

The value of ΔG° calculated above is indeed roughly the amount by which the free energy changes when a small, very stable protein molecule folds, as we shall see in the next few sections. The calculation we have done is very crude and contains many arbitrary assumptions, but it captures the importance of the hydrophobic effect in protein folding.

10.25 Calorimetric measurements allow the experimental determination of the free energy of protein folding

We now consider how the changes in energy and entropy that occur during protein folding are determined experimentally. Calorimetry, an experimental technique that measures how much heat is absorbed by a system, allows us to determine the values of the standard enthalpy change (ΔH°) and the standard entropy change (ΔS°) for protein folding reactions.

A schematic diagram of an instrument known as a **differential scanning calorimeter** is shown in the Figure 10.24. The instrument consists of two identical chambers, denoted A and B in Figure 10.24, which are in a thermally isolated container. Chamber A contains a solution of the protein of interest, at a known concentration and buffered at a fixed pH. Chamber B contains everything in chamber A (for example, the buffer solution), but without the protein molecules. By using heating elements and electronic controls, the temperature of the solutions in both chambers is gradually increased in lockstep, so that the temperature of chamber A is always the same as that of chamber B. This gradual increase in temperature is referred to as a “temperature scan.”

Because chamber A contains protein molecules and chamber B does not, more heat is transferred to chamber A than to chamber B in order to increase the temperature by the same amount in each. In other words, the heat capacity of chamber A is greater than that of chamber B (see Section 6.8). By analyzing the difference in heat capacity between the two chambers as the temperature is scanned, we can determine the heat capacity of the protein, and how it changes as the temperature increases.

10.26 The heat capacity of a protein solution depends on the relative population of folded and unfolded molecules, and on the energy required to unfold the protein

The differential heat capacity as a function of temperature for the small protein lysozyme is shown in Figure 10.25. The differential heat capacity is the excess heat capacity of the protein molecules (and associated water molecules and ions) with respect to buffer solution, and we shall simply refer to it as the heat capacity of the protein. The heat capacity of the protein illustrated in Figure 10.25 increases

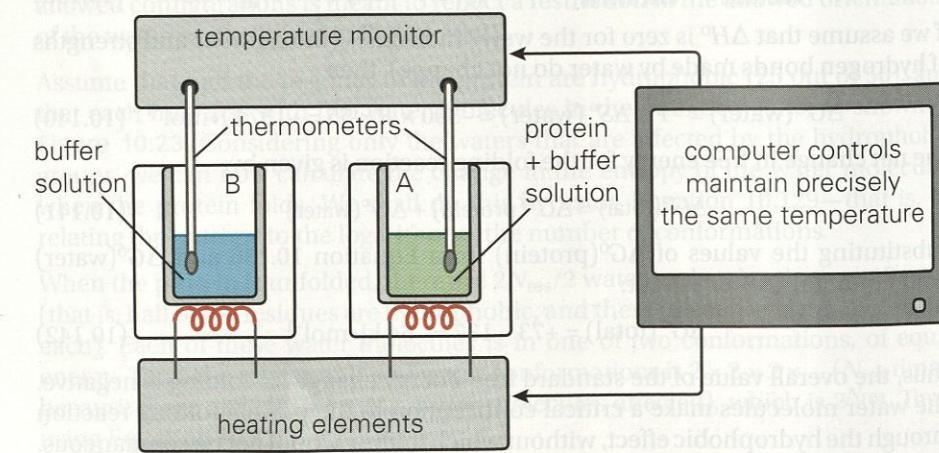
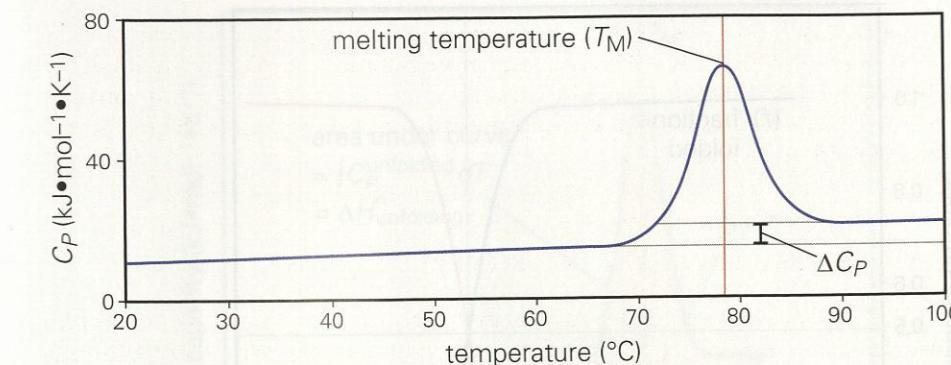


