Entropy-enthalpy compensation: Perturbation and relaxation in thermodynamic systems

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(Received 27 March 1996; accepted 20 August 1996)

The response of an equilibrium molecular system to perturbations depends on its environmental constraints. For example, the response of an equilibrium P, V, T system to a small temperature perturbation (specific heat) depends on whether the environmental constraint on the system is constant pressure or constant volume. In general, there are two classes of thermodynamic quantities of a system; one is completely determined by its equilibrium distribution, and the other also depends on how the distribution responds to macroscopic changes. The former class is independent of the environment of the thermodynamic system, while the latter class is a function of environmental constraints. In response to a small perturbation, the free energy change of an equilibrium system belongs to the first class but the entropy and enthalpy changes belong to the second. Therefore the thermodynamics of perturbation exhibit compensation between entropy and enthalpy of systems with different environments. The thermodynamic analysis presented here provides a framework for the interpretation of experimental observations of this phenomenon, and is illustrated by a real experimental example. © *1996 American Institute of Physics*. [S0021-9606(96)50544-1]

INTRODUCTION

Entropy-enthalpy compensation is a widely observed phenomenon in chemistry and biochemistry. It is found in a variety of circumstances, such as studies on hydrophobic reactions involving water, enzymatic reactions employing macromolecules, organic reactions dealing with modifications of small functional groups, and investigations on protein stability using site-directed mutagenesis (for reviews, see Refs. 1–5). Recent computational studies on solvation also have provided a modern scenario in which the compensatory thermodynamics occur.[6,7] In this paper, we propose that there is a unifying physical origin of this phenomenon in these seemingly unrelated systems. These systems can all be explained by a single equilibrium thermodynamic principle. A general statistical mechanical formalism based on the concept of statistical ensembles and thermodynamic constraints is presented. After a thermodynamic analysis, we illustrate our idea with a set of real experimental data on the binding of a calcium ion to various proteins. The binding thermodynamics show a compensation between entropy and enthalpy.

Consider a macroscopic system of N molecules, volume V, immersed in a heat bath at temperature T. Consider the same system with N and T, but in contact with a piston at a constant pressure, with the value of the pressure (p) chosen so that V^*, the mean volume of the system, has the same value as the V above. Then all the thermodynamic parameters are the same in the two cases.[8] However, the environmental constraints on the two systems are evidently different. We will show that interesting thermodynamic behavior arises from this difference.

The characteristic thermodynamic functions associated with the equilibria of these two systems are the Helmholtz (A) and Gibbs (G) free energies. Thermodynamic quantities like entropy (S) and chemical potential (μ) are the same between the two:

$$S=-\left(\frac{\partial A}{\partial T}\right)_{V,N}=-\left(\frac{\partial G}{\partial T}\right)_{p,N}$$

and

$$\mu=\left(\frac{\partial A}{\partial N}\right)_{T,V}=\left(\frac{\partial G}{\partial N}\right)_{T,p},$$

where a partial derivative has an experimental meaning; changing a certain thermodynamic quantity while keeping others constant. In fact, it can be shown that all the first-order partial derivatives of the free energy functions are equal between the two systems. However, these equalities do not hold for higher-order derivatives such as heat capacity, thermal expansivity, compressibility, partial molar entropy, and partial molar enthalpy. These quantities are also functions of the environmental constraints. This paper emphasizes the need to differentiate these two classes of thermodynamic quantities. This difference is the fundamental origin of the entropy-enthalpy compensation phenomenon: since chemical potential does not depend on the environmental constraint while partial molar entropy and enthalpy do, and since the sum of the latter two equals the chemical potential, a compensation between partial molar entropy and enthalpy arises during a comparison between the two different systems with different environmental constraints but same equilibrium.

