change $\Delta\overline{C}_{ij}$ is not zero an additional term,

$(\tau/\tau_0)^{-\Delta\overline{C}_{ij}/R} e^{(\Delta\overline{C}_{ij}/R\tau_0)(\tau-\tau_0)}$

needs to be included in eq. 21.

steric forms do not show appreciable intrinsic heat capacity differences and whose heats of ligand binding are small compared to the heats of allosteric transitions. Studies on the thermal denaturation of tRNAs generally satisfy these criteria whereas studies on the thermal denaturation of proteins almost certainly do not.

By assuming the heat of ligation is small compared to the heat of transition we focus attention on the average enthalpy change associated with transitions from one allosteric form to another. The average enthalpy change ($\Delta\bar{H}_i$) involved in the transition from the M_{00} reference state to the i-th allosteric state is in general obtained by summation over the liganded species of the i-th form as follows:

$$\Delta\bar{H}_i = \frac{\sum_j \alpha_{ij}\Delta\bar{H}_{ij}}{\sum_j \alpha_{ij}} \tag{23}$$

We assume for the special case under consideration that the heat capacity change is effectively zero. Then the equilibrium constant, L_i, for this transition may be written as:

$$L_i = L_i^0 e^{(-\Delta\bar{H}_i/R)(\tau-\tau_0)} \tag{24}$$

where L_i^0 is the effective equilibrium constant between the reference form (M_{00}) and the sum of all ligated i-th form species at τ_0.

In an actual experiment the total enthalpy of the macromolecular system is measured relative to the enthalpy of its native form at low temperature. In general, this form consists of a number of species with different amounts of ligand bound. We thus need a new reference state which includes all such species of the zeroth allosteric form. This is accomplished by normalizing the partition function given by eq. 6 by dividing by the sum of all zeroth form species, namely L_0. An asterisk (*) is used to denote quantities based on this total zeroth form reference state. With this reference state we define the partition function Q^* as:

$$Q^* = \frac{Q}{L_0} = \frac{\Sigma L_i}{L_0} = \Sigma L_i^* \tag{25}$$

In terms of this reference state, eq. 24 becomes:

$$L_i^* = L_i^{*0} e^{(-\Delta\bar{H}_i^*/R)(\tau-\tau_0)} \tag{26}$$

where now L_i^{*0} is the standard state ($\tau = \tau_0$) equilibrium constant and $\Delta\bar{H}_i^*$ is the change in enthalpy for the reaction between the i-th and zeroth forms. The fraction α_i of macromolecules in the i-th form is given as:

$$\alpha_i = \frac{L_i^{*0} e^{-\Delta\bar{H}_i^*(\tau-\tau_0)/R}}{\Sigma L_i^{*0} e^{-\Delta\bar{H}_i^*(\tau-\tau_0)/R}} \tag{27}$$

The total enthalpy $\bar{H}$ minus the enthalpy of the zeroth form is given as:

$$\bar{H} - \bar{H}_0 = \frac{\Sigma\Delta\bar{H}_i^* L_i^{*0} e^{-\Delta\bar{H}_i^*(\tau-\tau_0)/R}}{\Sigma L_i^{*0} e^{-\Delta\bar{H}_i^*(\tau-\tau_0)/R}} \tag{28}$$

The apparent excess heat capacity of the system is found by differentiating the enthalpy with respect to T:

$$\bar{C} - \bar{C}_0 = \frac{\tau^2}{R}\left[\frac{\Sigma\left(\Delta\bar{H}_i^*\right)^2 L_i^{*0} e^{-\Delta\bar{H}_i^*(\tau-\tau_0)/R}}{\Sigma L_i^{*0} e^{-\Delta\bar{H}_i^*(\tau-\tau_0)/R}} - \frac{\left(\Sigma\Delta\bar{H}_i^* L_i^{*0} e^{-\Delta\bar{H}_i^*(\tau-\tau_0)/R}\right)^2}{\left(\Sigma L_i^{*0} e^{-\Delta\bar{H}_i^*(\tau-\tau_0)/R}\right)^2}\right] \tag{29}$$

Eq. 29 reflects the heat capacity effects which are associated with alterations in the distribution of allosteric forms brought about by a change in temperature. Even in this simplified case, where the enthalpy changes between forms are assumed to be independent of temperature, two parameters are needed to describe the binding of enthalpy to each form in the system: the relative enthalpy of the i-th form ($\Delta\bar{H}_i^*$) and the value of L_i^{*0} at τ_0. It is also worth noting that both the equilibrium constant L_i^{*0} and the enthalpy change $\Delta\bar{H}_i^*$ depend on the chemical ligand activity.