Figure 10.24 Schematic diagram of a differential scanning calorimeter. The instrument consists of two identical chambers, labeled A and B. Chamber A contains the protein, along with a buffer solution. Chamber B contains only the buffer. During the course of the experiment, the temperature of each chamber is kept precisely the same, while the temperature in both chambers is increased by attached heating elements. The excess heat transferred to chamber A relative to chamber B is monitored and is related to the heat capacity of the protein.



very gradually as the temperature is increased from 20°C to 70°C. At that point, the heat capacity increases sharply, reaches a maximum value at ~80°C and then decreases rapidly. At temperatures above ~85°C, the heat capacity reaches a stable value, but one that is higher than that of the protein solution at lower temperature. The variation in the heat capacity of the protein as a function of temperature is known as a **melting curve**.

The unfolding of the protein can be described as an equilibrium between two states, the folded state (denoted F) and the unfolded state (denoted U)—see Equations 10.120–10.123 and Figure 10.13. For the calorimetric experiment, we start with folded protein molecules and gradually unfold them as the temperature is increased. It is therefore convenient to consider the unfolding reaction (Equation 10.122).

The standard free-energy change for the unfolding reaction is given by:

$$\Delta G_{\text{unfolding}}^\circ = \Delta H_{\text{unfolding}}^\circ - T\Delta S_{\text{unfolding}}^\circ \quad (10.143)$$

As the temperature increases, more and more of the protein unfolds. At a certain temperature, the value of ΔG° becomes equal to zero, and the population of folded and unfolded molecules is equal. This temperature is known as the **melting temperature of a protein** (T_M), and it corresponds to the peak value of the heat capacity (see Figure 10.25).

Recall from Chapter 6 that the heat capacity of the folded protein is lower than that of the unfolded protein, as can also be seen in Figure 10.25. Thus, at the lower end of the temperature scale, where the folded protein is more stable, the measured heat capacity is essentially that of the folded protein. Since the experiment is carried out at constant pressure, the heat capacity is denoted C_p^F , where the superscript “F” refers to the folded protein and the subscript “P” refers to constant pressure. Likewise, at the higher end of the temperature scale, most of the protein molecules are unfolded and the measured heat capacity corresponds to that of the unfolded protein (C_p^U).

C_p^F and C_p^U are related to the ability of folded and unfolded protein molecules, as well as associated solvent molecules, to take up energy by increasing their vibrations. It turns out that C_p^U and C_p^F depend weakly on temperature, and their difference is essentially independent of temperature. As the temperature increases, the fraction (f) of the molecules that are folded decreases, and the fraction of molecules that are unfolded ($1-f$) increases (Figure 10.26). Because folding and unfolding are highly cooperative, the value of f decreases very slowly until the temperature is just below T_M , at which point the value of f decreases sharply.

The measured heat capacity includes contributions from C_p^F and C_p^U , weighted by the fraction of each state present at any temperature:

$$C_p^{F+U} = f C_p^F + (1-f) C_p^U \quad (10.144)$$

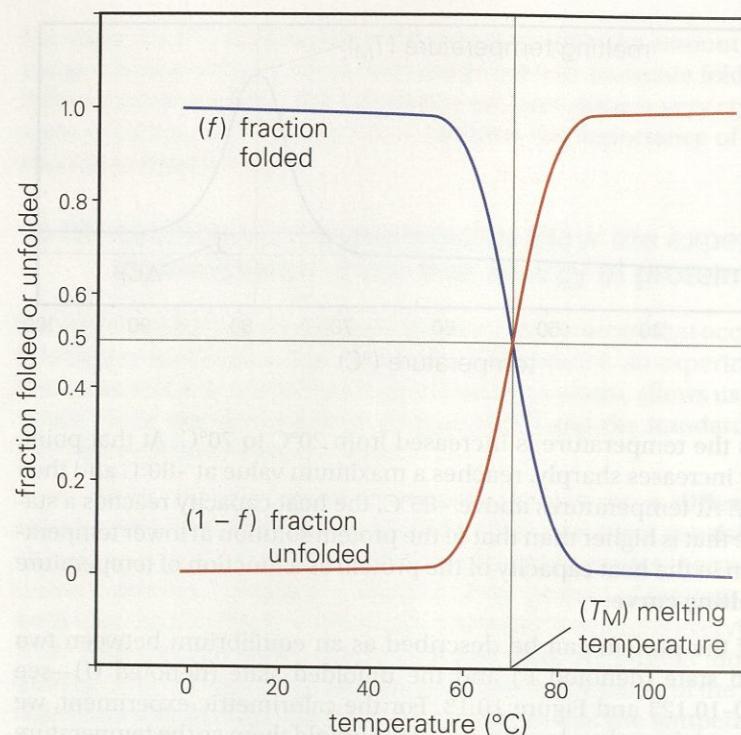
where f is the fraction of protein in the folded state. C_p^{F+U} is the combined heat capacity of the folded and unfolded proteins.