When a small perturbation is applied to a thermodynamic system, compensation occurs for the same physical reason given above but in a different guise. A small perturbation can be viewed mathematically as a differential, and the free energy change corresponding to the perturbation is

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analogous to a derivative, which will belong to the first class.[9–11] Hence, as the S and μ above, the free energy change associated with the perturbation is independent of the environmental constraints of the system. On the other hand, the changes in the entropy and enthalpy are related to higher-order derivatives, which will be sensitive to the environmental constraints of the system. Thus the compensation also arises in the case of comparing thermodynamic changes due to perturbation between systems with different environments.

One might wonder how environmental constraints, which are only boundary effects, could be significant to a true thermodynamic system which is very large. Consider a thermodynamic system responding to a microscopic (small) perturbation, a change on a single molecular level in a thermodynamic system of N molecules. In response to such a perturbation, some unconstrained (fluctuating) thermodynamic quantities will shift their mean values according to the Le Châtelier's principle. The magnitudes of these shifts are microscopic $(1/N)$, but they are amplified by the collective behavior of all the N molecules in the system. A microscopic perturbation which changes the free energy on the order of kT/N is capable of changing both entropy and enthalpy of the whole system on the order of kT, but these changes inevitably compensate each other! This qualitative difference in response to microscopic perturbation also calls for the recognition of the two classes of thermodynamic quantities. We will begin our analysis with a concrete example.

ENTROPY-ENTHALPY COMPENSATION BETWEEN DIFFERENT ENSEMBLES

Thermodynamic equilibrium can be described by the theory of statistical mechanical ensembles following Gibbs.[12,13] It is well known that different statistical ensembles with different environmental constraints provide identical values for thermodynamic quantities. To compute these thermodynamic quantities, the various statistical mechanical ensembles are merely theoretical devices, which lead to identical results. However, there are fundamental differences between the different environmental constraints. Taking constant volume and isobaric ensembles as examples, there is an additional type of fluctuation in the isobaric ensemble which is not present in the system with constant volume: the volume fluctuation. Even though the numerical values for entropy of the two systems are the same at the equilibrium, they comprise of different physical components

$$S_V(V^*)=S_p(p)=\sum_V P(V)S_V(V) - k\sum_V P_V(V)\ln P_V(V), \tag{1}$$

where $S_V(.)$ and $S_p(.)$ are entropies as functions of volume and pressure in the respective ensembles. $P(V)$ is the probability distribution for the volume in the isobaric ensemble. V^* is chosen so that

$$\sum_V VP(V)=V^*.$$

The second term on the right-hand side of Eq. (1) is the contribution to entropy from volume fluctuation in an isobaric system. Hence the two systems have same entropy at equilibrium *but from different physical origin*. When an identical perturbation is applied to these two systems, they respond differently. One familiar example is the heat capacity. C_p and C_V are distinctively different, even though both systems have the same macroscopic volume and pressure. It is well known that thermodynamic quantities like heat capacity, which are second-order derivatives of free energy functions, are intimately related to various types of fluctuation and their correlations. There are many other quantities which are similar to the heat capacity: thermal expansivity (α) and compressibility (κ), to name a few. Particularly relevant to our present discussion, partial molar entropy (s) and enthalpy (h) are in the same class. In general, these quantities are different when derived from different statistical ensembles: they reflect the underlying constraints of the ensembles. Mathematically, these thermodynamic quantities are second-order derivatives of free energy (source) functions.

Since s, h, and μ are related by

$$\mu=h-Ts,$$

where T is the temperature in Kelvin, there is entropy-enthalpy compensation between isobaric and canonical ensembles. The physical basis for the compensation is quite simple: at macroscopic equilibrium, almost by definition, temperatures, pressures, and chemical potentials of the two systems have to be equal, but there is no requirement for partial molar entropies and enthalpies. Similar arguments have been put forward before.[14–16]

Consider a concrete example involving only ideal gas. A canonical (T,V,N) ensemble and an isobaric (T,p,N) ensemble will be treated in parallel. Both chemical potentials have the same contribution from the momentum part which we have dropped to avoid clutter. After that, the chemical potentials for these two ensembles are

$$\mu_V=kT\ln(N/V), \quad \mu_p=kT\ln(p/kT),$$

and since $pV=NkT$ for ideal gas, it is obvious that $\mu_V=\mu_p$.

