Common Features of Protein Unfolding and Dissolution of Hydrophobic Compounds

KENNETH P. MURPHY, PETER L. PRIVALOV, STANLEY J. GILL*

Protein unfolding and the dissolution of hydrophobic compounds (including solids, liquids, and gases) in water are characterized by a linear relation between entropy change and heat capacity change. The same slope is found for various classes of compounds, whereas the intercept depends on the particular class. The feature common to these processes is exposure of hydrophobic groups to water. These observations make possible the assignment of the heat capacity change to hydrophobic solvation and lead to the description of protein stability in terms of a hydrophobic and a nonhydrophobic contribution. A general representation of protein stability is given by the heat capacity change and the temperature.

THE FORMATION AND STABILITY OF the structure of globular proteins is of importance in their role as catalysts and ligand transport molecules. Our understanding of the energetic features that determine structure is still at a relatively primitive state. We understand even less about the properties that control the highly cooperative nature of the formation of the native structure. The careful evaluation of the thermodynamics of the folding process is a first step in answering these questions. Scanning calorimetry (*1–3*) has provided basic information for the evaluation of changes in free energy (ΔG), entropy (ΔS), enthalpy (ΔH), and heat capacity (ΔC_p, constant pressure) and the establishment of two significant states in the denaturation process. Some of the general features of protein stability curves have been described recently by Becktel and Schellman (*4*) for the situation of temperature-independent heat capacity change and by Franks, Hatley, and Friedman (*5*) for the more general case, including temperature dependence of the heat capacity change.

Correlation of the thermodynamic properties of protein folding with data on hydrophobic model compounds has provided insight into the general importance of nonpolar interactions. One of the key features of nonpolar compounds dissolved in water is their unusually large heat capacity contribution (*6, 7*). The heat capacity is directly proportional to the surface area or number of solvated water molecules in the first solvation shell (*8*). Various views have been developed concerning the role that hydrophobic interactions play in determining the folded protein structure (*9–11*).

Privalov has noted that both the denaturational enthalpy and entropy changes for a variety of globular proteins extrapolate to a common value (per unit residue) near 110°C (*1*). Baldwin (*10*) recognized that this is the same temperature for which the entropy of dissolution of liquid hydrocarbons (*12*) is zero. He suggested that the hydrophobic contribution in stabilizing the native protein could be evaluated from the change in the heat capacity combined with temperature parameters characterizing the dissolution of liquid hydrocarbons. His observation was consistent with a property noted by Sturtevant (*13*), that the ratio of the entropy change to the heat capacity change for the dissolution of a variety of hydrophobic compounds is a constant. These features are also consistent with a general equation of state developed for the dissolution process of liquid hydrophobic compounds (*14*).

The 110°C reference temperature is where hydrophobic interaction, judged by the free energy of dissolution of liquid hydrocarbons, is at its maximum value and is determined entirely by enthalpic contributions. Baldwin proposed that the hydrophobic free energy in proteins could be evaluated from these properties (*10*). On the other hand, Privalov and Gill suggested that the hydrophobic free energy change could be more accurately delineated from the thermodynamic properties of protein denaturation, namely, from the heat capacity–dependent contribution to the free energy of protein unfolding (*11, 15*). Formally both proposals are based on the idea that the heat capacity change phenomenologically defines the hydrophobic effect and that the change in heat capacity is due entirely to exposure of nonpolar groups to the solvent water. However, these formulations lead to contrasting conclusions concerning the effect of hydrophobic interactions on protein stability: the liquid hydrocarbon model suggests that hydrophobic interactions always lead to stabilization, whereas the analysis of general heat capacity effects found for globular proteins suggests that hydrophobic interactions lead to destabilization. In order to explore the differences in these proposals we have examined the thermodynamic properties of dissolution for a variety of compounds (gases, liquids, and solids) containing hydrophobic groups for which high-precision information, particularly values of the heat capacity change, is available.

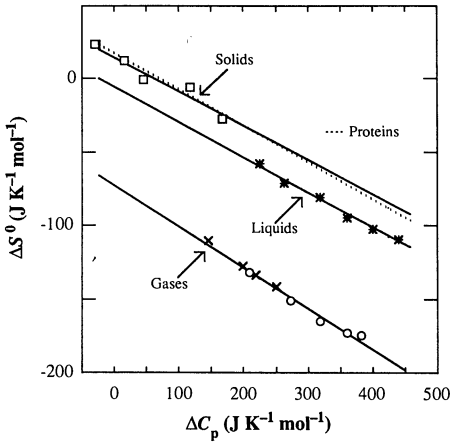

Fig. 1. Entropy change of dissolution versus heat capacity change at 25°C for apolar gases (x, rare gases; ○, saturated hydrocarbon gases), liquid hydrocarbons, and solid cyclic dipeptides. The elements and compounds are listed in the footnote of Table 1. The solid lines are the least-squares fit of the data with a slope of ln ($298/T_S^*$). The dotted line represents thermal denaturation data on 11 proteins as listed in Fig. 2A.