Figure 10.25 Heat capacity (C_p) of a small protein (lysozyme) as a function of temperature. The difference between the value of C_p at high temperature (when the protein is fully unfolded) and low temperature (when the protein is fully folded) is denoted ΔC_p . The peak in the heat capacity curve occurs at the melting temperature (T_M). (Adapted from W. Pfeil and P.L. Privalov, *Biophys. Chem.* 4: 23–32, 1976.)

Melting temperature of a protein

At the melting temperature, T_M , half the protein molecules in a solution are unfolded. The standard free-energy change for folding, ΔG° , is zero at $T = T_M$ for a monomeric protein. Melting temperatures of different proteins vary over a wide range.

Figure 10.26 The fraction of protein molecules that are folded or unfolded, as a function of temperature. There is generally a sharp transition from the fully folded to the fully unfolded state. The melting temperature (T_M) is the temperature at which half the protein molecules are folded.



The expected behavior of C_p^{F+U} is shown in Figure 10.27. The change in C_p^{F+U} with temperature does not explain the prominent peak in the heat capacity, centered at the melting temperature (T_M). This peak arises from an additional process that takes up energy as the temperature is increased, which is the conversion of protein molecules from the folded form to the unfolded one. It takes energy to break the interactions that stabilize the folded protein and this leads to an additional heat capacity term, denoted $C_p^{\text{unfolding}}$ (Figure 10.28). As the temperature approaches T_M , more and more protein molecules take up heat as they unfold, leading to increased heat capacity (see Figure 10.28). Once the temperature crosses T_M , most of the protein molecules are already unfolded, and the heat capacity decreases. Hence, the observed heat capacity is a sum of three terms:

$$C_p^{\text{observed}} = C_p^{F+U} + C_p^{\text{unfolding}} = f C_p^F + (1-f) C_p^U + C_p^{\text{unfolding}} \quad (10.145)$$

Although we have focused on the protein molecules in this discussion, it should be stressed that the measurements correspond directly to differences in enthalpies of all interactions, including those between the protein and the solvent.

10.27 The area under the peak in the melting curve is the enthalpy change for unfolding at the melting temperature

If we integrate the value of $C_p^{\text{unfolding}}$ over the narrow temperature range that spans the peak in the melting curve, we can determine the total amount of heat taken up as the folded protein is converted to the unfolded form:

$$\int C_p^{\text{unfolding}} dT = H_{\text{unfolded}} - H_{\text{folded}} = \Delta H_{\text{unfolding}} \quad (10.146)$$

$\Delta H_{\text{unfolding}}$ is essentially the change in enthalpy for the conversion of all of the folded protein to the unfolded form. Since we know the concentration of the protein, we can readily calculate the enthalpy change per mole of protein (ΔH°). Since most of the protein molecules undergo the unfolding process over a narrow range of temperature near T_M , we equate ΔH° with the enthalpy change for unfolding at the melting temperature, $\Delta H_{\text{unfolding}}(T_M)$.

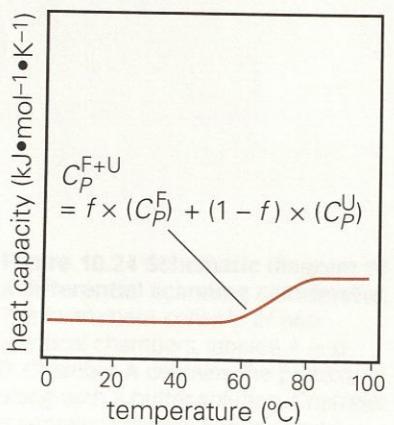


Figure 10.27 Contributions of C_p^F and C_p^U to the heat capacity of a protein. The red line shows the heat capacity due to the folded and unfolded proteins. It does not include the contribution that arises from the heat required to convert the folded form to the unfolded form.

