Entropy-enthalpy compensation: Conformational fluctuation and induced-fit

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A localized change in a protein, which occurs as a result of either ligand binding or single amino acid substitution, necessarily encounters the conformational fluctuation of the rest of the protein. Both the entropy and the enthalpy associated with the change consist of contributions from fluctuations in the atoms surrounding the localized site, but they compensate. A novel thermodynamic ensemble with a fluctuating boundary is proposed for studying the energetics of localized changes in proteins. Using an ideal gas as illustration, it is shown that the entropy-enthalpy compensation reflects the flexibility of the surrounding structures—its fluctuations contribute a term to the entropy and the conformational change associated with the induced fit contributes a term to the enthalpy. © *1998 American Institute of Physics*. [S0021-9606(98)51146-4]

INTRODUCTION

Entropy-enthalpy compensation is a widely observed phenomenon in biochemistry. A recent thermodynamic analysis has shown that the compensation phenomenon is intimately related to the types of constraints on the boundary of a thermodynamic system, and there is a unifying physical origin of this phenomenon in various molecular systems.[1] In the same study, it was also suggested that fluctuations of the boundary of a molecular system contribute to the system's partial molar entropy and enthalpy, but in such a way that they compensate. This note provides a simple derivation of this conjecture by introducing a novel *fluctuating boundary ensemble*, and uses a system of an ideal gas as an illustration. A thermodynamic framework is proposed for the interpretation of energetic measurements on protein-ligand interactions and changes in protein stability upon site-directed mutagenesis.[2] A general statistical mechanical derivation can be carried out similarly. It would involve more cumbersome algebra but would not provide further physical insight. Hence, for this presentation, we simply use the ideal gas for illustrating the basic idea rather then presenting the general derivation.

IDEAL GAS SYSTEM WITH FLUCTUATING BOUNDARY

Consider a box of ideal gas with N molecules under temperature T and external pressure p through a piston. This is traditionally known as an isobaric ensemble. We further introduce a harmonic spring behind the piston (Fig. 1). The spring constant η characterizes the flexibility of the boundary of the system, and the spring equilibrium length is chosen such that the piston is at the same equilibrium position with or without the spring. Clearly, when $\eta=0$ this system is reduced to the isobaric system (i.e., constant pressure), while when $\eta=\infty$ this system has a constant volume $V_0=NkT/p$. The spring, therefore, prescribes a fluctuating boundary (piston), and the fluctuating boundary ensemble (FBE) is a generalization of the traditional canonical and isobaric ensembles.

By introducing the FBE, we consider a thermodynamic system which contains constant number of molecules (atoms) whose positions are under constant influence from the environment through the boundary. A part of the energy of the system is simply pV for the ideal gas. In general this term is related to the mean force acting on the system by the surroundings. However, there is additional energy from the interaction between the system and the fluctuating surroundings: $\eta(V-V_0)^2/2$. Hence the total energy for the system is: $pV+\eta(V-V_0)^2/2$, and the partition function for the FBE is

$$Q(p,T,N)=\int_0^\infty \frac{V^N}{N!}e^{-\frac{pV}{kT}-\frac{\eta(V-V_0)^2}{2kT}}\,dV, \tag{1}$$

where $V^N/N!$ is the canonical partition function for the system. The chemical potential is

$$\mu=-kT\left(\frac{\partial \ln Q}{\partial N}\right)_{p,T}=kT\ln\left(\frac{N}{\bar{V}}\right), \tag{2}$$

where $\bar{V}$ is the mean thermodynamic volume (the overline represents the ensemble average),

$$\bar{V}=\frac{1}{Q}\int_0^\infty V\frac{V^N}{N!}e^{-\frac{pV}{kT}-\frac{\eta(V-V_0)^2}{2kT}}\,dV. \tag{3}$$

To obtain Eq. (2) from (1) we have made use of the thermodynamic limit for large N, which gives a product of two Gaussian distributions for the volume

$$\frac{V^N}{QN!}\exp\left\{-\frac{pV}{kT}-\frac{(V-V_0)^2}{2kT}\right\}\approx\frac{1}{Q}\exp\left\{-\frac{(V-v_1)^2}{2\sigma_1^2}-\frac{(V-v_2)^2}{2\sigma_2^2}\right\}, \tag{4}$$

where

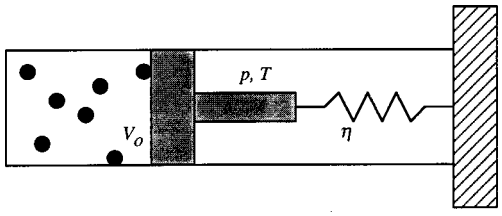

FIG. 1. An illustration of a box of an ideal gas in the fluctuating boundary ensemble. In addition to the external pressure p, there is also a harmonic spring acting on the piston, which fluctuates. The equilibrium position of the piston is chosen such that $V_0 = NkT/p$.

$$v_1 = NkT/p, \quad v_2 = V_0, \quad \sigma_1^2 = N(kT/p)^2, \quad \sigma_2^2 = kT/\eta.$$

