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Protein hydration and volumetric properties

Catherine Royer [a,*], Roland Winter [b]

[a] Centre de Biochimie Structurale INSERM U1054, CNRS UMR5048, Université Montpellier 1&2, Montpellier, France
[b] Department of Physical Chemistry, Technical University of Dortmund, Dortmund, Germany

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ABSTRACT

Pressure effects on proteins stem from volumetric differences between their conformational states. These differences implicate rigid structure-based solvent excluded void volumes, although hydration and thermal expansivity differences between states may also play a role. Defining quantitatively the contributions of hydration and solvent excluded voids to protein volumetric properties and thermal expansivities remains a major challenge. Experimental information concerning thermal expansivity can be gained from pressure perturbation calorimetric studies (PPC). We review here recent results from PPC that suggest that while hydration plays a significant role in the volumetric properties of unfolded states of proteins, the volumetric properties of folded states are defined by structural and energetic properties of the folded chain.

1. Introduction

1.1. The molar volume of proteins

As natural polymers selected for function throughout evolution, proteins display remarkable physical chemical properties. These properties, which include their unique three dimensional structures, their stability, their conformational dynamics on multiple scales of amplitude and time, are all coupled to varying degrees, and result from the specific amino acid sequence of which they are composed. Evolutionary pressure has resulted in specific sequences for each protein that confer the level of function appropriate to the niche of the organism in which the protein is found. Understanding the relationships between sequence and these properties has fascinated protein chemists for decades. One physical chemical property of proteins that has received considerably less attention over the years than those cited earlier is the molar volume. Indeed, the factors contributing to the volumetric properties of proteins have long eluded understanding. It has been known since 1914 [1] that the application of pressure can lead to the unfolding of structured proteins. Since then, numerous studies in the literature have relied on the pressure effect to study protein dissociation or unfolding [2]. This effect of pressure necessarily arises because the molar volume of the dissociated or unfolded states is smaller than that of the associated or folded states. These changes in volume observed for conformational transitions in proteins are coupled to their other physical chemical properties. Like them, the magnitudes of protein volume changes result from the specific amino acid sequence, and their characterization and understanding, likewise, should yield important information about the physical basis for protein structure, stability and function.

The volume of a protein solution can be decomposed into the volume of the solvent and that of the protein. The volume of the protein per se, includes the volume of its constitutive atoms, V_a and the volume of the solvent excluded voids present inside the folded structure, V_v, multiplied by the number of moles of protein in the solution, n_p. The volume of the solvent includes the molar volume of the bulk solvent V_{sb} (multiplied by the number of moles of bulk solvent, n_{sb}) and the molar volume of the solvent molecules in interaction with the protein, V_{sp}, which can be very different from that of bulk water (multiplied by the number of moles of interacting solvent, n_{sp}). The total volume of the protein solution is then given by

$$V_{tot} = \left(V_a x n_p\right) + \left(V_v x n_p\right) + \left(V_{sb} x n_{sb}\right) + \left(V_{sp} x n_{sp}\right)$$

where $n_{sp} + n_{sb} = n_s$, the total number of moles of solvent.

1.2. Temperature effects and the p–T phase diagram of proteins

Like all solutions, and all materials, the volume of protein solutions varies with temperature due to variations in either the volume of the protein, the volume of the bulk water, the volume of the hydrating water and/or a change in the number of interacting and bulk water molecules, with the total number of solvent molecules, n_s, remaining constant. An expansion (positive or negative) with temperature due to a change in the number of solvent molecules interacting with the protein requires that the molar volume of interacting solvent molecules be different than that of the bulk, ($V_{sp} \neq V_{sb}$).

* Corresponding author.
E-mail address: catherine.royer@cbs.cnrs.fr (C. Royer).