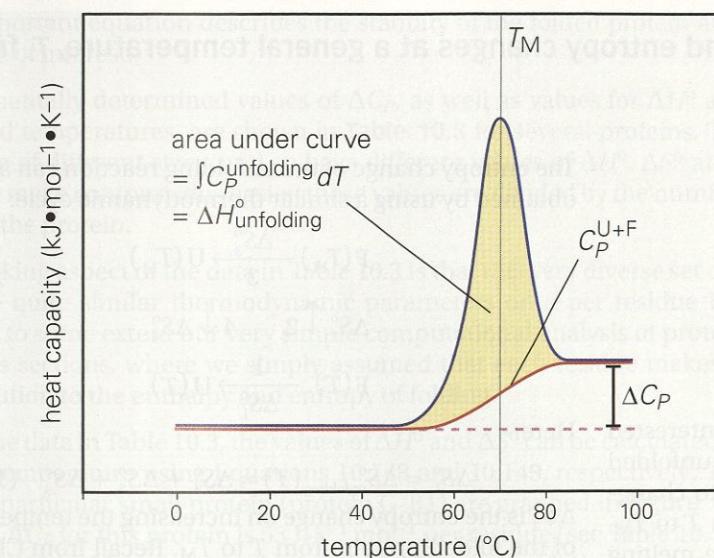


Figure 10.28 Determination of the standard enthalpy change of unfolding for a protein. Integrating the melting curve and subtracting the heat capacity of the unfolded and folded proteins yields the enthalpy change for unfolding (yellow shaded area).

At the melting temperature:

$$K(\text{at } T_M) = \frac{[U]}{[F]} = 1$$

so

$$\Delta G^\circ(T_M) = -RT \ln K(T_M) = 0$$

for a monomeric protein. Hence:

$$\begin{aligned} \Delta G^\circ_{\text{unfolding}}(\text{at } T_M) &= \Delta H^\circ_{\text{unfolding}} - T_M \Delta S^\circ_{\text{unfolding}} = 0 \\ \Rightarrow \Delta S^\circ_{\text{unfolding}} &= \frac{\Delta H^\circ_{\text{unfolding}}}{T_M} \end{aligned} \quad (10.147)$$

Thus, the calorimetric experiment gives us the values of ΔH° and ΔS° for the unfolding reaction at the melting temperature.

10.28 The heat capacities of the folded and unfolded protein allow the determination of ΔH° and ΔS° for unfolding at any temperature

When we considered the dissociation of water, we found that the values of ΔH° and ΔS° for that process were independent of temperature (see Section 10.14). But, as we noted, reactions that involve the hydrophobic effect, such as protein folding, do not behave this way. Although the calorimetric experiment gives us the values of ΔH° and ΔS° at the melting temperature, how do we determine these values at some other temperature (for example 25°C)? Because ΔH° and ΔS° are temperature dependent, we cannot simply apply the van't Hoff analysis (Section 10.14). Fortunately, the calorimetric data also give us the value of the difference between heat capacities of the folded and unfolded proteins ($\Delta C_p = C_p^U - C_p^F$), and this allows us to calculate how both ΔH° and ΔS° depend on temperature (Box 10.2 for details):

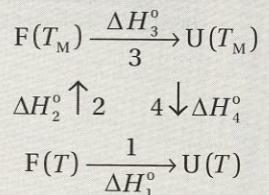
$$\Delta H_{\text{unfolding}}^\circ(T) = \Delta H_{\text{unfolding}}^\circ(T_M) + \Delta C_p(T - T_M) \quad (10.148)$$

$$\Delta S_{\text{unfolding}}^\circ(T) = \Delta S_{\text{unfolding}}^\circ(T_M) + \Delta C_p \ln \left(\frac{T}{T_M} \right) \quad (10.149)$$

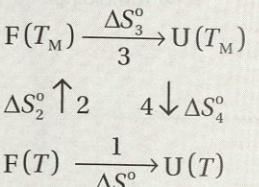
These equations tell us that if ΔC_p were zero (that is, if the folded and unfolded proteins had the same heat capacity), then $\Delta H_{\text{unfolding}}^\circ$ and $\Delta S_{\text{unfolding}}^\circ$ would be independent of temperature. But, in fact, experimental measurements of protein

Box 10.2 Determining the enthalpy and entropy changes at a general temperature, T , from heat capacity measurements

Consider the following thermodynamic cycle:



The entropy change of the unfolding reaction can also be obtained by using a similar thermodynamic cycle:



Here:

$$\Delta S_1^o = \Delta S_{\text{unfolding}}^o(T) = \Delta S_2^o + \Delta S_3^o + \Delta S_4^o \quad (10.2.6)$$

ΔS_2^o is the entropy change on increasing the temperature of the folded protein from T to T_M . Recall from Chapter 7 that the change in entropy is related to dq , the heat taken up during the process that is carried out very slowly (reversibly):

$$dS = \frac{dq}{T} \quad (10.2.7)$$

Since $dq = C_p dT$, we can write:

$$dS = \frac{C_p dT}{T} \Rightarrow \Delta S = \int \frac{C_p dT}{T} = C_p \int \frac{dT}{T} \quad (10.2.8)$$