However, derivatives of μ, which yield s and h, are quite different for these two ensembles

$$s_V=-\left(\frac{\partial\mu}{\partial T}\right)_{V,N}=-k\ln(N/V),$$

$$s_p=-\left(\frac{\partial\mu}{\partial T}\right)_{p,N}=-k\ln(p/kT)+k,$$

$$h_V=\left(\frac{\partial(\mu/T)}{\partial(1/T)}\right)_{V,N}=0, \quad h_p=\left(\frac{\partial(\mu/T)}{\partial(1/T)}\right)_{p,N}=kT.$$

There are an additional $1k$ of entropy and $1kT$ of enthalpy for the isobaric system, and they compensate each other in evaluating μ.

The physics behind the additional entropy and enthalpy can be clarified if we consider the following thought experi-

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ment: introduce one additional molecule into a system at temperature T, with number of molecules N, and volume being constrainted at V. Then relax the constraint, replacing it with an external pressure at p, the exact same pressure as for the volume-constrained system. Macroscopically, there is no alternation due to this change; hence T, V, N, μ, and p do not change. However, microscopically, several things happen when the volume-constraint is relaxed, since due to the additional molecule, the volume of the system increases by $\Delta V/V = 1/N$. This volume change is negligible on the macroscopic scale. But there are N molecules which all experience this volume increase, and since each contributes to the entropy increase by $k\Delta V/V$, the total entropy increase is k. On the other hand, increasing volume by ΔV will do work against the external pressure which is proportional to N at equilibrium. This work, kT to be exact, increases the enthalpy of the system (the internal energy for an idea gas depends only on T, thus does not change in the isothermal expansion).

To generalize the above result beyond the ideal gas, we start with canonical and isobaric ensembles with Helmholtz and Gibbs free energies as source functions respectively. All the other thermodynamic quantities can be derived as derivatives of these source functions. It can be shown that the first-order derivatives are all identical at macroscopic equilibrium. For example

$$G(T,p,N) = A(T,V(T,p,N),N) + pV(T,p,N), \tag{2}$$

$$\left(\frac{\partial G}{\partial T}\right)_{p,N} = \left(\frac{\partial A}{\partial T}\right)_{V,N} + \left(\left(\frac{\partial A}{\partial V}\right)_{T,N} + p\right)\left(\frac{\partial V}{\partial T}\right)_{p,N} = \left(\frac{\partial A}{\partial T}\right)_{V,N} = -S, \tag{3}$$

$$\left(\frac{\partial G}{\partial N}\right)_{T,p} = \left(\frac{\partial A}{\partial N}\right)_{T,V} + \left(\left(\frac{\partial A}{\partial V}\right)_{T,N} + p\right)\left(\frac{\partial V}{\partial N}\right)_{T,p} = \left(\frac{\partial A}{\partial N}\right)_{T,V} = \mu. \tag{4}$$

The key for these relations is the equilibrium condition

$$p = -\left(\frac{\partial A}{\partial V}\right)_{T,N}. \tag{5}$$

These first-order derivatives are mean values for microscopic quantities, and the second-order derivatives are related to their fluctuation. For example

$$C_p = -T\left(\frac{\partial^2 G}{\partial T^2}\right)_{p,N} = -T\left(\frac{\partial^2 A}{\partial T^2}\right)_{V,N} - 2T\left(\frac{\partial^2 A}{\partial T \partial V}\right) \times \left(\frac{\partial V}{\partial T}\right)_{p,N} - T\left(\frac{\partial^2 A}{\partial V^2}\right)\left(\frac{\partial V}{\partial T}\right)^2_{p,N} = C_V - T\left(\frac{\partial p}{\partial V}\right)\left(\frac{\partial V}{\partial T}\right)^2 = C_V + \frac{VT\alpha^2}{\kappa}, \tag{6}$$

where α and κ are the thermal expansivity and compressibility. The fact that C_p is always greater than C_V can be understood as the contribution of additional volume fluctuation in isobaric system. Similarly

$$s_p = -\left(\frac{\partial^2 G}{\partial T \partial N}\right) = s_V - \left(\frac{\partial p}{\partial T}\right)\left(\frac{\partial V}{\partial N}\right) = s_V + \frac{\alpha v}{\kappa}, \tag{7}$$

where v is the partial molar volume. Note that s_p is always greater than s_V.