As noted, plots of enthalpy (per residue) of denaturation against temperature yield a common extrapolated value near 110°C (*1*) and the entropy of dissolution of liquid hydrocarbons gives a common extrapolated value near 112°C (*10*). An equivalent way of representing such data is to plot the enthalpy change or the entropy change versus the heat capacity change at a given temperature T for the group of compounds under consideration. If linear representation is observed, then the condition of a common reference temperature and intercept is met. Thus, if the heat capacity change at a temperature T for a given process has a value ΔC_p, then the temperature dependence of the thermodynamic function, X, is described with reference to a hypothetical temperature T_X^*:

$$\Delta H = \Delta H^* + \Delta C_p\,(T - T_H^*) \quad (1)$$

$$\Delta S = \Delta S^* + \Delta C_p \ln (T/T_S^*) \quad (2)$$

Plots of ΔH versus ΔC_p or ΔS versus ΔC_p at a temperature T will have a slope given by $(T - T_H^*)$ or $\ln(T/T_S^*)$ and an intercept of ΔH^* or ΔS^*. This linear representation has the advantage of removing the possible effects of a temperature dependence of ΔC_p.

In Fig. 1 we show four sets of standard entropy data, based on high-precision calo-

K. P. Murphy and S. J. Gill, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309.
P. L. Privalov, Institute of Protein Research, Academy of Sciences of the U.S.S.R., Moscow Region, U.S.S.R.

*To whom requests for reprints should be addressed.

rimetric and solubility studies, plotted by the use of this procedure: (i) dissolution of liquid hydrocarbons (*12*), (ii) dissolution of saturated hydrocarbon gases (*16–20*), (iii) dissolution of rare gases (*16, 21, 22*), and (iv) dissolution of crystalline cyclic dipeptides (diketopiperazines) (*23, 24*). In Fig. 2 both entropy (A) and enthalpy (B) data are plotted versus heat capacity change for protein denaturation at 25°C for 11 proteins that have been examined by scanning calorimetry (*11*). The notable features of these plots are the linearity and the virtually identical slopes.

The slope of the entropy plots allows determination of T_S^* for the dissolution processes of model compounds and for the denaturation of proteins. The nearly parallel slopes observed for these processes allow them to be fitted simultaneously to straight lines under the condition that T_S^* is the same for all of the data sets and yields a T_S^* of 112° ± 3°C. The results are summarized in Table 1. Temperatures in this range characterize molecular events of solvation processes of apolar substances. It has been suggested from thermodynamic features (*25*) and specific models (*8, 26*) that at this temperature approximately one-half of the hydrogen bonds of water in the solvation shell are broken (*27*).

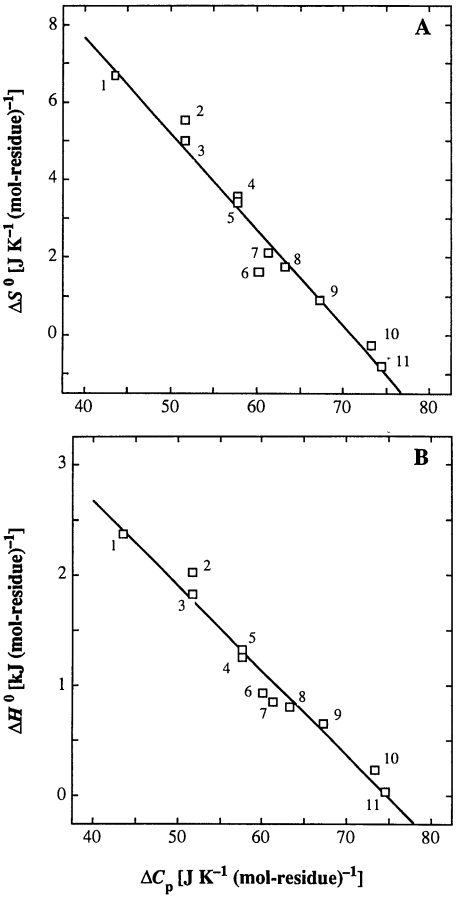

Fig. 2. Linear correlation of thermodynamic properties for denaturation at 25°C for the following proteins as tabulated by Privalov and Gill [table 1 in (*11*)]: 1, ribonuclease A; 2, egg white lysozyme; 3, fragment K4 of plasminogen; 4, α-chymotrypsin; 5, β-trypsin; 6, papain; 7, *Staphylococcus* nuclease; 8, carbonic anhydrase; 9, cytochrome c; 10, pepsinogen; and 11, myoglobin. (**A**) Entropy of denaturation versus heat capacity change. (**B**) Enthalpy of denaturation versus heat capacity change.