Thus,

$$\Delta S_2^o = C_p \int_T^{T_M} \frac{dT}{T} = C_p \ln \left(\frac{T_M}{T} \right) \quad (10.2.9)$$

Likewise,

$$\Delta S_4^o = C_p \ln \left(\frac{T}{T_M} \right)$$

ΔS_3^o is the entropy change of unfolding at the melting temperature, denoted as $\Delta S_{\text{unfolding}}^o(T_M)$, which is determined experimentally. Combining these equations we get:

$$\Delta S_{\text{unfolding}}^o(T) = \Delta S_{\text{unfolding}}^o(T_M) + \Delta C_p \ln \left(\frac{T}{T_M} \right) \quad (10.2.10)$$

The standard free-energy change for unfolding at a temperature T is therefore given by:

$$\begin{aligned} \Delta G_{\text{unfolding}}^o(T) &= \Delta H_{\text{unfolding}}^o(T_M) - T \Delta S_{\text{unfolding}}^o(T_M) \\ &\quad + \Delta C_p \left[T - T_M - T \ln \left(\frac{T}{T_M} \right) \right] \end{aligned} \quad (10.2.11)$$

Here, the process labeled 1 is the one we are interested in, where the folded protein is converted to the unfolded form at temperature T . Process 2 corresponds to changing the temperature of the folded protein from T to T_M . Process 3 is the unfolding of the protein at the melting temperature, T_M . Finally, process 4 involves changing the temperature of the unfolded protein from T_M back to T .

Because enthalpy is a state function, we can write:

$$\Delta H_{\text{unfolding}}^o(T) = \Delta H_1^o + \Delta H_2^o + \Delta H_3^o + \Delta H_4^o \quad (10.2.1)$$

ΔH_2^o and ΔH_4^o can be readily determined from the measured heat capacities of the folded and unfolded proteins, respectively:

$$\Delta H_2^o = \int_T^{T_M} C_p dT = C_p(T_M - T) \quad (10.2.2)$$

and

$$\Delta H_4^o = \int_{T_M}^T C_p dT = C_p(T - T_M) \quad (10.2.3)$$

ΔH_3^o is the enthalpy of unfolding at the melting temperature, which is measured experimentally:

$$\Delta H_3^o = \Delta H_{\text{unfolding}}^o(T_M) \quad (10.2.4)$$

By substituting these values for ΔH_2^o , ΔH_3^o , and ΔH_4^o into Equation 10.2.1, we get:

$$\begin{aligned} \Delta H_{\text{unfolding}}^o(T) &= \Delta H_{\text{unfolding}}^o(T_M) + C_p^U(T - T_M) + C_p^F(T_M - T) \\ &= \Delta H_{\text{unfolding}}^o(T_M) + (C_p^U - C_p^F)(T - T_M) \end{aligned}$$

$$\Delta H_{\text{unfolding}}^o(T) = \Delta H_{\text{unfolding}}^o(T_M) + \Delta C_p(T - T_M) \quad (10.2.5)$$

where ΔC_p is the difference between the heat capacities of the unfolded and folded proteins.

unfolding demonstrate that the value of ΔC_p always has a positive value for proteins, as can be seen for lysozyme in Figure 10.25.

Combining Equations 10.148 and 10.149, we can calculate the standard free-energy change of unfolding ($\Delta G_{\text{unfolding}}^o$) at any temperature, T :

$$\begin{aligned} \Delta G_{\text{unfolding}}^o(T) &= \Delta H_{\text{unfolding}}^o(T) - T \Delta S_{\text{unfolding}}^o(T) \\ \Delta G_{\text{unfolding}}^o(T) &= \Delta H_{\text{unfolding}}^o(T_M) + \Delta C_p(T - T_M) \\ &\quad - T \left(\Delta S_{\text{unfolding}}^o(T_M) + \Delta C_p \ln \left(\frac{T}{T_M} \right) \right) \end{aligned} \quad (10.150)$$

This important equation describes the stability of the folded protein at any temperature of interest.

Experimentally determined values of ΔC_p , as well as values for ΔH^o and ΔS^o at specified temperatures, are shown in Table 10.3 for several proteins. These proteins are of different sizes, and so have different values of ΔH^o , ΔS^o and ΔC_p . In order to make comparisons easier, these values are divided by the number of residues in the protein.

One striking aspect of the data in Table 10.3 is that this very diverse set of proteins all have quite similar thermodynamic parameters on a per residue basis. This justifies to some extent our very simple computational analysis of proteins in the previous sections, where we simply assumed that each residue makes a similar contribution to the enthalpy and entropy of folding.