It is pertinent to examine the question of how small the heat of ligation must be, in comparison to the heats of transition, for the assumption of equal heat capacities of all forms to be valid. We utilize an MWC model [16] which assumes that the macromolecule exists in $q + 1$ allosteric forms each of which binds the ligand X independently with a

binding constant (κ_i) that depends solely upon the allosteric form. The partition function may be written as:

$$Q^{\mathrm{MWC}} = \sum_{i=0}^{q} (\beta_{i0})(1 + \kappa_i a_x)^r \tag{30}$$

Note that in this formulation the effect of X ligand activity, a_x, is shown explicitly, and the sum of ligand species of a given allosteric form is represented by the polynomial. The allosteric equilibrium constant β_{i0}, and the intrinsic X binding constant κ_i, depend implicitly on temperature and pressure. This dependence is determined by the enthalpy and volume changes for the reactions which define these equilibrium constants. The enthalpy of the i-th allosteric form relative to that of the reference species may be obtained by the procedure specified by eq. 14 for each term in the summation of eq. 30 as follows:

$$\Delta \bar{H}_i = \Delta \bar{H}_{i0} - R \frac{\partial \ln(1 + \kappa_i a_x)^r}{\partial \tau}$$

$$= \Delta \bar{H}_{i0} - R \frac{r \kappa_i a_x}{1 + \kappa_i a_x} \frac{\partial \ln \kappa_i}{\partial \tau} \tag{31}$$

where $\Delta \bar{H}_{i0}$ is the enthalpy change associated with going from the reference M_{00} state to the unligated i-th allosteric form. The enthalpy change for binding X to an individual site in the i-th form, $\Delta \tilde{h}_i$, is given by the van't Hoff equation [10] employing the intrinsic constant κ_i. The effect of temperature and X ligand activity on $\Delta \bar{H}_i$ can be seen explicitly by integrating the van't Hoff expression for κ_i, substituting the result into eq. 31, and expanding the exponential terms about a reference temperature T_0. The result to first order in temperature change, ΔT, is:

$$\Delta \bar{H}_i = \Delta \bar{H}_{i0} + \frac{r \kappa_i^0 a_x \Delta \tilde{h}_i}{1 + \kappa_i^0 a_x} + \frac{r \kappa_i^0 a_x (\Delta \tilde{h}_i)^2 \Delta T}{(1 + \kappa_i^0 a_x)^2 R T T_0} + \cdots \tag{32}$$

This expression separates the total enthalpy change associated with the allosteric form, $\Delta \bar{H}_i$, into three components. The first term of eq. 32 is simply the enthalpy change associated with the allosteric transition in the absence of ligand. The second term describes contributions to the enthalpy change which are due directly to ligand binding effects. The third term represents heat capacity effects due to changes in the amount of ligand bound with temperature. When this third term is sufficiently small compared to the first two terms then we meet the criteria assumed in the derivation of eqs. 24–30. This essentially means that if the heat of ligand binding $\Delta \tilde{h}_i$ is of the order of RT and the fractional temperature range $\Delta T/T$ is less than $1/10$, then the formulation given by eq. 29 is applicable.

4. The independent transition model

Independent ligand binding reactions and independent thermal transitions are the simplest ways to model the thermodynamics of a macromolecular system. Thermal melting curves of tRNA have been analyzed using this framework extensively. The independent transition model assumes that the macromolecule consists of a set of n independent segments or domains, each of which exists in only two conformations (such as native or denatured). The total number of allosteric states of such a macromolecule then consists of all combinations of the independent substates. Assuming each substate is uniquely characterized by a set of thermodynamic parameters then the total number of allosteric states will be 2^n. The partition function for this case is obtained by multiplication of the partition functions for each individual domain. The partition function for the ith domain (two states) is:

$$q_i = 1 + l_i \tag{33}$$

where l_i represents the equilibrium constant for the melting of the ith domain. The value of l_i will depend, as we have seen, upon the X ligand activity, the temperature, and the pressure. The essential feature we wish to illustrate is the temperature dependence of l_i. This is shown explicitly as follows:

$$q_i = 1 + l_i^0 e^{-\Delta h_i (\tau - \tau_i^0)/R} \tag{34}$$

where l_i^0 is the equilibrium constant for the i-th

transition at τ_i^0 (and will depend in general upon ligand activity). The enthalpy change for the transition of the i-th segment is Δh_i and we have assumed that it is independent of temperature. If the reference reciprocal temperature, τ_i^0, is chosen at the midpoint temperature of the ith transition then $l_i^0 = 1$. In this case the reference reciprocal temperature is denoted by τ_i^m.

