Heat Capacity of Protein Folding

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ABSTRACT We construct a Hamiltonian for a single domain protein where the contact enthalpy and the chain entropy decrease linearly with the number of native contacts. The hydration effect upon protein unfolding is included by modeling water as ideal dipoles that are ordered around the unfolded surfaces, where the influence of these surfaces, covered with an "ice-like" shell of water, is represented by an effective field that directs the water dipoles. An intermolecular pair interaction between water molecules is also introduced. The heat capacity of the model exhibits, the common feature of small globular proteins, two peaks corresponding to cold and warm unfolding, respectively. By introducing ad hoc vibrational modes, we obtain quantitatively good accordance with experiments on myoglobin.

INTRODUCTION

A protein is a large polymer consisting of many thousands of atoms and may, therefore, from a physical point of view, be regarded as a macroscopic system (Privalov, 1992). Anfinsen (1973) proved that the one-dimensional sequence of amino residues uniquely determines the three-dimensional conformation of the protein. Furthermore, he concluded that the native (folded) state is the state of the lowest free energy. Consequently, given a polypeptide sequence, a microscopic analysis of its enthalpy and degrees of freedom, followed by a statistical mechanical evaluation, should reveal the thermodynamic properties of a given protein.

Proteins are known to fold on time scales from milliseconds to seconds, which apparently seems to be in great contrast to a naive statistical analysis based on the vast number of conformations in the energy landscape, which indicates an astronomical folding time. This is called the Levinthal paradox (Levinthal, 1968). Later analysis by Levitt and Warshel (1975) shows that this paradox does not exist when assuming that the folding follows a much simpler conformation space than the complete space considered by Levinthal. In this work we circumvent this paradox by supposing some kind of folding pathway (Baldwin and Rose, 1999; Wang and Shortle, 1995; Hansen et al., 1998a,b, 1999; Bakk et al., 2000, 2001a,b), which means that the folding process, starting from a denatured (unfolded) conformation, follows a specific sequence of folding steps in the free energy landscape until the native state is reached.

Proteins consist of 20 different amino acids with a great diversity with regard to size, polarity, and charge. By considering the electrostatics, it is possible to argue that the small number of charges does not contribute significantly to the stability of the native conformation (Richards, 1992). Thus, two kinds of surfaces remain most relevant, the apolar (hydrophobic) and the polar surfaces (Privalov, 1992).

In this work we make a model of a small single-domain protein by supposing that the protein consists of energetically equal contacts that lower their energy when they are closed, and the energy decreases linearly with the number of "native-like" contacts. In addition, we introduce water molecules, modeled as ideal electrical dipoles in an external effective field that represent the influence or ordering effect of the unfolded apolar surfaces on the water. Besides, there are pair interactions between water molecules. In a self-consistent mean-field treatment they add to the effective field (Høye and Stell, 1980; Ma, 1985). By performing a statistical mechanical evaluation we then find thermodynamic functions, such as the free energy and the heat capacity of the protein. In the end we assign ad hoc vibrational modes in the IR region and compare the heat capacity to experimental results on metmyoglobin (Privalov et al., 1986).

THE PROTEIN MODEL

In this section we will present the statistical model, which is a further development of earlier models by Hansen et al. (1998a, 1999) and Bakk et al. (2000, 2001a,b). The chain-chain interactions, although reformulated, are used as in these models, but the protein-water interactions are new compared to these models. Thus, we will spend the greater part of this section discussing protein-water and water-water interactions.

Internal forces in the protein

For simplicity, the protein is regarded as consisting of N contacts (Plaxco et al., 1998). A contact is a conformation with a specified free energy. Beyond this specification the model does not have a detailed connection to the structure of proteins. However, the general character of the model makes it possible to reveal various key features about the specific mechanism of protein folding thermodynamics that can lead to cold and warm unfolding. Each contact is supposed to be a specific point on a folding pathway (Baldwin and Rose, 1999; Wang and Shortle, 1995; Hansen et al., 1998a,b, 1999; Bakk et al., 2000, 2001b). The pathway is also in accordance with a "folding funnel" (Leopold et al., 1992), where each contact now represents the intersection between a level or a "contour line" in the free energy landscape and one of the possible multiple pathways (Galzitskaya and Finkelstein, 1999).