Given the data in Table 10.3, the values of ΔH^o and ΔS^o can be calculated as a function of temperature using Equations 10.148 and 10.149, respectively. The results for one particular small protein (protein G-B1) are graphed in Figure 10.29. The value of ΔC_p for this protein is $53 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ per residue (see Table 10.3). Protein G-B1 has 56 residues, and so the value of ΔC_p for the whole protein is $56 \times 53 \approx 3000 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1} = 3 \text{ kJ}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. Consider a change in temperature from 20°C (293 K) to 30°C (313 K). According to Equation 10.148, the value of $\Delta H_{\text{unfolding}}^o$ would then change as follows:

$$\begin{aligned} \Delta H_{\text{unfolding}}^o(T=313\text{K}) - \Delta H_{\text{unfolding}}^o(T=293\text{K}) &= \\ \Delta C_p(313-293) &= 3.0 \times 10 = 30 \text{ kJ}\cdot\text{mol}^{-1} \end{aligned} \quad (10.151)$$

Indeed, ΔH^o increases by $30 \text{ kJ}\cdot\text{mol}^{-1}$ over this temperature range for protein G-B1, as reflected in the graph in Figure 10.29. Note that ΔH^o and $T\Delta S^o$ change much more rapidly than their difference, which is ΔG^o (Figure 10.30).

How do these experimental results compare with our simple computational model for folding? Figure 10.30 shows the value of $\Delta G_{\text{unfolding}}^o$ as a function of temperature for G-B1. At room temperature (25°C , 298 K), the experimentally determined value of $\Delta G_{\text{unfolding}}^o$ is $+28 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta H_{\text{unfolding}}^o$ is $\approx +78 \text{ kJ}\cdot\text{mol}^{-1}$. In terms of the

Table 10.3 Thermodynamic parameters for unfolding of various proteins.

Protein	Molecular weight	$\Delta H^o(25^\circ\text{C})$ [$\text{kJ}\cdot\text{mol}^{-1}$ per residue]	$\Delta S^o(110^\circ\text{C})$ [$\text{J}\cdot\text{mol}^{-1}$ per residue]	ΔC_p [$\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ per residue]
Protein G-B1	7200	1.4	16.1	53
Parvalbumin	11,500	1.4	16.8	46
Cytochrome c	12,400	0.64	17.8	67
Ribonuclease A	13,600	2.4	17.8	44
Hen lysozyme	14,300	2.0	17.6	52
Staph. nuclease	16,800	0.85	17.5	61
Myoglobin	17,900	0.04	17.9	75
Papain	23,400	0.93	17.0	60
β -Papain	23,800	1.3	17.9	58
α -Chymotrypsin	25,200	1.1	18.0	58
Average		1.2 ± 0.7	17.4 ± 0.6	57 ± 9

This table presents values of ΔH^o , ΔS^o , and ΔC_p for several proteins. The temperatures for which ΔH^o and ΔS^o are calculated were chosen to reflect the enthalpy and entropy of the protein chain itself, without the hydrophobic interaction (see Baldwin, 1986, in Further Reading). The values scale with the size of the protein; in order to make comparison easier, the values have been divided by the number of residues in the protein to give a "per residue" value. (Adapted from P. Alexander et al., and P. Bryan, *Biochemistry* 31: 3597–3603, 1992. With permission from the American Chemical Society.)

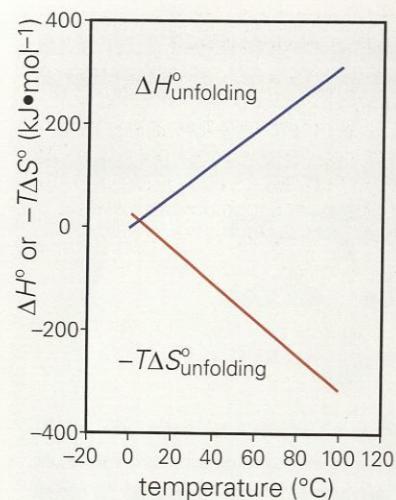


Figure 10.29 Temperature dependence of the enthalpy and entropy components of the free energy of unfolding. (Based on data for protein G-B1 in Table 10.3.)

folding reaction, $\Delta G_{\text{folding}}^{\circ}$ is therefore $-28 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta H_{\text{folding}}^{\circ}$ is $-78 \text{ kJ}\cdot\text{mol}^{-1}$. These values are comparable to the values of $\Delta G_{\text{folding}}^{\circ}$ and $\Delta H_{\text{folding}}^{\circ}$ we calculated for our highly simplified model:

$$\Delta G_{\text{folding}}^{\circ} = -64 \text{ kJ}\cdot\text{mol}^{-1}, \quad \Delta H_{\text{folding}}^{\circ} = -150 \text{ kJ}\cdot\text{mol}^{-1}$$