Parallel results can be obtained between the canonical ensemble and grand ensemble, where the number of molecules is allowed to fluctuate, and the source function is pV.

ENTROPY-ENTHALPY COMPENSATION IN LIGAND BINDING

Entropy-enthalpy compensation phenomenon in ligand-binding has been previously studied by several authors.[14–18] In this section, we try to derive similar results from the point of view of different ensembles, using the same formalism we developed in the previous section. One of our ensembles is equivalent to the ''frozen state'' introduced by Ben-Naim.[14]

Consider a two-component mixture in which component 2, a macromolecule, binds component 1, a ligand. We assume that the macromolecule has at least two different conformers, which bind the ligand differently. Therefore, intuitively, when a ligand molecule is added into the system, in addition to a binding process, the equilibrium distribution between the two conformers will shift due to the Le Châtelier's principle, *if* the fluctuation between two conformers are not constrainted. We will show that the chemical potential for the ligand are identical for ensembles with and without the constraint, but the partial molar entropy and enthalpy are not equal. Hence, the different constraints on the conformational fluctuation give rise to entropy-enthalpy compensation.

Denote the number of macromolecule N_2, and ligand N_1, and the number of macromolecule in conformer A and B by N_{2A} and N_{2B}; $N_{2A} + N_{2B} = N_2$. Consider the canonical ensembles (T,V,N_1,N_{2A}), (T,V,N_1,N_{2B}), and (T,V,N_1,N_2). At thermodynamic equilibrium, similar to Eq. (2), we have

$$G(T,p,N_1,N_2) = G_A(T,p,N_1,N_{2A}(T,p,N_1,N_2)) + G_B(T,p,N_1,N_{2B}(T,p,N_1,N_2)), \tag{8}$$

where $N_{2A} + N_{2B} = N_2$. And similar to Eq. (4) we have

$$\frac{\partial G(T,p,N_1,N_2)}{\partial N_2} = \frac{\partial G_A(T,p,N_1,N_{2A})}{\partial N_{2A}} = \frac{\partial G_B(T,p,N_1,N_{2B})}{\partial N_{2B}}. \tag{9}$$

We now calculate the chemical potential of ligand binding

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$$\mu_1=\left(\frac{\partial G}{\partial N_1}\right)_{N_2}=\left(\frac{\partial G_A}{\partial N_1}\right)_{N_{2A}}+\left(\frac{\partial G_B}{\partial N_1}\right)_{N_{2B}}+(\mu_2^A-\mu_2^B)\times\left(\frac{\partial N_{2A}(T,p,N_1,N_2)}{\partial N_1}\right). \tag{10}$$

Here $\mu_2^A=\partial G_A/\partial N_{2A}$, $\mu_2^B=\partial G_B/\partial N_{2B}$, and $\mu_2^A=\mu_2^B$. Hence the last term in Eq. 10 in fact is zero [see Eq. (4) for comparison]

$$\mu_1=\left(\frac{\partial G_A}{\partial N_1}\right)_{N_{2A}}+\left(\frac{\partial G_B}{\partial N_1}\right)_{N_{2B}}=\mu_1^A+\mu_1^B.$$

To obtain partial molar entropy of ligand binding

$$s_1=-\left(\frac{\partial^2 G}{\partial T\partial N_1}\right)_{N_2}=s_1^A+s_1^B+(s_2^A-s_2^B)\times\left(\frac{\partial N_{2A}(T,p,N_1,N_2)}{\partial N_1}\right). \tag{11}$$