Therefore,

$$\bar{V} = \frac{\sigma_2^2 v_1 + \sigma_1^2 v_2}{\sigma_2^2 + \sigma_1^2} = NkT\frac{p + \eta V_0}{p^2 + \eta NkT}, \tag{5}$$

which equals NkT/p exactly as the ideal gas law. Furthermore, it is shown that the chemical potential μ given in Eq. (2) is independent of the fluctuations in the boundary (η); it only depends on the mean force p acting on the system. From Eq. (2) we have partial molar entropy, s, and partial molar enthalpy, h,

$$s = -\left(\frac{\partial \mu}{\partial T}\right)_{p,N} = -k\ \ln\left(\frac{N}{\bar{V}}\right) + \frac{kT}{\bar{V}}\left(\frac{\partial \bar{V}}{\partial T}\right)_{p,N}, \tag{6}$$

$$h = -\left(\frac{\partial(\mu/kT)}{\partial(1/kT)}\right)_{p,N} = \frac{kT^2}{\bar{V}}\left(\frac{\partial \bar{V}}{\partial T}\right)_{p,N}, \tag{7}$$

so the compensating terms are directly related to the thermal expansivity α,

$$kT^2\alpha = \frac{kT^2}{\bar{V}}\left(\frac{\partial \bar{V}}{\partial T}\right)_{p,N} = \frac{kTp^2}{p^2 + \eta NkT}, \tag{8}$$

which can be related to the fluctuation in the volume of the system $\overline{(\Delta V)^2} = \sigma_1^2\sigma_2^2/(\sigma_1^2 + \sigma_2^2)$ as follows:

$$kT^2\alpha = kT\frac{\overline{(\Delta V)^2}_{\mathrm{FBE}}}{\overline{(\Delta V)^2}_{\mathrm{isobaric}}}, \tag{9}$$

when the system is constrained under constant volume, $\alpha = 0$; when it is constrained under constant p, $\alpha = 1/T$.[1] In general, with arbitrary η, the system is constrained in between constant volume and constant pressure, and the compensating terms are proportional to its volume fluctuation [Eq. (9)].

s and h are the partial molar entropy and enthalpy of the system, respectively. They are also the entropy and enthalpy *changes* due to introducing an additional molecule into the system. Equation (5) shows that when introducing the molecule into the system, there is also a volume change associated with the process, which can be considered as a conformational change in the language of protein physical chemistry. The volume change is proportional to the compensating terms

$$\Delta\bar{V} = \frac{kTp}{p^2 + \eta NkT}. \tag{10}$$

In fact, the compensating enthalpy term is nothing but the work associated with the $\Delta\bar{V}$,

$$p\Delta\bar{V} = \frac{kTp^2}{p^2 + \eta NkT} = h. \tag{11}$$

We now generalize these results to entropy and enthalpy measurements from protein systems.

DISCUSSION

A localized change in a protein, either a ligation or amino acid substitution, could be considered in terms of a thermodynamic system which is defined by all the atoms within a core of perhaps a few angstroms radius around the site. Outside this core, the rest of the protein would only be considered as the environment for the system. Since the conformation of the protein fluctuates, the boundary of the system can have a wide range of fluctuations; hence the volume of our system fluctuates.[3] The entropy and enthalpy of the ligand binding or substitution are analogous to the s and h in Eqs. (6) and (7). Accordingly, fluctuations of the entire protein manifest themselves in the entropy of the ligation or substitution. But inevitably this entropy term is compensated by a similar term in the enthalpy of the ligation or substitution. The enthalpy term is the stored energy in the protein due to the conformational change associated with the ligation or substitution [Eq. (11)]. Such conformational change is well known in proteins: the ligation (substitution) *induces* the fluctuating protein to conformations which *fit* the ligand (substituent) better.[4,5] Hence ligation and substitution in proteins with larger conformational flexibility will give larger entropy, but also exhibit larger conformational change upon the perturbation.[2] According to our model, one can use the compensating entropy for ligation or substitution as an indicator for the conformational flexibility of a protein around its perturbed site. Similarly, one can use the compensating enthalpy for ligation or substitution as an estimation for the conformational change of a protein upon the perturbation. This is consistent with the experimental observation that proteins with larger compensating terms upon calcium binding also exhibit larger conformational change upon the calcium binding.[6]

The significance of the fluctuating boundary ensemble is that it separates a localized site and its surroundings in a protein, and treats the surroundings as boundary effect with a few coarse-grained parameters, p, V_0, and η, which characterize the mean force, the mean volume, and the volume fluctuation of the rest of the protein. p, V_0, and η can be estimated from the protein before the localized change is made. We have shown that the free energy associated with the localized change, μ, is independent of η while both the entropy and enthalpy depend on the magnitude of η. The FBE provides a means for progressively computing the thermodynamic hierarchy of localized changes in proteins,[1] or in many other systems of condensed matter. For example, solvation is a well known process which exhibits thermody-

namic entropy-enthalpy compensation.[7] The FBE is also the natural statistical ensemble for studying the entropy of ligand binding to cavities in proteins.[8–10] On the experimental side, it is now possible, under favorable conditions, to quantitatively measure localized conformational fluctuations in a protein;[11,12] hence a quantitative experimental test for the present model is in our reach.

The present model also provides an intuitive hierarchical view of the thermodynamics of a localized change. The free energy associated with the change is determined by the contribution from all the neighboring atoms in the vicinity which define our system, as well as a mean force acting on the system from the atoms farther away. Beyond the operationally defined boundary, all the atoms will contribute to the free energy of the change through the mean force, and to the entropy (and enthalpy) of the change through fluctuations. The definition of the system is operational. The larger it is chosen, the more accurate a thermodynamic calculation, but also the more difficult the calculation will be.

Entropy-enthalpy compensation appears in many different forms. A thermodynamic system with entropy-enthalpy compensation in one form also leads to compensation in other forms. For example, compensation in folding equilibrium of a series of homologous proteins will lead to compensation in the ligand binding of these proteins. This perhaps is another reason why this phenomenon is observed so widely.

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