10.29 Folded proteins become unstable at very low temperature because of changes in ΔH° and ΔS°

There is one fundamental way in which the experimental measurements of protein folding differ from the result of our simple computational model. Note that the energy and entropy changes in the simple model are both *independent* of temperature (see Equations 10.133 and 10.134). The free energy of folding is given by:

$$\Delta G_{\text{folding}}^{\circ} = \Delta H_{\text{folding}}^{\circ} - T\Delta S_{\text{folding}}^{\circ}$$

Recall that in our model calculation, $\Delta S_{\text{folding}}^{\circ}$ is positive, which predicts that the stability of the folded protein decreases linearly with temperature, shown by the blue line in Figure 10.30. This contrasts markedly with the experimental situation, where the stability of the folded protein is maximal at a particular temperature and decreases as the temperature either increases or decreases with respect to this temperature. The temperature of maximum stability varies considerably for different proteins within the same organism. In addition, as you might expect, proteins from thermophilic organisms (ones that grow at elevated temperatures) remain folded at temperatures that would lead to the unfolding of most proteins from normal organisms.

To better understand why proteins lose stability at low temperatures, we will examine the values of $\Delta H_{\text{unfolding}}^{\circ}$ and $\Delta S_{\text{unfolding}}^{\circ}$ for protein G-B1. First, let us use the data in Table 10.3 to figure out what these values are. From Table 10.3, the value of $\Delta H_{\text{unfolding}}^{\circ}$ at 25°C (298 K) is $+1.4 \text{ kJ}\cdot\text{mol}^{-1}$ per residue. Since this protein has 56 residues, $\Delta H_{\text{unfolding}}^{\circ} = +1.4 \times 56 = +78.4 \text{ kJ}\cdot\text{mol}^{-1}$. From the graph of $\Delta G_{\text{unfolding}}$ versus temperature (see Figure 10.30), we see that $T_M = 85^\circ\text{C}$ for protein G-B1. Using Equation 10.148 and the known value of ΔC_p , we can calculate the value of $\Delta H_{\text{unfolding}}^{\circ}(T_M)$, which turns out to be $+258 \text{ kJ}\cdot\text{mol}^{-1}$. Recalling Equation 10.147, at the melting temperature:

$$\Delta S_{\text{unfolding}}^{\circ} = \frac{\Delta H_{\text{unfolding}}^{\circ}}{T_M} \quad (10.152)$$

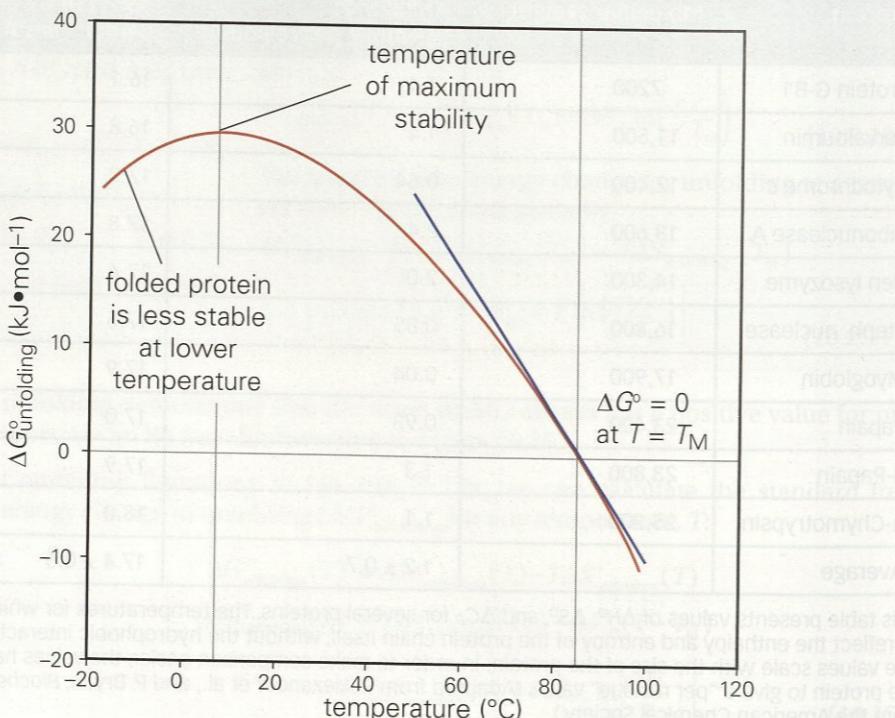


Figure 10.30 The change in the free energy of $\Delta G_{\text{unfolding}}^{\circ}$ as a function of temperature for a small protein (B-G1). This “protein stability curve” is based on the data in Table 10.3 and Equation 10.150. This curve shows that this protein has a maximum stability at about 7°C. The blue line reflects what the protein stability would be if ΔH and ΔS were temperature independent, and equal to their values at the melting temperature.

