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COMMUNICATIONS

Published on Web 12/09/2006

Denatured State Effects and the Origin of Nonclassical ϕ Values in Protein Folding

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Undoubtedly, the most powerful method for characterizing side-chain interactions in the transition state for protein folding is the ϕ value approach.[1−3] The method involves comparing the effect of conservative mutations on the equilibrium free energy of folding, $\Delta\Delta G°$, to their effects on the activation free energy, $\Delta\Delta G^{TS}$, for folding. The ratio, $\Delta\Delta G^{TS}/\Delta\Delta G°$, defines the ϕ value. The ϕ values have a simple interpretation if the mutation does not perturb the free energy level of the denatured state. In this case, $\Delta\Delta G^{TS}$ is just equal to the change in the free energy of the transition state, and $\Delta\Delta G°$ is just the change in the free energy of the native state (Figure 1a). The ϕ values then report on the relative development of side-chain interactions in a simple way. A ϕ value of 0 indicates that the interaction being probed is no more developed in the transition state than it is in the denatured state, while $\phi = 1$ indicates that the interaction is as well developed in the transition state as it is in the native state. In practice, most ϕ values are fractional, ranging between 0 and 1. Fractional ϕ values can arise from partially developed interactions or from multiple routes over the transition barrier.[1]

A small but noticeable number of ϕ values have been observed which are either less than 0 or greater than 1.[4−9] These ϕ values, sometimes referred to as noncanonical or nonclassical ϕ values, have several potential origins. A common view is that they result from non-native interactions. Serrano and co-workers showed that unusual ϕ values can be due to conformational strain in the hydrophobic core of proteins.[6] Dill and co-workers have proposed an interesting alternative explanation of nonclassical ϕ values.[5] On the basis of the results of lattice simulations, it was suggested that alternation of the relative flux through different pathways upon mutation generates nonclassical ϕ values. In this scenario, unusual ϕ values are evidence of multiple folding pathways, and it was argued that such noncanonical values could not be rationalized in the framework of a single reaction coordinate and sequential paths. Here we illustrate a simple alternative explanation for ϕ values less than 0 or greater than 1, namely, denatured state effects. If denatured state effects are considered, simple models of folding can account for nonclassical ϕ values without invoking changes in flux between multiple routes on the free energy landscape or formation of non-native interactions in the transition state. Consider the case where a mutation changes the free energy level of the denatured state as well as the free energy of the transition state and native state (Figure 1b). In this case, the ϕ value is still a well-defined quantity and is still equal to the ratio of $\Delta\Delta G^{TS}$ to $\Delta\Delta G°$, but now both of these terms contain a contribution from the change in the free energy level of the denatured state ensemble:

$$\phi = \frac{\Delta\Delta G^{TS}}{\Delta\Delta G°} = \frac{\Delta G^{TS} - \Delta G^{D}}{\Delta G^{N} - \Delta G^{D}} \quad (1)$$

where ΔG^{TS} is the change in the free energy of the transition state, ΔG^{N} is the change in the free energy of the native state, and ΔG^{D} is the change in the free energy of the denatured state ensemble (Figure 1b). If ΔG^{D} is equal to 0, then the expression for the ϕ value reduces to its simpler form.

The effects of denatured state interactions on three representative ϕ values were examined. First, consider the case of a mutation which alters the free energy of the transition state by 0.0 kcal mol^{-1} and the free energy change of the native state by 2.0 kcal mol^{-1}, that is, $\phi = 0$. Consider the effect upon this ϕ value when the mutation also alters the free energy of the denatured state ensemble. Figure 2 shows a plot of the resulting ϕ value versus the change in the free energy level of the denatured state ensemble, ΔG^{D}. Here, positive ΔG^{D} values represent unfavorable changes in the free energy of the denatured state ensemble upon mutation; that is, the mutation raises the free energy of the denatured state. Such effects could, for example, arise from the disruption of hydrophobic clusters in the denatured state by the mutation of a large hydrophobic residue to a small one, or by the elimination of favorable electrostatic interactions in the denatured state, or by unfavorable entropic effects caused by mutation. Conversely, a negative ΔG^{D} represents, in the notation adopted here, a decrease in the free energy of the denatured state ensemble, that is, stabilization upon mutation. Such a change might result from a mutation which enhanced hydrophobic clustering or introduced some favorable polar interaction. As the destabilization of the denatured state ensemble increases, the ϕ value decreases, resulting in negative ϕ values (Figure 2). Denatured state effects can also lead to ϕ values greater than 1.