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Upon folding the protein lowers its enthalpy by an amount ϵ_c for each contact that "folds" (Bryngelson and Wolynes, 1987). Let $i \in \{0, 1, \ldots, N\}$ be the number of contacts that are correctly folded. Thus the resulting energy for the "dry" or vacuum chain-chain interactions can be written

$$\mathcal{H}_i^c = -i\epsilon_c. \tag{1}$$

The specific value $i = 0$ means an unfolded (denaturated) protein, while $i = N$ is a complete folded (native) protein. To incorporate the rotational freedom or flexibility in the polypeptide backbone, we assign g_c degrees of freedom for each unfolded contact, where g_c is interpreted as the relative increase of the degrees of freedom for an unfolded contact compared to a folded one. Consequently the folding of i contacts corresponds to g_c^{N-i} degrees of freedom. It is worth noting that this chain-chain interaction model can be viewed as a further simplification of the model by Zwanzig et al. (1992) and Zwanzig (1995), which builds on the same ideas as Levitt and Warshel (1975) assuming a narrower conformation space than the complete space.

Hydration effect upon unfolding

In this section we will consider the hydration effect upon unfolding. However, the water-exposed native parts of the protein will contribute to the total heat capacity of the protein, regardless of the degree of folding. Privalov and Makhatadze (1992) conclude that the native hydration heat capacity effect for myoglobin contributes <0.4 JK^{-1} g^{-1}, and from Fig. 4 one sees that the total heat capacity of the folded state is >1.3 JK^{-1} g^{-1} for myoglobin in the temperature region considered. Thus, we ignore the hydration heat capacity due to the native protein, but nevertheless this should be included in a more accurate model. Furthermore, in the next section we introduce ad hoc vibrational modes where the above-mentioned minor effect may be regarded as being incorporated in these modes.

It is known from experiments that the heat capacity change upon aqueous dissolution of apolar molecules from their gaseous state is positive and proportional to the solute molecule concentration (Edsall, 1935; Privalov and Makhatadze, 1992), and this change decreases with increasing temperature (Privalov and Gill, 1988). Furthermore, a solution of an apolar substance in water is associated with a *negative* entropy change at room temperature, which decreases in absolute value with increasing temperature (Privalov, 1992). In other words, there seems to be an ordering of the water around the apolar surfaces. In sum, the hydration effect of an apolar molecule can be explained by a gradual melting of an ordered "ice-like" shell around these compounds (Frank and Evans, 1945). Melting of ice is a complex process whereupon conformational changes imply a change in the enthalpy and the entropy. In this paper we incorporate the hydration of the apolar surfaces by an extension of a model first proposed by Hansen et al. (1998a) and further developed by Bakk and Høye (submitted for publication).

The idea with the water interactions is to cover two basic properties of the unfolding solvation process. First, there is an ordering of water around unfolded parts of the protein. This is accompanied by decreased enthalpy and entropy upon hydration of apolar molecules. Second, there are interactions between the water molecules. These interactions tend to orient the molecules with respect to each other to form an "ice-like" structure. The water molecules form hydrogen bonds at tetrahedral angles with neighboring water molecules. These bonds are associated with location of positive and negative charges within the water molecules. This again results in large permanent electric dipole moments of these molecules. Thus, we approximate the water molecules by ideal electric dipoles as a simplification.

The idea of representing the solvent by dipoles in protein folding calculations was introduced by Warshel and Levitt (1976), and later applications on proteins by, e.g., Russell and Warshel (1985), Fan et al. (1999), and a recent application on different amino acids by Avbelj (2000).