The partition function, Q^{Indep}, for the entire macromolecule with n independent segments is given by the multiplication of all the contributions of the partition functions for each segment:

$$Q^{\text{Indep}} = \prod_{i=1}^{n} q_i \tag{35}$$

For the case given by eq. 34 with the definition of the midpoint reciprocal temperature we obtain:

$$Q^{\text{Indep}} = \prod_{i=1}^{n} \left(1 + e^{-\Delta h_i(\tau - \tau_i^m)/R}\right) \tag{36}$$

One observes by expansion of this expression that each term in the resulting sum represents an allosteric state whose properties are determined by the particular combination of the independent substate properties. The allosteric state is described by specifying those segments that have melted. The total enthalpy per mole of macromolecule ($\bar{H}$) is given as:

$$\bar{H} = \bar{H}_0 + \sum_{i=1}^{n} \frac{\Delta h_i e^{-\Delta h_i(\tau - \tau_i^m)/R}}{1 + e^{-\Delta h_i(\tau - \tau_i^m)/R}} \tag{37}$$

where $\bar{H}$ is the enthalpy of the unmelted native state. As might be expected, this result shows that the enthalpy of the system is the sum of the segment enthalpy changes weighted by the fraction of the particular segment melted.

The theoretical heat capacity is found by differentiation of eq. 37 with respect to T:

$$\bar{C} = \bar{C}_0 + \frac{\tau^2}{R} \sum_{i=1}^{n} \frac{(\Delta h_i)^2 e^{-\Delta h_i(\tau - \tau_i^m)/R}}{\left(1 + e^{-\Delta h_i(\tau - \tau_i^m)/R}\right)^2} \tag{38}$$

where $\bar{C}_0$ is the heat capacity of the reference state. The simplicity of this equation results from the fact that each segment transition contributes separately to the total heat capacity. In the general allosteric situation, the heat capacity (eq. 29) cannot in general be separated into contributions from each specific allosteric state. In this case, the thermal properties of the various states are interwoven with each other.

5. Applications

5.1. Scanning calorimetry

We wish to show how our general theoretical development applies to the analysis of specific experimental data. In view of the extensive experimental work done on the melting of transfer RNA [5,17], this system is particularly well suited for analysis in terms of the models developed here. Privalov and Filimonov [5] have presented extensive results on the thermal denaturation of various tRNAs. They give their results in terms of transition enthalpies and midpoint temperatures as obtained by fitting the experimental data to the independent transition model. Since they show that this analysis reproduces the data within experimental error, it is possible to reconstruct the experimental curves from their published parameters. We shall consider these reconstructed melting curves to be equivalent to the original experimental data. The original data are estimated to be reproducible within an error of a few percent [5].

It is informative to analyze these data from the more general thermal state treatment given by eq. 29. From this viewpoint we seek to determine the minimum number of allosteric forms that are required to describe the heat capacity data in accordance with the macromolecular partition function given by eqs. 25 and 26. All the states are ordered according to their enthalpy content with the reference state being set equal to zero. Each state is characterized by a particular enthalpy difference, $\Delta \bar{H}_i^*$, and an equilibrium constant, L_i^{*0}, at the reciprocal reference temperature τ_0.

The choice of the minimum number of enthalpy states is essentially governed by the fit between the theoretical and experimental heat capacity curves. Trial cases with different numbers of allosteric forms are used to generate best-fit heat capacity curves. This procedure is terminated when the

error of the fit is approximately equal to the actual experimental error.

To illustrate this analysis we have taken the independent transition enthalpies and temperatures obtained by Privalov and Filimonov [5] for the $tRNA^{Phe}$ in 20 and 150 mM NaCl and generated heat capacity differences for integral temperature values from 0 to 100°C using eq. 38. This gives the means for reconstructing the original observations. We then found by nonlinear least-square analysis the best values for the enthalpy changes $\Delta \bar{H}_i^*$ and associated L_i^{*0} parameters for cases with various numbers of allosteric forms using eq. 29. The criteria of fit determined from the least-squares analysis is the standard error of a point. The value of this measure decreases as more allosteric forms are included in the fitting procedure. The results are shown in tables 1 and 2. From the data of Privalov and Filimonov the actual experimental error appears to be about 1% of the maximum excess heat capacity value. Thus, it is seen that the analysis with six allosteric forms fits the data well within this criterion. Fig. 3 illustrates the curves obtained for the cases of five and six forms. It is seen that the system with five forms gives an inadequate fit whereas that with six gives a curve that is virtually indistinguishable from the constructed experimental curve.