Here $s_1^A=\partial\mu_1^A/\partial T$ and $s_1^B=\partial\mu_1^B/\partial T$ are partial molar entropies of binding for conformer A and B binding, respectively. $s_2^A=\partial\mu_2^A/\partial T$ and $s_2^B=\partial\mu_2^B/\partial T$ are partial molar entropies of conformers A and B, respectively. Similar results have been previously derived.[14–16]

ENTROPY-ENTHALPY COMPENSATION FOR SMALL PERTURBATION

The discussion in previous two sections is focused on the differences between ensembles which have different environmental constraints. The entropy-enthalpy compensation phenomenon shows up in partial molar entropy and enthalpy. In this section, we discuss the thermodynamics when a system is perturbed. Introducing one molecule into a thermodynamic system (dissolution) can be viewed as a small perturbation on the system.[19] We now show that any form of small perturbation will lead to entropy-enthalpy compensation to some degree. A single-residue mutation in a protein is another example of such small perturbation. Figure 1 shows an example of entropy-enthalpy compensation in the thermodynamics of calcium binding to various of proteins with a similar local binding site. In this example, the binding of a calcium ion to a local structural site in a protein is modulated by the tertiary structure of the whole protein, which serves as the environmental perturbation.

The physical reason of this general result is simple: in response to small perturbations on a thermodynamic system, the corresponding changes in free energy are related to the first-order derivatives of the free energy which is independent of environment. The corresponding changes in entropy and enthalpy, however, are second-order derivatives of free energy and they are functions of the environmental constraints on the system. For example, one can perturb the temperature and the volume, or even the molecular interaction function in a canonical ensemble

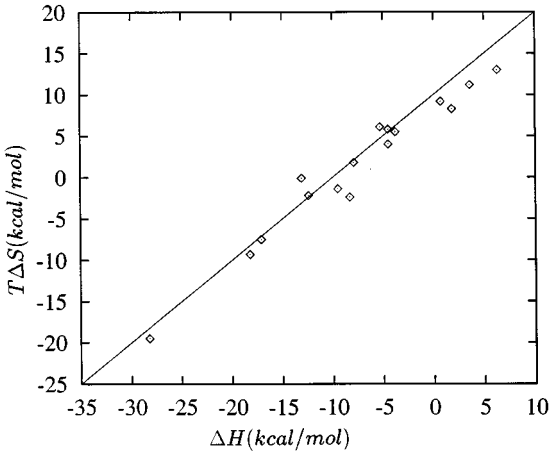

FIG. 1. An example, taken from Ref. 17, shows the compensation between entropy and enthalpy. The corresponding entropy ($T\Delta S$) and enthalpy (ΔH) of calcium binding to each of the set of 16 proteins are plotted. The correlation shows a nice line of slope unity. This indicates, according to our model, that the tertiary structures of the proteins only play a secondary role - the environmental perturbation - on the binding thermodynamics. For the ligand binding problem, the compensatory term is given in Eq. (11). The difference in the partial molar binding entropy (enthalpy) among different conformations of each protein is responsible for the compensation.

$$A=-kT\ln\left(\int dre^{-E(r)/kT}\right)=A(T,V,E(r))=U-TS, \tag{12}$$

where we treat Helmholtz free energy as a functional of intermolecular interaction $E(r)$ which is defined on 3^N dimensional phase space. U is the thermodynamic internal energy. We therefore have

$$\left(\frac{\partial A}{\partial T}\right)_V=\left(\frac{\partial U}{\partial T}\right)_V-T\left(\frac{\partial S}{\partial T}\right)_V-S=C_V-\left(T\frac{C_V}{T}+S\right)=-S \tag{13}$$

so the compensated term is C_V, the heat capacity, and we expect large compensation for system with $C_V\gg S$. This result is the differential form of ''Benzinger's discovery''[5] which states that only part of total change in entropy and internal energy contribute to the change in free energy, and the compensated term is associated with heat (C_V).[18]

