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Letter

Protein volume changes on cosolvent denaturation

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Abstract

A thermodynamic relationship is obtained which links the effect of a cosolvent on the denaturation equilibrium of a protein to the effect of the cosolvent on the change in partial molar volume (pmv) of a protein on denaturation. The relationship uses the concept of preferential interactions and is exact for an infinitely dilute protein. Analysis of the literature data on protein volume changes suggests that many of the observed volume changes are thermodynamically inconsistent with the corresponding free energy changes, especially at low cosolvent concentrations. It is argued that the most reasonable explanation for this involves cosolvent induced changes in the degree of protein–protein association.

Keywords: Protein volume; Cosolvent; Protein denaturation; Protein aggregation; Preferential interactions

The change in volume of a protein upon denaturation is an important property that may play a significant role in understanding the denaturation process [1,2]. Recently, several studies have reinvestigated the change in protein volume accompanying denaturation or stabilization in the presence of chaotropic (urea, GdmCl) or kosmotropic (some salts, sugars, polyols) cosolvents [3–7]. Some of these results [7], together with earlier studies [8–10], suggest that the change in volume on denaturation becomes more positive in the presence of chaotropes. Here we show that such data are thermodynamically inconsistent with a cosolvent-induced favorable denaturation free energy change for an infinitely dilute protein solute in which protein–protein interactions are assumed to be negligible.

There are several ways to measure differences in protein volumes. Early approaches used changes in density or dilatometric studies to determine the change in volume on transfer of the native protein from pure water to a denaturing solution [11]. Volume changes determined in this manner correspond to,

$$\Delta V = f_D \overline{V}_D(\rho_c) + f_N \overline{V}_N(\rho_c) - \overline{V}_N(0) \quad (1)$$

where f_D is the fraction of denatured state at a particular molar cosolvent concentration ρ_c, and $\overline{V}_i$ is the partial molar volume (pmv) of the different states, assuming a two state equilibrium (N⇄D). The results obtained for urea and GdmCl cosolvents generally display an oscillating pattern with an increase in ΔV at low cosolvent concentrations, followed by a sharp fall in the transition region, and a final increase for post transition concentrations [8–10]. The increases at low and high cosolvent are attributed to cosolvent binding to the protein, while the sharp decrease corresponds to the transition from native to denatured states [8,10].

Alternatively, protein volume changes can be extracted from studies of pressure denaturation [2]. As the partial molar volume (pmv) of the denatured state is typically smaller than the native state [2], increases in pressure shift the equilibrium in favor of the denatured state. The corresponding volume change is given by,

$$\Delta \overline{V}_s = \overline{V}_D(\rho_c) - \overline{V}_N(\rho_c) \quad (2)$$

The relatively small volume changes observed are difficult to measure and require an extrapolation of the effects of high pressure on the denaturation equilibrium. The effects of cosolvents on pressure denaturation have been studied with both increases and decreases in the volume change being

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observed [2,4–6]. All interpretations of the experimental data on protein volume changes have ignored protein–protein interactions and are therefore equivalent to assuming the cosolvent effects refer to an infinitely dilute protein.

Here we outline the thermodynamic relationship between the effect of a cosolvent on the denaturation equilibrium and the corresponding effect on the pmv values of the native and denatured proteins. The effects of a cosolvent on the denaturation equilibrium can be expressed in terms of the preferential interaction of a cosolvent (c) and water (w) with a protein solute (s). We will adopt the same approach as Parsegian et al. [12] for describing the thermodynamics of an infinitely dilute protein, for which one can write the following two Gibbs–Duhem relationships for the chemical potentials (μ_i) at constant pressure (p) and temperature (T),

$$d\mu_s^{\infty} + N_c d\mu_c + N_w d\mu_w = 0 \tag{3a}$$

$$n_c d\mu_c + n_w d\mu_w = 0 \tag{3b}$$

the former applied to the solution in the vicinity of the protein which has a composition of N_c and N_w molecules, while the latter refers to the bulk solution which has a composition of n_c and n_w water molecules. Elimination of $d\mu_w$ from Eq. (3a) using Eq. (3b) leads to the following thermodynamic description for the effect of a cosolvent on the denaturation equilibrium ($K=\rho_D/\rho_N$),

$$-\left(\frac{\partial \Delta\mu_s^{0,\infty}}{\partial \rho_c}\right)_{T,p} = RT\left(\frac{\partial \ln K}{\partial \rho_c}\right)_{T,p} = RT\frac{\Delta v_{sc} a_{cc}}{\rho_c} = m \tag{4}$$

The above equation is exact for an infinitely dilute protein solute existing in one of two major states, if the ratio of internal partition functions is independent of cosolvent concentration [13]. Here, $a_{cc}=(\partial \ln a_c/\partial \ln \rho_c)_{T,p}$ where a_c is the cosolvent activity. The value of a_{cc} is always positive and, due to the infinitely dilute protein approximation, can be determined from studies of the solution mixture in the absence of protein [14]. The value of $\Delta v_{sc}=v_{Dc}-v_{Nc}$ measures the difference in preferential interaction of the cosolvent between the denatured (v_{Dc}) and native (v_{Nc}) states [13,15,16]. The preferential interaction describes the deviation of the solution composition in the vicinity of the protein from that in the bulk solution and can be written $v_{sc}=N_c-\rho_c/\rho_w N_w$. For denaturation to occur with a given cosolvent then Δv_{sc} must be primarily positive. The symbol m is the slope of the free energy plot within the linear extrapolation approximation ($\Delta\Delta G=-m\rho_c$) [17].