Substituting the values of $\Delta H_{\text{unfolding}}^{\circ}$ and T_M , we find that $\Delta S_{\text{unfolding}}^{\circ}$ is $0.72 \text{ kJ}\cdot\text{K}^{-1}\text{mol}^{-1}$ for protein G-B1, at the melting temperature.

We can now readily calculate the value of $\Delta G_{\text{unfolding}}^{\circ}$ for protein G-B1 at any temperature by using these values, assuming either that $\Delta H_{\text{unfolding}}^{\circ}$ and $\Delta S_{\text{unfolding}}^{\circ}$ are temperature independent, or that they vary with temperature due to ΔC_p . In the latter case, we have to use Equation 10.150 to relate $\Delta G_{\text{unfolding}}^{\circ}$ to $\Delta H_{\text{unfolding}}^{\circ}$ and $\Delta S_{\text{unfolding}}^{\circ}$. The results of both calculations are graphed in Figure 10.30. When the ΔC_p term is included, we see that $\Delta G_{\text{unfolding}}^{\circ}$ has a maximum value at $\sim 7^\circ\text{C}$ and decreases at *both* higher and lower temperatures. In contrast, using temperature-independent values for the changes in enthalpy and entropy, the value of $\Delta G_{\text{unfolding}}^{\circ}$ continues to increase as temperature gets lower.

The loss of protein stability at lower temperature is known as **cold denaturation**, and it runs counter to intuition. We can see that the curvature in the protein stability curve (free energy vs. temperature) arises as a consequence of the terms involving ΔC_p in Equation 10.150. These terms, which describe the increased heat capacity of the unfolded protein, are not explained by our simple model for protein folding. ΔC_p is observed to be positive for protein-unfolding reactions and this behavior arises because of changes in the energetic interactions between hydrophobic residues in the protein and water molecules (neglected in our simple treatment). The origin of the ΔC_p term is still not completely understood, but it is found to be correlated empirically with the increased exposure of hydrophobic sidechains in the unfolded protein. In proteins with low stability and large values of ΔC_p , the free-energy curve can drop sufficiently at low temperature that it crosses $\Delta G_{\text{unfolding}}^{\circ} = 0$, the point at which the folded protein becomes unstable and unfolds. Such proteins do not fold at low temperature.

Summary

This chapter introduced the concept of the chemical potential, μ , which is the rate of change of free energy with respect to the number of molecules. The chemical potential is essentially the free energy per molecule, or per mole. For ideal dilute solutions, a key result is that the chemical potential is related to the logarithm of the concentration. If the concentrations of a molecule in two solutions are C_1 and C_2 , then the difference in chemical potential for that molecule is given by:

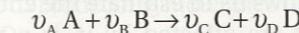
$$\Delta\mu = \mu_2 - \mu_1 = RT \ln\left(\frac{C_2}{C_1}\right) \quad (10.32)$$

The chemical potential for a molecule under standard conditions (typically, 1 M solution) is denoted μ° . Equation 10.32 allows us to calculate the chemical potential of the molecule at a nonstandard concentration (C):

$$\mu = \mu^\circ + RT \ln\left(\frac{C}{C^\circ}\right) = \mu^\circ + RT \ln C \quad (10.33)$$

In Equation 10.33, we have assumed that the standard concentration, C° , is 1 M, and have omitted it in the final expression.

For a general chemical reaction of the form:



where v_A , v_B , v_C , and v_D are the stoichiometric coefficients, the value of the standard free-energy change for the reaction, ΔG° , is given by:

$$\Delta G^\circ = v_C \mu_C^\circ + v_D \mu_D^\circ - v_A \mu_A^\circ - v_B \mu_B^\circ \quad (10.54)$$

where μ_A° , μ_B° , μ_C° , and μ_D° , are the chemical potentials (molar free energies) of the A, B, C, and D molecules, respectively, under standard conditions (typically 1 M solutions). The equilibrium point for the reaction is defined by the following condition:

$$\Delta G^\circ = -RT \ln K_{\text{eq}} \quad (10.52)$$

where K is the equilibrium constant for the reaction. The value of K is related to the concentrations, [A], [B], [C], and [D] at equilibrium as follows:

$$K = \frac{[C]^{v_C} [D]^{v_D}}{[A]^{v_A} [B]^{v_B}} \quad (10.51)$$

Cold denaturation

Cold denaturation refers to the unfolding of proteins induced at low temperature. This arises from the ΔC_p term in the protein stability equation, which correlates with the burial of hydrophobic residues in the folded state of the protein.