Table 1

Enthalpy and temperature parameters for thermal state treatment of $tRNA^{Phe}$ melting in 0.150 M NaCl

Parameters were determined by least-square analysis of heat capacity data from ref. 5 on $tRNA^{Phe}$. Error: This is the standard errors of a point for the fit divided by the maximum value of the heat capacity of the data. Values for $\Delta \bar{H}_i^*$ are expressed as J/mol and for T_i as K. For convenience T_i is defined as $T_i = \Delta \bar{H}_i^* / (R \ln L_i^{*0})$ and $\tau_0 = 0$. This means that the reference state is chosen to be a very high temperature. The value of T_i gives the temperature at which $L_i^{*0} = 1$ in eq. 26.

State i	4-state model		5-state model		6-state model		7-state model	
	$\Delta \bar{H}_i^*$	T_i	$\Delta \bar{H}_i^*$	T_i	$\Delta \bar{H}_i^*$	T_i	$\Delta \bar{H}_i^*$	T_i
1	407258	321.12	298519	318.82	197649	315.72	194815	315.70
2	897329	326.18	691130	324.14	449623	320.01	441110	319.90
3	1310000	331.01	1031400	328.26	776445	324.08	716774	324.60
4			1310000	332.78	1064780	327.70	801134	324.75
5					1310000	331.93	1066640	327.82
6							1310000	331.93
Error	0.19		0.049		0.00095		0.00030	

Table 2

Enthalpy and temperature parameters for thermal state treatment of $tRNA^{Phe}$ melting in 0.020 M NaCl

Parameters were determined by least-square analysis of heat capacity data from ref. 5 on $tRNA^{Phe}$. Error: This is the standard errors of a point for the fit divided by the maximum value of the heat capacity of the data. Values for $\Delta \bar{H}_i^*$ are expressed as J/mol and for T_i as K.

State i	4-state model		5-state model		6-state model		7-state model	
	$\Delta \bar{H}_i^*$	T_i	$\Delta \bar{H}_i^*$	T_i	$\Delta \bar{H}_i^*$	T_i	$\Delta \bar{H}_i^*$	T_i
1	457773	312.95	223232	301.72	190961	299.19	187998	299.09
2	886084	317.16	617757	309.27	453993	306.64	381719	307.10
3	1252000	321.89	968116	313.9	722787	310.0	498676	307.82
4			1252000	318.34	1010530	313.82	729380	310.80
5					1252000	317.75	1011390	313.80
6							1252000	317.71
Error	0.17		0.046		0.00012		0.00010	

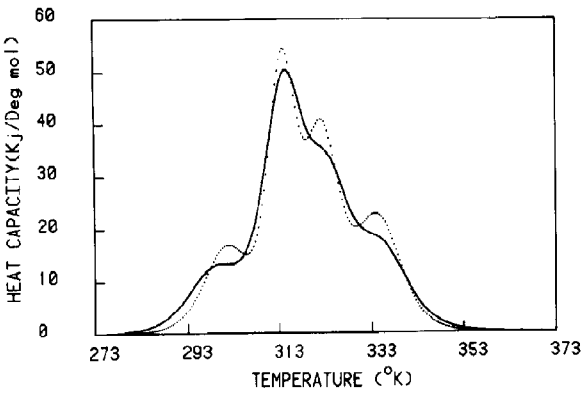

Fig. 3. A comparison of fit between the general allosteric state analysis (eq. 29) with 5 states (·····) and the calorimetrically determined melting curve (———) of $tRNA^{Phe}$ in 20 mM NaCl solution as determined by Privalov and Filimonov [5]. The parameters for the fits are listed in table 2. The inclusion of a sixth state provides a theoretical curve which is indistinguishable from the experimental data.