Now consider a mutation which leads to a fractional ϕ value, for example, a mutation which alters the free energy of the native state and transition state by 1.0 and 2.0 kcal mol^{-1}, respectively (ϕ

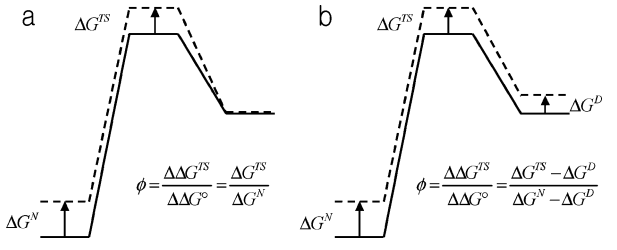

Figure 1. Schematic free energy diagram for two state protein folding. (a) The free energy of the denatured state is assumed not to change upon mutation. (b) The free energy of the denatured state is assumed to change upon mutation. In both cases, the destabilization of the transition state is the same, but the apparent ϕ is different. ΔG^{N} represents the change in the free energy of the native state, ΔG^{TS} the change in the free energy of the transition state, and ΔG^{D} the change in the free energy of the denatured state ensemble.

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10.1021/ja0669878 CCC: $33.50

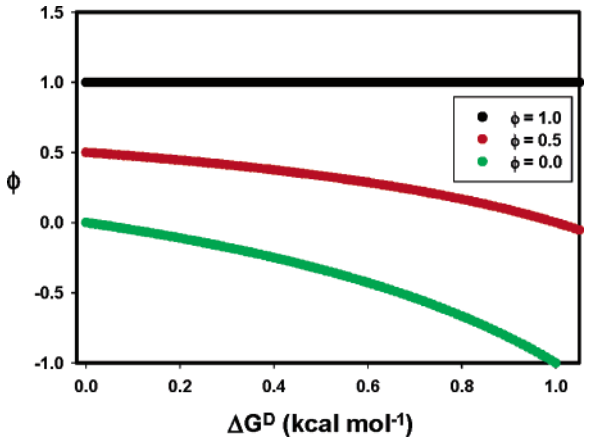

Figure 2. Effects upon observed ϕ values of changes in the free energy of the denatured state ensemble, ΔG^{D}. Positive values of ΔG^{D} correspond to destabilization of the denatured state ensemble upon mutation. Equation 1 was used to calculate ϕ values. ΔG^{N} was set to 2 kcal mol^{-1} for all three cases. The destabilization of the transition state, ΔG^{TS}, was assumed to be 2 kcal mol^{-1} for $\phi = 1$, 1 kcal mol^{-1} for $\phi = 0.5$, and 0 kcal mol^{-1} for $\phi = 0$.

$= 0.5$). Again, as ΔG^{D} increases, the ϕ value decreases (Figure 2). In this case, the ϕ value spans the range from 0.5 to 0.0 as ΔG^{D} varies from 0.0 to 1 kcal mol^{-1}. Further increases in ΔG^{D} again lead to negative ϕ values. Finally, consider a mutation which changes the free energy of the native and transition states by the same amount (i.e., $\phi = 1.0$). In this case, the ϕ value does not change upon modulating denatured state interactions. It is clear from eq 1 that the ϕ value becomes extremely sensitive to perturbations of the free energy of the denatured state ensemble when ΔG^{D} is comparable to the destabilization induced in the native and transition states by mutation. Thus special care is necessary when interpreting ϕ values when the stability difference between wild-type and mutant ($\Delta\Delta G^{\circ}$) is small.

There is increasing evidence that the denatured state ensemble can contain energetically important interactions and that mutations can exert energetically significant effects on the denatured state that are comparable to or larger than their effects on the native state or transition state.[10−19] Thus the possibility exists that denatured state effects could lead to ϕ values greater than 1 or less than 0.

Acknowledgment. This work was supported by a grant from the NIH (GM 70941) to D.P.R.

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JA0669878