Similarly, we have

$$\left(\frac{\partial A}{\partial V}\right)_T=\left(\frac{\partial U}{\partial V}\right)_T-T\left(\frac{\partial S}{\partial V}\right)_T=\left(T\left(\frac{\partial S}{\partial V}\right)_T-p\right)-T\left(\frac{\partial S}{\partial V}\right)_T=-p \tag{14}$$

and the compensated term is

$$T\left(\frac{\partial S}{\partial V}\right)_T=T\left(\frac{\partial p}{\partial T}\right)_V=-T\frac{(\partial V/\partial T)_p}{(\partial V/\partial p)_T}=\frac{T\alpha}{\kappa}. \tag{15}$$

When $\alpha/\kappa\gg p/T$, we expect large compensation.

The most interesting result is to differentiate A with respect to $E(r)$. This is called functional differentiation which is similar to partial differentiation. And we have

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$$\frac{\delta A}{\delta E(r)} = \frac{\delta U}{\delta E(r)} - T\frac{\delta S}{\delta E(r)}$$
$$= \left(\rho^\circ(r) + \frac{\langle E\rangle - E(r)}{kT}\rho^\circ(r) \right)$$
$$- T\frac{\langle E\rangle - E(r)}{kT^2}\rho^\circ(r) = \rho^\circ(r), \qquad (16)$$

where $\rho^\circ(r) = (e^{-E(r)/kT})/(\int e^{-E(r)/kT}dr)$ is the Boltzmann distribution function, $\langle E\rangle = \int E(r)\rho^\circ(r)dr$ is the mean energy. It is clear that compensated terms are always associated with some types of fluctuation: C_V, α, κ, and $\langle E\rangle - E(r)$.

Parallel results can be obtained starting with the Gibbs free energy instead the Helmholtz free energy.

THERMODYNAMIC HIERARCHY OF PERTURBATION AND PERTURBED SYSTEM

We now elaborate on thermodynamic response to a small perturbation. Many different problems can be cast in this framework. For example in the problem of solvation for a rigid solute, the solvation free energy is the change in free energy of the solution upon introducing the solute molecule into solvent system at a *fixed* position.[19] This process is equivalent to introduce an additional term $\Delta E(r)$ to potential function $E(r)$, for which the change in free energy is[9–11,19]

$$\Delta A = -kT\ \ln\left(\int dr e^{-\Delta E(r)/kT}\rho^\circ(r) \right),$$

where $\rho^\circ(r) = (e^{-E(r)/kT})/(\int dr e^{-E(r)/kT})$. We immediately notice that at a given temperature, ΔA can be obtained by knowing $\rho^\circ(r)$ at the *fixed* temperature of the unperturbed system, no temperature dependence of $\rho^\circ(r)$ is required. This is in sharp contrast with changes in other thermodynamic quantities like entropy

$$\Delta S = -\partial\Delta A/\partial T = k\ \ln\int dr e^{-\Delta E(r)/kT}\rho^\circ(r)$$
$$+ \frac{\int dr(\Delta E/T)e^{-\Delta E(r)/kT}\rho^\circ(r)}{\int dr e^{-\Delta E(r)/kT}\rho^\circ(r)}$$
$$+ kT\frac{\int dr e^{-\Delta E(r)/kT}\rho_T{}^\circ(r)}{\int dr e^{-\Delta E(r)/kT}\rho^\circ(r)}. \qquad (17)$$

Note that in the third term, $\rho_T{}^\circ(r) = \partial\rho^\circ(r)/\partial T$ is now required to obtain ΔS. The last term in Eq. 17 is the contribution of relaxation in the system, as has been identified in previous studies on solvation.[6,7] This result is very instructive, it indicates that the ultimate reason for relaxation in a distribution is its temperature dependence, i.e., non-zero heat capacity. From this, we see why entropy-enthalpy compensation has been discussed as a temperature law which is intimately related to heat capacity (Benzinger, 1971; J.A. Schellman, 1990, unpublished work).