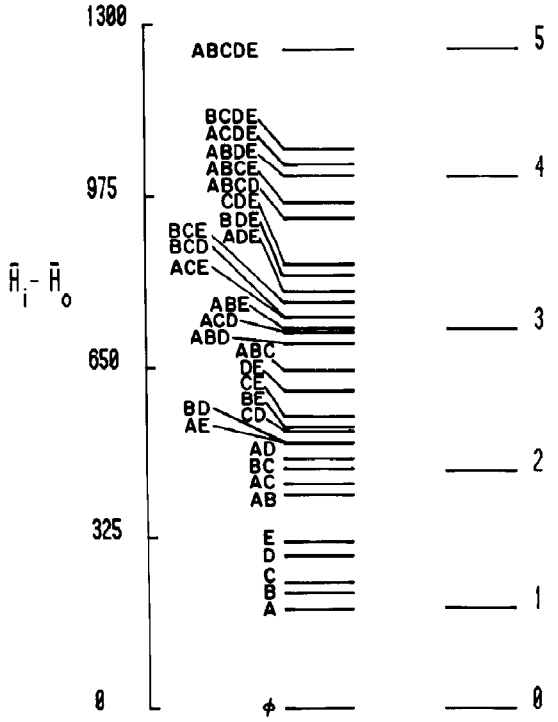

Fig. 4. Enthalpy level diagram for the independent transition model (left) and for the six allosteric state treatment (right) for $tRNA^{Phe}$ (20 mM NaCl) [5]. The independent thermal transition enthalpies are denoted as follows: A transition, 188 kJ; B transition, 218 kJ; C transition, 239 kJ; D transition, 289 kJ; E transition, 318 kJ. The allosteric state levels are given by the six-state model of table 2.

The question arises as to how the six-form allosteric model given here can represent the same data which was originally obtained from the independent transition model with 2^5 different allosteric forms. Fig. 4 illustrates the comparison between the enthalpy states for the independent transition model for $tRNA^{Phe}$ and the six energy levels of the six-state allosteric model. There is no obvious relation between these two enthalpy level diagrams. Since the two schemes both fit the experimental data, one expects to find an underlying connection. This was found by considering the fractions of the various allosteric species as a function of temperature. The fraction of macromolecules in the i-th form, α_i, is given by eq. 27. The fractional occupancy of any level in the independent model is found by expanding the partition function given by eq. 36 and noting that each term is proportional to the concentration of that particular species. The fraction is computed by normalizing to the sum of all species. The fractional occupancy * for both schemes is shown in fig. 5. One observes that only a few of the 32 states of the independent transition model are significantly populated. The population of these states as a function of temperature closely corresponds to the population of the six states found in the general allosteric treatment.

As seen, the experimental data can be described by either model. The underlying correspondence between the independent transition model and the general allosteric approach is due to the selection of the significant states in each case. The general allosteric method described above selects the sig-

* It is worth noting here that the maximum amount of any species occurs at the temperature corresponding to the point at which the enthalpy added to the system is equal to the enthalpy of that state. This result is analogous to the general rule in ligand binding that the maximum amount of i-th species occurs at the extent of reaction corresponding to i moles of ligand bound.

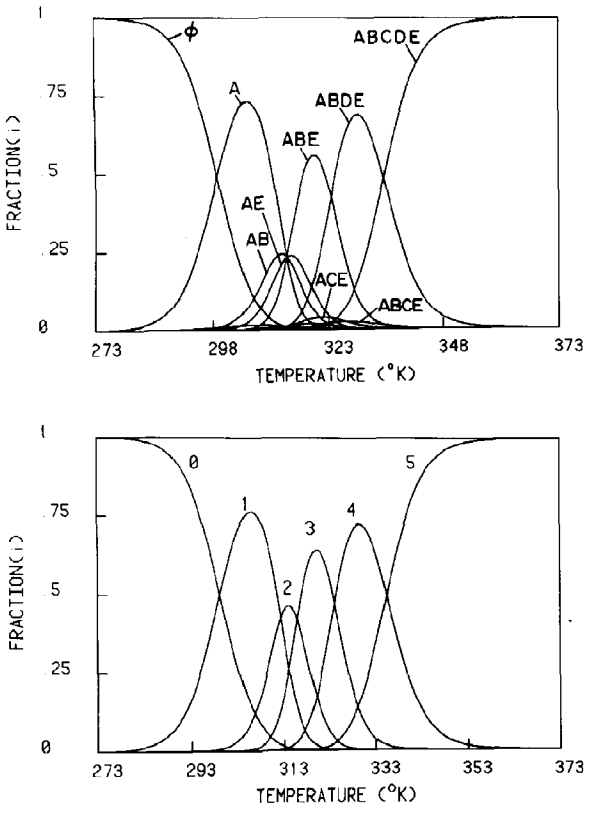

Fig. 5. The species fractions as a function of temperature computed for the independent transition model (top) and the six allosteric state formulation (bottom). The top figure labels correspond to the combinations of independent transitions given in fig. 4 and the bottom figure labels correspond to the states given in table 2.

nificant allosteric states directly, whereas the independent transition model finds the same significant states while implying the existence of many other unnecessary forms. The general approach also allows one to avoid making any assumption about the interactions or lack of interactions between various regions of the macromolecule.