Apolar surfaces, in combination with hydrogen bonds, make it favorable for the water to make "ice-like" shell structures around these surfaces. The influence of these apolar surfaces we thus will model by an electric field $\mathbf{E}$, which also has a structuring effect as it rectifies the dipole moments. This field yields an interaction for each dipole

$$\mathcal{H}_E = -\mathbf{E} \cdot \mathbf{s} = -E \cos \vartheta. \tag{2}$$

Here $\mathbf{s}$ is the dipole moment of the molecule where we put $|\mathbf{s}| = 1$ for simplicity and ϑ is the angle between $\mathbf{E}$ and $\mathbf{s}$.

Besides, there will be pair interactions between neighboring molecules with the total energy

$$\mathcal{H}_p = -\tfrac{1}{2} \sum_{i,j} J_{ij} \mathbf{s}_i \cdot \mathbf{s}_j, \tag{3}$$

where J_{ij} is the coupling constants between water molecule i and neighboring molecules j. The factor 1/2 prevents double counting of interactions. In our model these can be regarded as interactions between the water dipole moments. We find reasons to take such interactions into account as formation of ice also represents a directional ordering of water molecules, and we want to investigate their influence. In the Appendix we calculate the partition function for one water molecule Z_w (see Eq. 15) by a mean-field approximation (Høye and Stell, 1980; Ma, 1985) where the field E is replaced by an effective field $E_e = E + bm$.

The resulting term i in the canonical partition function for the protein has i contact energies ϵ_c, and it has g_c degrees of freedom and M "bound" water molecules, each contributing a factor Z_w at all of the $N - i$ unfolded contacts, thus

$$Z_i = g_c^{N-i} e^{i\beta\epsilon_c} (4\pi)^{Mi} (Z_w)^{M(N-i)}, \tag{4}$$

where the factor 4π is the Z_w for $E = 0$ for the Mi "unbound" bulk water molecules, and $\beta = 1/T$, where Boltzmann's constant k_B is absorbed in the absolute temperature T such that the energy quantities can be expressed in terms of the temperature unit K (Kelvin).

Eq. 4 further can be rewritten as

$$Z_i = e^{\beta N \epsilon_c} (4\pi)^{MN} r^{i-N}, \tag{5}$$

where the function r, when inserting the Z_w from the Appendix (see Eq. 15), is

$$r = \left[a e^{\beta\mu} e^{1/2\beta bm^2} \frac{\beta E_e}{\sinh \beta E_e} \right]^M, \tag{6}$$

with $a = 1/g_c^{1/M}$ and $\mu = \epsilon_c/M$. The parameters a and μ will depend upon the chemical environments (pH, denaturant concentration, etc.).

The canonical partition function for the system is now simply the sum over Z_i for the various contact conformations along the folding pathway

$$Z = \sum_{i=0}^{N} Z_i = e^{\beta N \epsilon_c} (4\pi)^{MN} \frac{1 - r^{-(N+1)}}{1 - r^{-1}}. \tag{7}$$

From the definition of the internal energy, $U = -\partial(\ln Z)/\partial\beta$, it is clear from Eq. 7 that the exponential contributes with a constant factor to U, thus the heat capacity, $C = -\beta^2 \partial U/\partial\beta$, will only depend on the function r in Eq. 6. The parameters, for a fixed system size ($N = 100$ in this work), are: a, b, μ, E, and M.

Vibrational modes

As the results will show, the model above yields a heat capacity that lacks certain features. Experimentally, the heat capacity by hydration increases markedly with T, and the "valley" in the folded region is well above zero. Thus, to account for these latter features, we will introduce ad hoc vibrational modes to reproduce the physics in a reasonable way. These may be

regarded as effective internal modes of the protein due to couplings between neighboring atoms, and they can be considered as harmonic oscillators. The quantization of the latter yield the energy levels

$$\mathcal{H}_{\mathrm{h}}(n) = (n + \tfrac{1}{2})h\nu, \tag{8}$$

where $h = 6.63 \cdot 10^{-34}$ Js is Planck's constant and ν is the frequency. Summing over all energy levels gives us the partition function for the vibrational modes for N_{h} independent harmonic oscillators

$$Z_{\mathrm{h}} = \left(\sum_{\mathrm{n}=0}^{\infty} e^{-\beta \mathcal{H}_{\mathrm{h}}(n)} \right)^{\mathrm{N_h}} = (2 \sinh(d/T))^{-\mathrm{N_h}}, \tag{9}$$

where $d = h\nu/(2k_{\mathrm{B}})$. We suppose, as a very simple assumption, that the vibrational modes are independent of the degree of folding, thus the partition function for the system including these is

$$Z' = Z_{\mathrm{h}} Z, \tag{10}$$

where Z is the partition function in Eq. 7.