The similar slopes found for the entropy–heat capacity plots (Fig. 1) indicate the dominant role that water plays in determining the thermodynamics of hydration of these compounds. The intercept values correspond to the process involving no change in heat capacity and can be regarded as the nonhydrophobic contribution of the entropy change. The observed difference in the entropy of dissolution between liquids and gases of 80 J mol^{-1} K^{-1} corresponds to the general prediction of Trouton's rule (*28*). The correspondence between the model solid compounds and the proteins reflects the inherently similar melting and solvation features that are common to these processes and supports the contention that the protein core is described by properties of organic solids (*29–31*).

The plots of enthalpy and entropy versus heat capacity change for protein unfolding (Fig. 2, A and B) are linear and give essentially the same value for the convergence temperature. In marked contrast, liquid hydrocarbons do not show the same convergence temperature for both the enthalpy and entropy functions; the enthalpy of dissolution is zero near room temperature, whereas the entropy of dissolution is zero near 112°C. These differences in the properties of globular proteins and liquid hydrocarbons lead to the two different views about the role of hydrophobic effects in stabilizing proteins. This can be seen in terms of the standard free energy change ΔG°:

$$\Delta G^\circ = \Delta H^* - T\Delta S^* + \Delta C_p\left[(T - T_H^*) - T\ln(T/T_S^*)\right] \quad (3)$$

The heat capacity term represents the hydrophobic contribution to the free energy change, namely,

$$\Delta G^\circ_{hyd} = \Delta C_p\left[(T - T_H^*) - T\ln(T/T_S^*)\right] \quad (4)$$

Although other contributions to the heat capacity change in protein denaturation have been suggested (*13*), the model compound studies suggest that these other contributions are largely insignificant.

In the liquid hydrocarbon case this is the only term that contributes to the free energy of dissolution and furthermore is always positive at practical experimental temperatures. For proteins, however, T_S^* and T_H^* are equal and ΔG°_{hyd} is always less than or equal to zero. Thus within proteins the hydrophobic effect, by this thermodynamic description and with currently available data on globular proteins, is found to be destabilizing as suggested (*11*) in a less comprehensive analysis. The liquid hydrocarbon properties, used by Baldwin as a measure of hydrophobic interactions in proteins (*10*), now seem inappropriate. The close similarity of properties of solid model compounds (*24*) to those found in proteins suggests that the solid nature of the protein core plays a significant role in the formation of the folded state.

Table 1. Linear representation and entropy change versus heat capacity change at 25°C for protein denaturation and dissolution processes of model compounds with hydrophobic groups. Errors shown are 1 SD of fit for a given parameter.

Process	Slope	ΔS^* for T_S = 112°C
Protein denaturation	−0.25 ± 0.01	18 ± 1
Liquid hydrocarbon dissolution*	−0.24 ± 0.01	−0.5 ± 3.1
Gas dissolution, rare gases,† and alkanes‡	−0.28 ± 0.01	−78.5 ± 2.5
Solid dissolution (hydrophobic cyclic dipeptides)§	−0.23 ± 0.04	16 ± 1

*Compounds include benzene, toluene, ethylbenzene, cyclohexane, pentane, and hexane. †Neon, argon, krypton, and xenon. ‡Methane, ethane, propane, butane, and isobutane. §Cyclo-(glycyl-glycyl), cyclo-(alanyl-glycyl), cyclo-(alanyl-alanyl), cyclo-(leucyl-glycyl), and cyclo-(valyl-valyl).

From Eq. 3 one can obtain a general view of protein stability in terms of temperature and hydrophobicity as represented by the heat capacity change. A contour plot, normalized to the number of amino acid residues, of the stability of globular proteins as a function of T and ΔC_p is shown in Fig. 3. This plot is based on a standard state reference pH = 7.0 in the absence of denaturants or other ligands. Inclusion of these effects will shift the contours of the free energy surface primarily in a vertical manner through general ligand linkage contributions. This surface, when applied to a specific protein with n amino acids, retains the same contour line features but the spacing between contour levels is multiplied by a factor of n. The bold line, where $\Delta G^\circ/n$ is zero, remains unchanged.