The Gibbs–Duhem equations can be applied to any partial molar quantity and not just the partial molar Gibbs free energies (chemical potentials) [18]. Therefore, one can write the following conditions for the pmv values,

$$d\overline{V}_s^{\infty} + N_c d\overline{V}_c + N_w d\overline{V}_w = 0 \tag{5a}$$

$$n_c d\overline{V}_c + n_w d\overline{V}_w = 0 \tag{5b}$$

Performing the same manipulations as before but using the pmv values of the protein, cosolvent, and water results in the relationship,

$$\left(\frac{\partial \Delta\overline{V}_s^{\infty}}{\partial \rho_c}\right)_{T,p} = -\Delta v_{sc}\left(\frac{\partial \overline{V}_c}{\partial \rho_c}\right)_{T,p} \tag{6}$$

which describes the variation in the protein volume change upon denaturation in terms of the same difference in preferential interaction (Δv_{sc}) as used in Eq. (4), together with the derivative of the cosolvent pmv. The pmv values of the common denaturants (urea and GdmCl) increase with cosolvent concentration [19]. Hence, if a cosolvent is observed to denature a particular protein, then Δv_{sc} will be positive for most concentrations and therefore, according to Eq. (6), the difference in pmv between the native and denatured states should become more negative. The opposite is true for stabilizing cosolvents as long as the derivative of the cosolvent pmv is positive. To our knowledge, this relationship has not been used before when interpreting protein volume changes. A survey of the literature suggests that this relationship is not always obeyed with many volume changes in the opposite direction and sometimes an order of magnitude too large [7–9]. Furthermore, one would expect a reasonable correlation between urea or GdmCl m values and the observed values of ΔV or $\Delta\overline{V}_s$. No such correlation is evident [10,20,21].

The above principles can be illustrated using the example of lysozyme. A recent extensive study of lysozyme denaturation by thermal and dialysis techniques provides good data for examining Eq. (6) [22]. Properties of aqueous urea solutions (a_{cc} and $\overline{V}_c$) were taken from previous studies [23,24]. Urea denaturation of lysozyme at pH 2 and 293 K results in a value of m=9.2 kJ/mol/M and a transition midpoint at 3.7 M urea. This provides the values of f_D and also Δv_{sc} via Eq. (4). The dialysis data quantifies the preferential interaction of a cosolvent (3) with a protein (2) and is equivalent to [22],

$$\Gamma_{23} = f_D v_{Dc} + f_N v_{Nc} \tag{7}$$

which can then be used with Δv_{sc} to determine the values of v_{Dc} and v_{Nc} as a function of concentration. Finally, the change in pmv on denaturation of lysozyme in the absence of cosolvents has been estimated by pressure denaturation to be -20 cm^3/mol [25], while the volume of native lysozyme is approximately 10 067 cm^3/mol. Using the above data one can determine the values of $\overline{V}_N$ and $\overline{V}_D$ as a function of urea concentration according to Eq. (6). The results are displayed in Fig. 1.

As expected, the preferential interaction of urea with denatured lysozyme is larger than for the native state. The corresponding change in pmv of the native state with urea concentration is small and negative and reaches a value of -5 cm^3/mol at 8 M urea. The change in pmv of the denatured state is larger and reaches a value of -44 cm^3/mol at 8 M urea. The difference in pmv on unfolding