In general, for an unperturbed system, we need $\rho^\circ(r)$ to calculate the free energy A; first-order derivatives of $\rho^\circ(r)$ to calculate the entropy S and energy U; second-order derivatives of $\rho^\circ(r)$ to calculate the thermal expansivity α, compressibility κ, and heat capacity C_V; and third-order derivatives to calculate the temperature and pressure dependences of α, κ, and C_V. Similarly for a small perturbation, we need $\rho^\circ(r)$, its first, and the second derivatives to calculate ΔA, ΔU and ΔS, and $\Delta\alpha$, $\Delta\kappa$, ΔC_V, respectively. [We can reach the same conclusions on the Gibbs free energy G in a (T,p,N) ensemble.] Therefore, if we only need to calculate molecular solubility, a model which well represents H and S for pure solvent would be as satisfactory as a model which further represents α, κ, and C_p of pure solvent well. In the case of aqueous solvent,[20] the multi-state model for water has been successful up to α, κ, and C_p, but fails to deal with temperature and pressure dependences of them. In fact, some of the peculiar behaviors of hydrophobic solvation can be understood through the peculiar properties of pure water (H. Qian, manuscript in preparation).

The thermodynamic hierarchy of perturbation and perturbed system we discussed could have important implications for theoretical and computational studies of various biological processes. When a thermodynamic system is perturbed, it is shown that only a crude molecular model is required if one is only interested in the changes in free energy of the perturbation. This is why a hard sphere solvent model can fit the hydrophobic solubility data well.[21] However, with such crude molecular model, the calculation for higher order thermodynamic quantities such as entropy and heat capacity will be misleading, and the overall physical pictures might not be correct. This shortcoming, however, should not prevent researchers from developing a crude model as first-order approximation. The present work provides a theoretical basis for the validity of such models, even though it might fail to predict higher order thermodynamic quantities.

DISCUSSION

Thermodynamic equilibrium is a macroscopic concept. The epitome of macroscopic equilibrium between two systems is their equal chemical potential, pressure, and temperature. There still could be many subtle differences between such equilibrated systems, for example, partial molar entropy and enthalpy, which are the macroscopic manifestations of microscopic fluctuation. Hence, when two macroscopically equilibrated system are compared, or a single molecule is transferred from one system to another, (partial molar) entropy and enthalpy compensate, which reflects different constraints on microscopic fluctuation.

When a thermodynamic system is perturbed, the system's response can be dissected into two parts: a direct interaction between the perturbation and unperturbed system, and a redistribution of subsystems which are in thermal equilibrium, due to Le Châtelier's principle. The latter process is entropy-enthalpy compensating. Our analyses also reveal the situations under which the compensation would be expected.

Water is well known for its dynamic character because of the versatility of hydrogen bonding, which contributes to its large heat capacity and other peculiar thermodynamic

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properties in contrast to organic solvents. Since the hydrophobic solvation can be treated as a perturbation to the water system, it is no surprise that the compensation phenomenon is often observed.

The systematic study of an organic reaction with small functional group modifications is a good example for perturbation. Hence experimentally observed compensations are consistent with our analyses.