At a given temperature the stability of proteins decreases with increasing hydrophobicity, indicated by increasing ΔC_p values. For a protein with ΔC_p greater than 50

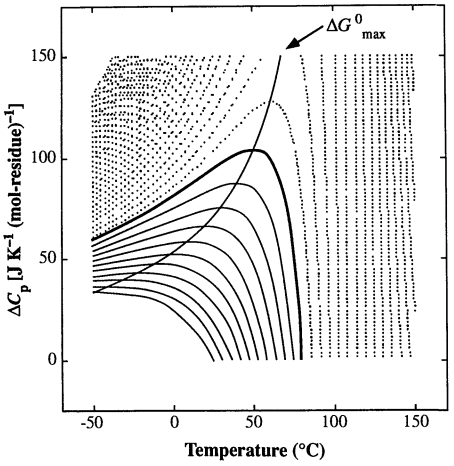

Fig. 3. Generalized map of the free energy change of protein stability per unit amino acid residue as a function of heat capacity change and temperature. The bold solid line represents the level at which $\Delta G^o/n$ is zero for the denaturation reaction of the native protein (N → D). The spacing between contour lines is 100 J mol^{-1}, with solid lines indicating positive values and dotted lines indicating negative values. The contour map was generated from Eq. 3 with ΔH^* = 6.4 kJ (mol-residue)$^{-1}$, ΔS^* = 18.1 J K^{-1} (mol-residue)$^{-1}$, and T^* = 112°C. The solid line labeled ΔG^o_{max} indicates where ΔG^o for a protein with a given ΔC_p goes through its maximum value as the temperature is raised.

J K^{-1} (mol-residue)$^{-1}$, the stability rises to a maximum value and then decreases with increasing temperature. The decrease in stability with decreasing temperature is a direct manifestation of increased hydrophobic solvation at lower temperatures and gives rise to the phenomenon of cold denaturation. At high temperatures, a decrease in stability occurs as a result of the dominance of the nonhydrophobic entropy contribution, expressed in $T\Delta S^*$. The standard state stability surface given in Fig. 3 summarizes the universal thermodynamic features found in proteins and provides a first-order representation of the thermal stability of globular proteins.

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Activation of Proto-Oncogenes: An Immediate Early Event in Human Cytomegalovirus Infection

ISTRAN BOLDOGH,* SAZALY ABUBAKAR, THOMAS ALBRECHT†

A rapid increase in the RNA levels of the proto-oncogenes c-*fos*, c-*jun*, and c-*myc* was detected after human cytomegalovirus infection. Neither inactivation of viral infectivity with ultraviolet irradiation (with or without psoralen), nor inhibition of translation with cycloheximide or anisomycin adversely affected the enhanced expression of proto-oncogenes, even though these treatments substantially reduced or eliminated the detection of immediate early viral antigens. The increase in the RNA levels of the proto-oncogenes was prevented in the presence of α-amanitin or actinomycin D. Thus, expression of these oncogenes appears to be induced by events occurring before the onset of viral protein synthesis, perhaps by the interaction of viral particles with the cell surface.

HUMAN CYTOMEGALOVIRUS, A VIrus with demonstrated oncogenic potential in vitro (*1*), has long been recognized for its ability to stimulate cellular DNA synthesis and cell proliferation (*2*). Infection with human cytomegalovirus (HCMV) generates cellular responses similar to those described for activation of cells by growth factors such as platelet-derived growth factor (*3*) including: increased cellular levels of the secondary messengers inositol trisphosphate (IP_3) and 1,2-diacylglycerol (DG), Ca^{2+} influx, increased cytosolic Ca^{2+} activity, and increased cellular levels of adenosine 3′,5′-monophosphate (cAMP) [reviewed in (*4*)]. It has also been demonstrated that HCMV particles that have been inactivated and appear to be incapable of giving rise to the synthesis of either immediate early (IE) or early viral antigens play an important role in the stimulation of HCMV-induced cell proliferation (*5*). These findings suggest that defective or inactivated HCMV particles have the capacity to initiate cellular replication.

The molecular interactions associated with the activation of cell proliferation have been the subject of extensive recent studies through analysis of the induction of proto-oncogenes in quiescent cells stimulated to proliferate by the addition of serum or growth factors. From the temporal pattern of their expression, it has been suggested that proto-oncogenes play a pivotal role in the regulation of cell proliferation (*6*). Since stimulation of proto-oncogene expression could be involved in cell activation by HCMV as previously described (*4*), we wished to determine whether HCMV infection affected the expression of selected proto-oncogenes. We now show that one of the consequences of HCMV infection is a striking increase in the levels of RNA derived from the proto-oncogenes c-*fos*, c-*jun*, and c-*myc*.

Quiescent human embryonic lung (LU) cells infected with HCMV demonstrated a rapid, transient increase in the levels of RNA for the proto-oncogenes c-*jun*, c-*fos*, and c-*myc* between 20 and 120 min postin-

Department of Microbiology, The University of Texas Medical Branch, Galveston, TX 77550.

*Permanent address: Department of Microbiology, Medical University of Debrecen, Debrecen H-4012, Hungary.

†To whom correspondence should be addressed.