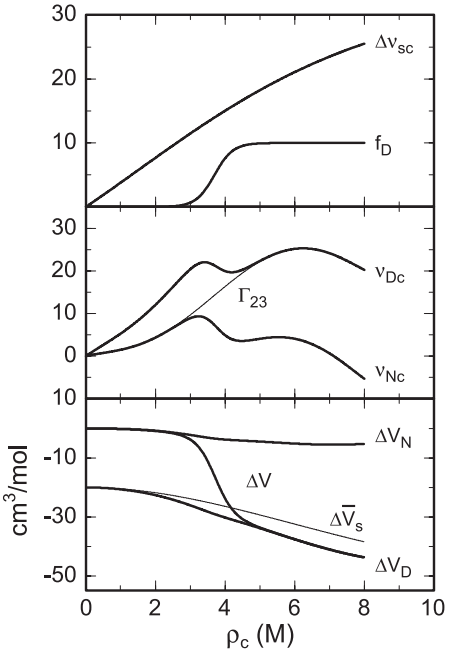

Fig. 1. Experimental data for the urea denaturation of lysozyme from combined thermal denaturation and dialysis studies at 293 K and pH 2 taken from Ref. [22]. The top panel displays the difference in preferential interaction (Δv_{sc}) and the fraction of protein in the denatured state ($f_D \times 10$) as a function of urea concentration. The middle panel displays the total preferential interaction (Γ_{23}) and the preferential interaction between urea and the native (v_{Nc}) and denatured (v_{Dc}) states as a function of urea concentration. The bottom panel displays the change in pmv of the native and denatured states ($\Delta V_i = \overline{V}_i(\rho_c) - \overline{V}_N(0)$) and the volume change on transfer (ΔV) as a function of urea concentration. The data were obtained using a fit to the aqueous urea partial molar volume data ($\overline{V}_c = 44.2 + 0.258\rho_c - 0.009\rho_c^2$ cm^3/mol) and then integrating Eq. (6).

varies from -20 cm^3/mol in pure water to -21 cm^3/mol at 1 M urea, and finally -39 cm^3/mol in 8 M urea. For comparison, the pmv of water is ≈ 18 cm^3/mol. Hence, the volume changes are relatively small, the equivalent of just 1–2 water molecules, with high urea concentrations resulting in a 100% increase in the magnitude of the volume change. The change in volume on transfer from water to aqueous urea solution is also displayed in Fig. 1. A sharp decrease in transfer volume in the transition region is evident and in agreement with the experimental data. However, any increase in volume at low or high cosolvent is absent. This is directly related to the fact that v_{Nc} at low urea concentrations, and v_{Dc} at high urea concentrations, are both positive. Similar trends would be expected for GdmCl denaturation.

It is possible that dialysis data determining v_{Nc} at low urea or GdmCl concentrations (which is difficult to measure) could be negative and thereby lead to an initial positive value for ΔV and $\Delta\Delta\overline{V}_s$. However, typical volume changes appear to be too large. For example, ΔV values are nearly linear in cosolvent concentration at low concentrations and are dominated by the change in pmv of the native state as f_N=1 in Eq. (1). Using a reasonable value of ΔV=80 cm^3/mol for transfer to 2 M urea or GdmCl solution [8–10], leads to a protein pmv derivative of 40 cm^3/mol/M. The derivative of the urea cosolvent pmv at 2 M is 0.22 cm^3/mol/M (or 0.53 cm^3/mol/M for GdmCl). Hence, an average value of $v_{Nc} \approx -180$ for urea (or ≈ -75 for GdmCl) is required to generate such a relatively large volume change. Clearly, this appears unreasonable [15]. Hence, the previous explanations of the initial volume increase (as simply due to cosolvent binding) appear to be incorrect as the observed changes are both too large and in the wrong direction.

One can also rule out the possibility of pH effects, or the presence of intermediates in the folding pathway, as leading to the observed deviations. The positive volume changes are observed at low cosolvent concentration where the population of intermediates will be negligible. The value of v_{Nc} does vary with pH with a larger exclusion of urea observed at lower pH for lysozyme [22]. However, these variations are too small to explain the corresponding volume changes. Therefore, in our opinion, the discrepancy is probably due to the infinitely dilute protein approximation. This then suggests that the initial positive volume increases are related to the contribution of protein–protein interactions which vary with cosolvent concentration. There is some evidence to support this conclusion. Several studies have inferred that low concentrations of urea destabilize protein aggregation [26–28]. If protein–protein interactions are decreased on the addition of urea, then the pmv of the protein should increase (for example, see Eq. (10) of Ref. [29]). This could help to reconcile the difference between the predicted changes based on Eq. (6) and the experimentally observed data.

The mechanism of the effect of urea (or GdmCl) on protein association is unknown. The effect on protein aggregation is not a simple surface tension (or cosolvent exclusion) effect, as urea and GdmCl increase the surface tension of water. The most probable explanation lies in the interaction of urea with hydrophobic groups on the surface of the protein [30]. In this case, urea binding would remove hydrophobic patches on the protein surface which then decreases the probability of protein aggregation. The same effect could also operate at high urea concentrations after denaturation and exposure of interior hydrophobic groups. Finally, it should be noted that the use of the phrase *protein aggregation* does not necessary imply the formation of highly populated dimers, trimers, or higher aggregates. Rather, the distribution of proteins around each other deviates from that expected for a random distribution in solution. This distinction is directly analogous to the difference between the classical descriptions of direct cosolvent binding and the thermodynamic concept of preferential interactions [15].

In summary, a link between the effects of cosolvents on the denaturation equilibrium and the changes in pmv values of the denatured and native states has been established for an infinitely dilute protein solute. The relationship suggests that if a cosolvent leads to protein denaturation, and the pmv of the cosolvent increases with cosolvent concentration,

then the change in pmv for the protein must become more negative with increasing cosolvent concentration. Analysis of the literature data indicate that the observed volume changes for several proteins deviate from this relationship, and that the initial increase in transfer volume is too large to be explained by preferential interactions with the cosolvent. It is suggested that changes in protein–protein interactions represent the most probable explanation for these deviations, and should therefore be used when explaining the experimental data.

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