From our analysis, it is clear that compensation is likely to occur in systems with large fluctuation and high density of states. Hence, biological molecules like protein are ideal systems for studying entropy-enthalpy compensation. Figure 1 shows an example from a thermodynamic study of calcium binding by proteins. A localized small-ligand binding event could be considered in two different ensembles, one with no constraints, and the other with the constraint that all atoms at a shell of perhaps a few angstroms radius around the binding site remain fixed in their unligated position during the ligation event. If the ligand is small, or if it fits into a protein structure in a ''lock-and-key'' fashion, then the difference in the atomic positions of the constrained atoms in the two ensembles is small, of order δ. Since in the unconstrained and unligated protein, there is no net force on these atoms, the difference in free energy between the constrained and the unconstrained systems can only be of order δ^2. However, a protein is held in its compact folded configuration by a complex combination of hydrophobic, polar, and van der Waals interactions.[22] The ordering of water and of the rotational motions involve large entropy changes, and the forces even on interior atoms in a protein have significant entropic components as well as enthalpic ones. The zero net force condition is maintained by a precise cancellation between the entropic and the enthalpic components of the force, both of which are nonzero (given by partial derivatives analogous to those in the earlier section). The ligation event will thus involve compensating enthalpy and entropy effects of order δ when the constraint is released, just as in the case of the ideal thermodynamic ensemble described earlier. Plots like that of Fig. 1 arise because proteins which differ in the periphery have different compensating effects of order δ. Recent mutational studies of protein stabilities also encounter entropy-enthalpy compensation. These experiments can be discussed along a similar line.[23]

When dealing with experimental data, there is a major difference between the entropy-enthalpy compensation and a linear entropy-enthalpy relationship in general. Our theory for the compensation requires a linear ΔH vs. $T\Delta S$ plot *with slope 1* (see Fig. 1). For those plots with slopes other than unity, the origin of the correlation is not explained by the present theory. For further discussion, see Ref. 5.

When developing molecular models from thermodynamic measurements, the concept of thermodynamic hierarchy is of particular importance. The first term in Eq. (16) is easy to obtain theoretically while the second term is more difficult. This fact indicates that to calculate free energy, one does not need to know how a system responds to perturbation, but only how it is in the unperturbed state. As first-order approximation, a model which cannot yield correct temperature and pressure dependencies can still provide a reasonable free energy.

The thermodynamic analysis we presented on the general ligand binding is similar to the work by Grunwald.[16] Various special analyses with specific models have been in the literature for quite a long time.[14,15] These works emphasize solvation processes in particular. In these solvation analyses,[14–16] a multi-state model for water was used. The validity of such a simplistic model for water has been seriously questioned;[20] therefore a more general analysis with a more realistic model for water is desirable. Our analysis shows that, however, even though the properties of water are extremely complex, calculating free energies (stabilities) of systems in water should be still possible without a detailed water theory. It should be expected that the analysis based on a crude water model cannot predict the entropy and enthalpy of the systems. Nevertheless, it could predict the stability in a relatively accurate way. This is reminiscent of the successes of applying scaled particle theory to aqueous solution[24] and the more recent work of Lee.[21,25,26]

A very similar mathematics of the behavior of components of a variational quantity lies behind the paradox posed by A.W. Overhauser (personal communication) in 1960 as follows:

''Sodium is a free-electron metal to an excellent approximation. In this description, if an electronic excitation is generated at zero temperature by taking an electron from the Fermi surface and placing it in a state of a little larger wave vector with an increase in energy ΔE, the change in the electronic kinetic energy $\Delta(KE)$ will be very nearly equal ΔE, and the change in the Coulomb energy will be roughly zero. Yet, the virial theorem for a system with only Coulomb force (like all real solids) clearly states $\Delta(KE) = -\Delta E$, and change in potential energy $\Delta V = +2\Delta E$.''

The resolution of this paradox by one of us (J.J.H.) shortly later lies in the fact that the intuition and mathematics of the solid state physicist is based on systems at constant *volume*, while this virial theorem holds at zero *pressure*. The expansion which takes place due to the electronic excitation has a negligible effect of order $\Delta E/N$ on the total energy, but an effect of order ΔE on the total kinetic energy and on the total potential energy. This paradox was another introduction to the compensation ideas.

ACKNOWLEDGMENTS

We thank John Schellman for discussion and carefully reading the manuscript, Rufus Lumry for discussion and kindly providing a preprint on similar subject, and Ben Widom for correspondence. H.Q. also thanks Linda Kurz and Arieh Ben-Naim for introducing him to the problem many years ago, and the Program in Mathematics and Molecular Biology at the University of California at Berkeley, which is supported by NSF grant DMS8720208, for a fellowship.

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