The influence of NaCl on the melting curve can be analyzed by applying the linkage rules developed by Wyman [12]. At a given temperature, the equilibrium constant between the reference form and the i-th form species, L_i^*, is given by eq. 26. The amount of NaCl absorbed upon the transition from the reference state to the i-th state, $\Delta n_{\mathrm{NaCl}}(i)$, is given by the linkage rule as follows:

$$\left[\frac{\partial \ln L_i^*}{\partial \ln a_{\mathrm{NaCl}}}\right]_T = -\Delta n_{\mathrm{NaCl}}(i) \tag{39}$$

where a_{NaCl} is the activity of the NaCl in solution at temperature T. To apply this expression to the results given in tables 1 and 2 we approximate the partial derivative term in eq. 39 by [ln L_i^* (150 mM) − ln L_i^* (20 mM)]/(ln 150 − ln 20) where the salt activities are approximated by concentrations. The results of this calculation are plotted as $\Delta n_{\mathrm{NaCl}}(i)$ vs. T in fig. 6. The most significant feature is the negative value found for $\Delta n_{\mathrm{NaCl}}(i)$ in all transitions. This indicates that NaCl is released upon melting.

The Manning [18] theory of counterion condensation on highly charged polyelectrolytes predicts that double-stranded nucleic acid with its higher charge density will bind a greater number of counterions (Na^+) than the single-strand form with its lower charge density. Thus, the melting of double-stranded regions in tRNA should cause a release of Na^+ from the tRNA molecule. Qualitatively, this agrees with the negative values of $\Delta n_{\mathrm{NaCl}}(i)$ observed by application of the linkage equation.

The idea of counterion condensation implies

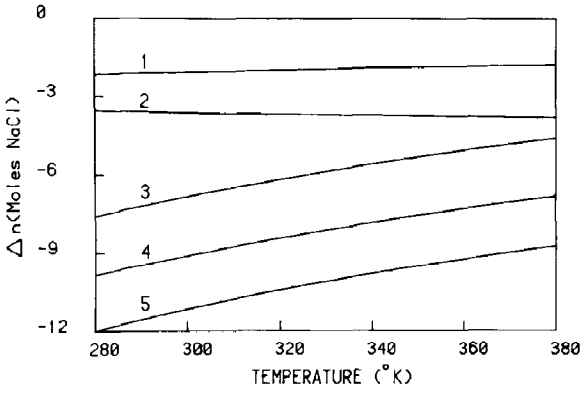

Fig. 6. The calculated change in the moles of NaCl bound for the allosteric transition of $tRNA^{Phe}$ between the reference and i-th state, $\Delta n_{\mathrm{NaCl}}(i)$, as a function of temperature. The lines labeled 1–5 correspond to the transition from the zeroth form reference state to the labeled state. The average NaCl concentration is approx. 0.1 M.

that a large effective binding constant applies to the interaction between the electrolyte salt and tRNA. This means that the heat capacity term in eq. 32 becomes negligible under typical solution conditions. We also note that the second term in eq. 32 accounts for the contribution of the heat of counterion binding to a given allosteric form. This contribution need not be small.

5.2. *Isothermal titration calorimetry – single site reaction*

The second area of application we wish to illustrate is the use of the general theoretical formulation given above for the prediction of heats of chemical ligation in allosteric reactions.

The case of single site binding reactions to several allosteric forms has been recently discussed by Eftink et al. [7] who showed that significant heat capacity changes can be attributed to ligand binding effects. Thermal titrations of the L-arabinose-binding protein [19], which has a single sugar-binding site, show large heat capacity changes for the L-arabinose-binding reaction. The authors of this study chose to treat the heat capacity change as a phenomenological observation. However, there is a strong implication that an allosteric transition is involved with the binding reaction. X-ray structural data show different structural forms for the protein, when sugar is either bound or not bound [20]. The observed heat effects may be easily formulated in terms of the allosteric model. The simple MWC formulation given by eq. 30 is used and must be normalized by the sum of terms representing the unligated species. For the case of two allosteric forms, each of which binds only one ligand, we find that the stoichiometric binding constant, β_1, is then given as:

$$\beta_1 = \frac{\kappa_0 + \beta_{10}\kappa_1}{1 + \beta_{10}} \tag{40}$$

Here the allosteric equilibrium constant is β_{10}, and the intrinsic sugar binding constants to the zeroth and first allosteric forms are κ_0 and κ_1. Each of these parameters depends upon temperature through the enthalpy changes of the underlying reaction processes which define each term (see eq. 10). The phenomenological observation that the stoichiometric reaction has a significant heat capacity change, $\Delta\bar{C}_p$, requires the inclusion of this term in the description of the temperature behavior of β_1 (see footnote, p. 5). Assuming that the heat capacity changes for the underlying allosteric reaction processes are negligible one may use the first portion of eq. 21 to express the temperature dependence for β_{10}, κ_0, and κ_1. The overall result for β_1 in terms of either the phenomenological parameters or the allosteric reaction parameters is:

$$\begin{aligned}\beta_1 &= (\beta_1)_0 e^{-(\Delta H^0/R)(\tau-\tau_0)} e^{(\Delta\bar{C}_p/R)\tau_0(\tau/\tau_0)} (\tau/\tau_0)^{-\Delta\bar{C}_p/R} \\ &= \left\{(\kappa_0)_0 e^{-(\Delta\bar{H}_0/R)(\tau-\tau_0)} \right. \\ &\quad \left. + (\beta_{10})_0 e^{-(\Delta\bar{H}_{10}/R)(\tau-\tau_0)} (\kappa_1)_0 e^{-(\Delta\bar{H}_1/R)(\tau-\tau_0)}\right\} \\ &\quad \times \left\{1 + (\beta_{10})_0 e^{-(\Delta\bar{H}_{10}/R)(\tau-\tau_0)}\right\}^{-1}\end{aligned} \tag{41}$$

Here the equilibrium constants at the reference reciprocal temperature τ_0 are denoted by $(\,)_0$. The enthalpy changes for the various reaction processes at the reference reciprocal temperature τ_0 are given by $\Delta\bar{H}^0$, $\Delta\bar{H}_0$, $\Delta\bar{H}_1$, and $\Delta\bar{H}_{10}$. The partial derivative of the logarithm of β_1 with respect to τ gives $-\Delta\bar{H}_{obs}/R$. Thus, one sees that both the observed enthalpy change and the binding constant β_1 depend on temperature as governed by the six underlying allosteric reaction parameters: $(\kappa_0)_0$, $\Delta\bar{H}_0$, $(\kappa_1)_0$, $\Delta\bar{H}_1$, $(\beta_{10})_0$, and $\Delta\bar{H}_{10}$. The experimental data for the equilibrium binding constant, $(\beta_1)_0$, are given by Clark et al. [21] and values for $\Delta\bar{H}^0$ and $\Delta\bar{C}_p$ have been determined by Fukada et al. [19]. These data may be employed in a manner analogous to that used in the previous section on scanning calorimetric data; namely, one generates a set of values for β_1 for each temperature between 8 and 30°C. Then one determines the best-fit values of the six underlying allosteric reaction parameters by a nonlinear least-squares procedure. The results are shown in fig. 7. The error of the fit using this two-state allosteric model is 0.1% of the binding constant values and indicates the model is adequate. Since the standard error estimated for each fitted parameter is found to be significantly smaller than the parameter itself, this indicates that each parameter is needed for the description of the data by the two-state model. Although this