RESULTS AND DISCUSSION

Whether the protein is folded or unfolded can now be analyzed in a straightforward way by regarding the ratio $r = Z_{\mathrm{i}+1}/Z_{\mathrm{i}}$. The contribution Z_{i} to the full partition function Z may be regarded as the partition function for a protein with i contacts folded. Thus, a free energy difference $\Delta_{\mathrm{n}}^{\mathrm{d}}F$ between the denatured (d) and the native (n) protein can be expressed as

$$\Delta_{\mathrm{n}}^{\mathrm{d}}F = -T(\ln Z_0 - \ln Z_{\mathrm{N}}) = TN \ln r, \tag{11}$$

which determines the stable conformation and gives a direct interpretation of the function r. For $\Delta_{\mathrm{n}}^{\mathrm{d}}F > 0$ ($r > 1$) the native conformation is thermodynamically stable, while for $\Delta_{\mathrm{n}}^{\mathrm{d}}F < 0$ ($r < 1$) the denatured conformation is stable. The value $r = 1$ is critical and in a small region around this value the protein switches between the two conformations.

In Fig. 1 we have plotted $\Delta_{\mathrm{n}}^{\mathrm{d}}F$ as function of the temperature for different values of the "chemical potential-like" parameter μ, while the other parameters are fixed. We see that for the three largest values of μ considered, according to Eq. 11 there is an interval in the middle where the native conformation is stable, while for low and high temperatures the denatured protein is preferred. In other words, one has two unfolding transitions, a cold one and a warm one. This cold and warm unfolding seems to be a common feature of small globular proteins (Privalov, 1990; Chen and Schellman, 1989).

The smallest value $\mu_4 = 1.743$ in Fig. 1 is a critical one, where the maximum of the stability function is at $\Delta_{\mathrm{n}}^{\mathrm{d}}F = 0$, where the unfolded an folded states have equal probabilities, i.e., the lower curve of Fig. 2 has a maximum of 0.5. Qualitatively, the parabolic plots in Fig. 1 correspond well to the experiments of Privalov et al. (1986) on sperm whale metmyoglobin, where such conformational free energy differences were measured.

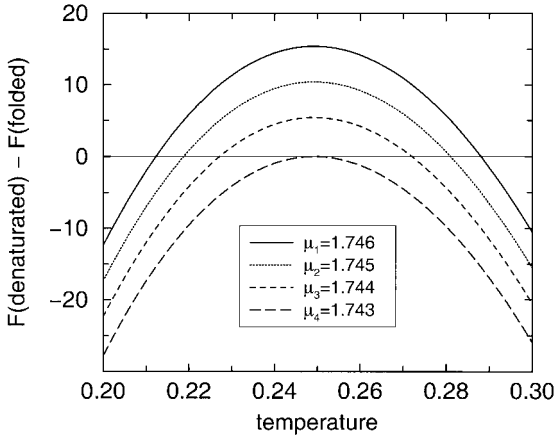

FIGURE 1 Temperature dependence of the denaturated and native protein free energy difference $\Delta_{\mathrm{n}}^{\mathrm{d}}F$ (see Eq. 11) for different μ. Other parameters according to Eq. 6 are $a = 0.12$, $b = 2.0$, and $M = 10$, with E equal to 1.