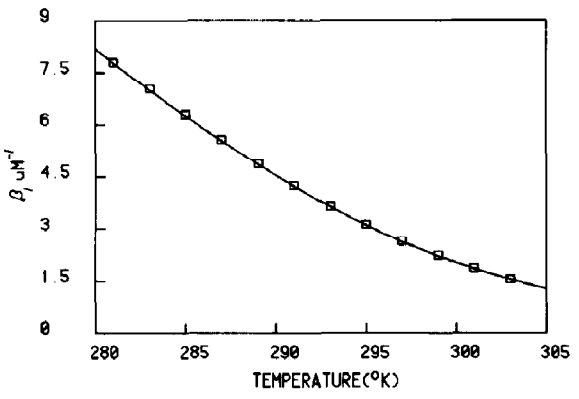

Fig. 7. Temperature dependence of the binding constant for L-arabinose to L-arabinose-binding protein. (□) Phenomenological values of the constant calculated from the literature values of β_1 at 25°C of 2.40×10^6 M^{-1} [21], $\Delta\bar{H}^0$ at 25°C of -15.26 kcal/mol [19], and $\Delta\bar{C}_p$ of -436 cal/mol per K [19]. (———) Results obtained using the two-state allosteric model given by eq. 41. The values obtained for the allosteric parameters along with their estimated standard errors are as follows: $(\kappa_0)_0 = 4.8\times10^6 \pm 8\times10^4$ M^{-1}, $\Delta\bar{H}_0 = -5.8\pm0.1$ kcal/mol; $(\kappa_1)_0 = 5\times10^5 \pm 1\times10^5$ M^{-1}, $\Delta\bar{H}_1 = -5.2\pm3.6$ kcal/mol; $(\kappa_{10})_0 = 1.29\pm0.05$, $\Delta\bar{H}_{10} = 21.66\pm0.62$ kcal/mol.

is the simplest allosteric model that might be chosen, it still requires six parameters for the description of the ligand and thermal binding properties of the system.

This analysis has used purely phenomenological data in the form of an equilibrium constant and enthalpy change under standard state conditions, along with the heat capacity change for the reaction. As shown these data can be fitted to a simple two-state allosteric model. The important result of this analysis is that the allosteric model is able to account fully for the thermal and ligand binding properties for this system. This analysis can be extended to include effects of reversible thermal denaturation which occur at higher temperatures.

5.3. Isothermal titration calorimetry – multiple site reactions

Thermal titrations of more complex ligand binding situations usually present a more difficult problem. We wish to describe some of the general features for such processes in terms of the preceding development. Eq. 30, when normalized by $\Sigma\beta_{i0}$, gives a generalized MWC partition function with the reference state defined by the sum of unligated forms. Differentiation of the logarithm of this expression with respect to either the reciprocal temperature or the logarithm of ligand activity allows the enthalpy change or the amount of chemical ligand bound to be evaluated as a function of ligand activity. The observed heat of ligation per mole of macromolecule may be expressed most naturally in terms of the free ligand activity. As an example we show the results of calculations using parameters obtained in earlier calorimetric and ligand binding studies on the reaction of CO and trout I hemoglobin [22]. Values of the enthalpy change due to the addition of ligand to reach given activity are shown in in fig. 8 for several temperatures. This mode of presentation is analogous to the way ligand binding curves are often represented. One similarity between these two binding phenomena is the saturation effects exhibited at high ligand activity. The main difference between ligand and enthalpy binding is shown in this example by the presence of both positive and negative enthalpy changes. This contrasts with ligand binding where only positive changes in ligation occur with increasing ligand activity. Another difference is that ligand binding

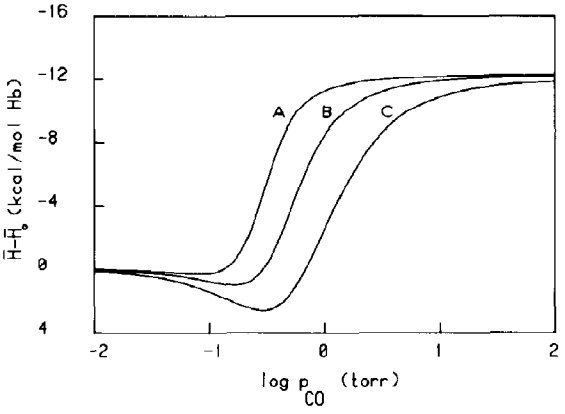

Fig. 8. Enthalpy change per mole of Trout I hemoglobin versus logarithm of CO ligand activity at various temperatures: (A) 278 K, (B) 298 K, (C) 318 K. These curves were calculated from enthalpy and equilibrium constant values given by Barisas and Gill [22] using eqs. 14 and 30.

is characterized by integer stoichiometry whereas thermal binding is governed by noninteger enthalpy changes (see fig. 2). Because of these differences the analysis of thermal binding curves requires both the equilibrium constants and their associated reaction enthalpy changes whereas ligand binding curves require only the equilibrium constants. Thus, in general the description of thermal binding curves at a given temperature requires twice the number of parameters as that required for ligand binding curves.

6. Conclusion

In conclusion we see by these three specific applications how the general partition function formulation given above may be used to analyze and simulate a variety of situations which involve heat and chemical ligand binding phenomena. When heat effects are ascribed to specific macromolecular and chemical ligand binding reactions then one notes a strong parallel with the description of chemical ligand binding. In this way it becomes useful to regard such heat and chemical ligand effects in terms of a generalized concept of 'binding', where the physical parameters (enthalpy and volume) play a role which is formally analogous to that of chemical ligation.

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