The picture of cold and warm unfolding against different values of μ is substantiated by a glance at Fig. 2, which shows the mean number of folded contacts relative to the system size given by

$$n = \frac{\sum_{\mathrm{i}=0}^{\mathrm{N}} iZ_{\mathrm{i}}}{N\sum_{\mathrm{i}=0}^{\mathrm{N}} Z_{\mathrm{i}}} = \frac{r}{N} \frac{Nr^{\mathrm{N}+1} - (N+1)r^{\mathrm{N}} + 1}{(1 - r^{\mathrm{N}+1})(1 - r)}. \tag{12}$$

For a complete denatured protein $n = 0$, while for a native one $n = 1$.

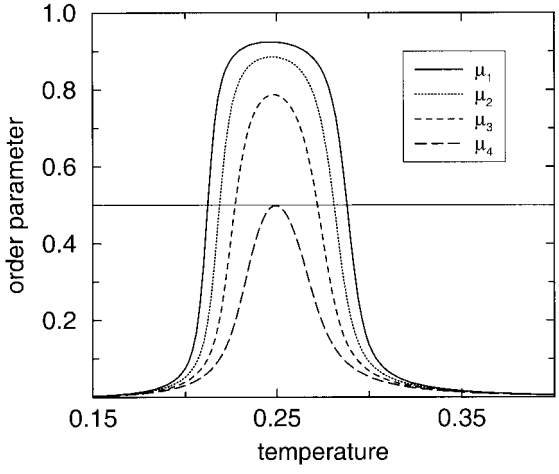

FIGURE 2 Temperature dependence of the order parameter n for the corresponding parameter set used in Fig. 1. Note that the maximum for μ_4 is $n = 0.5$, which corresponds to $\Delta_{\mathrm{n}}^{\mathrm{d}}F = 0$ (see Fig. 1).

It is now interesting to study the heat capacity, as is done in Fig. 3. We obtain two peaks, which show both cold and warm unfolding. The peaks vanish as μ decreases toward its critical value. This feature is in accordance with the experiments by Privalov et al. (1986) on small globular proteins.

By choosing $d = 494$ K and $N_h = 5981$ as the number of oscillators per protein in Z' (Eqs. 9 and 10), we see in Fig. 4 that the model is *qualitatively* in good correspondence with experimental data on myoglobin. We note the specific choice $d = 494$ K corresponds to a wavelength $1.5 \cdot 10^{-5}$ m, which is in the IR region.

Finally, in this section it can be noted that the coupling between the water molecules (see Eq. 3) resulting in the parameter b does not have significant influence upon the qualitative behavior of our results. In the calculations we use a non-zero value of b, but replacing it by zero while adjusting other parameters leads to minor changes of the results obtained.

CONCLUSION

We have studied a single-domain protein, which is supposed to follow a specific folding pathway. The chain-chain contact enthalpy and entropy increase linearly with the degree of folding. Each individual water molecule is modeled as a dipole in an external electrical field. Between the dipoles there are interactions that are incorporated in a mean-field approximation.

We find that the protein is folded in an intermediate temperature region, while it becomes denaturated at low and high temperatures. This cold and warm unfolding behavior is in accordance with experiments on small globular proteins (Privalov et al., 1986; Privalov, 1990; Chen and Schellman, 1989), and is also seen in earlier models by Hansen et al. (1998a, 1999), Bakk et al. (2000, 2001a,b), and Bakk (2001).

By introducing effective or ad hoc vibrational modes, we find that the model yields a quantitatively good representation of the heat capacity of myoglobin that undergoes unfolding transitions at low and high temperatures (Privalov et al., 1986; Privalov, 1990).

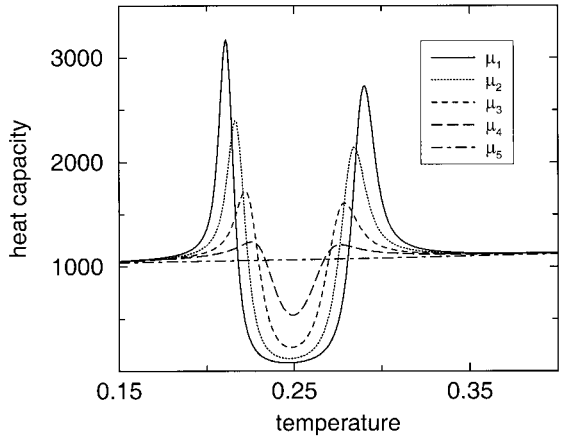

FIGURE 3 Heat capacity for different μ based upon Eq. 7 for the corresponding parameters used in Fig. 1, and in addition we have drawn the heat capacity for $\mu_5 = 1.700$, which is the pure hydration contribution of the denatured protein. Note the smoothing of the peaks for decreasing μ.

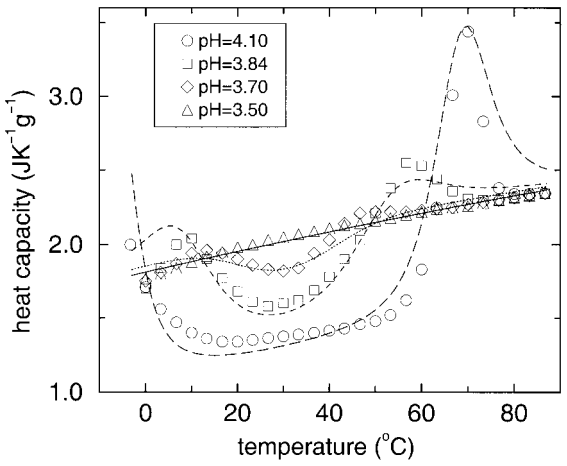

FIGURE 4 Heat capacity at different μ based upon Z' in Eq. 10, where $a = 0.1118$, $N_H = 5981$ (oscillators per protein), and $M = 15.7$, and in units of temperature: $d = 494$ K, $b = 2600$ K, and $E = 1300$ K. Experimental data from Privalov et al. (1986) on metmyoglobin at the different pH values (and corresponding μ in our model): pH = 4.10 (μ = 2290.3 K), pH = 3.84 (μ = 2288.5 K), pH = 3.70 (μ = 2287.4 K), and pH = 3.50 (μ = 2275.0 K), where pH = 3.50 corresponds to a denatured protein. At pH = 4.10 the protein is folded between 20°C and 50°C, and has an unfolding transition around 70°C.

APPENDIX

The assumed pair interaction $-\Sigma_j J_{ij}\mathbf{s}_i \cdot \mathbf{s}_j$ between the dipole moment of water molecule i and its neighbors j is in a mean-field consideration approximated by the term $-b\mathbf{m} \cdot s_i$, where $\mathbf{s}_j$ is replaced by its average $\langle \mathbf{s}_j \rangle = \mathbf{m}$ and $b = \Sigma_j J_{ij}$. Such an approximation thus accounts for neglecting correlations between neighboring dipoles. The factor $b\mathbf{m}$ can now be regarded as an added electric field by which one obtains an effective (mean) electric field (Høye and Stell, 1980; Ma, 1985)

$$E_e = E + bm \tag{13}$$

that acts upon independent (or free) dipoles. However, when adding effective fields on all dipoles, interactions are counted twice, which is compensated by an energy $1/2N_w bm^2$ for a system counting of N_w water molecules. Thus, in a mean-field treatment the pair interaction energy in Eq. 3 is approximated by

$$\mathcal{H}_p \rightarrow -b\mathbf{m} \cdot \sum_i \mathbf{s}_i + \tfrac{1}{2} N_w bm^2. \tag{14}$$

The partition function for one water molecule becomes

$$Z_w = e^{-1/2\beta bm^2} Z_w^e, \tag{15}$$

$\beta = 1/T$ where Boltzmann's constant k_B is absorbed in T and

$$Z_w^e = 2\pi \int_0^\pi d\vartheta \sin\vartheta e^{\beta E_e \cos\vartheta} = 4\pi \frac{\sinh \beta E_e}{\beta E_e}. \quad (16)$$

The polarization (or "magnetization") m is now obtained as (Ma, 1985)

$$m = \frac{\partial \ln Z_w^e}{\partial(\beta E_e)} = \coth \beta E_e - \frac{1}{\beta E_e}, \quad (17)$$

Inserting Eq. 13 in Eq. 17, one obtains the relation between m and E.

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