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Since the 1970s it has been known [3–6] that appropriate modeling of experimental p–T phase diagrams for two state protein folding (Fig. 1) requires accounting for a difference in the molar volumes between folded (F) and unfolded (U) states, ΔV_u, and in their thermal expansivity, $\Delta\alpha_u$ in addition to the difference in enthalpy, entropy and heat capacity. Differences in their isothermal compressibility, $\Delta\beta_u$, have also been invoked, although the effect of this parameter on the phase diagram is rather small. In contrast, the non-zero value for the change in thermal expansivity upon unfolding results from a strong temperature dependence of the volume change of unfolding, $\Delta\alpha_u = (d\Delta V_u/dT)_p$. Indeed, at low temperatures, ΔV_u is generally negative (the application of pressure leads to unfolding under these conditions), but decreases in absolute value approaching zero as temperature increases and can become positive at high temperature [3,5,7–11]. This indicates that the thermal expansivity of the unfolded state is larger than that of the folded state. Based on temperature dependent densitometry measurements on Staphylococcal nuclease and the values of ΔV_u obtained at several temperatures (rather than fitting the p–T phase diagram for unfolding) we constructed an experimentally based volume diagram for the folded and unfolded states of this protein [12] (Fig. 2). We showed that the expansivity of the folded state was large and positive at low temperature but decreased significantly as temperature increased. The unfolded state expansivity could only be measured directly at temperatures above the unfolding transition temperature and was rather constant and somewhat higher than the value measured for the folded state just prior to the transition. This revealed that the expansivity of the folded state, α_f, was not constant with temperature, although p–T diagrams can be described by a temperature independent $\Delta\alpha_u$.

2. Pressure perturbation calorimetry

2.1. PPC of model compounds

Since this work was published, we and others have continued to investigate the temperature dependent volumetric properties of proteins using a technique called pressure perturbation calorimetry, introduced by Lin and coworkers [13••]. This approach involves coupling small increases in pressure (0.5 MPa) to the protein solution in a differential calorimetric scan, and measuring the heat released or absorbed upon pressure perturbation at each temperature ($dQ/dp = T(dS/dp)$). Via the Maxwell relation between the variation in entropy with pressure at constant temperature, $(dS/dp)_T$, and the variation in volume

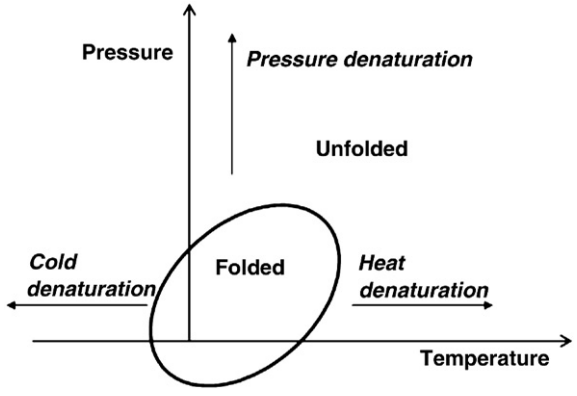

Fig. 1. Theoretical temperature–pressure phase diagram for proteins. At atmospheric pressure temperature unfolding generally occurs between 323 and 353 K, except for proteins from extremophiles. On the pressure axis, at room temperature, unfolding, depending upon the volume change, typically occurs in the range of 300–800 MPa.

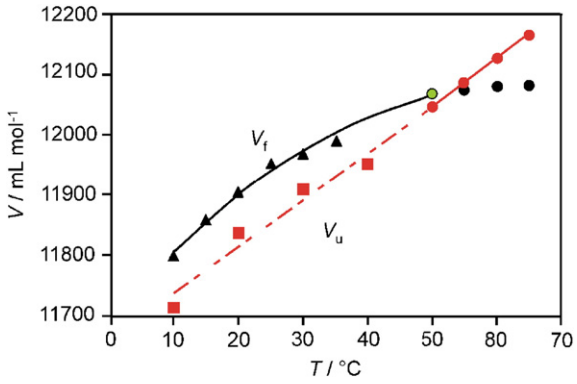

Fig. 2. Experimentally determined volumetric properties of the folded and unfolded states of staphylococcal nuclease. (Figure modified from Mitra et al. [27•]) Black triangles and red circles are direct densitometry measurements [12], red squares are unfolded state volumes obtained by taking the volume change at each temperature determined by pressure-dependent fluorescence [10] and subtracting this from the molar volume determined by densitometry. Green point is taken from the volume change at the transition temperature from PPC measurements [25•].

with temperature at constant pressure, $-(dV/dT)_P$, one calculates the thermal expansivity, α.

$$\alpha(T) = \frac{1}{V}\left(\frac{dV}{dT}\right)_p = \left(\frac{1}{TV}\right)\Delta Q / \Delta p \quad (1)$$

If an unfolding transition occurs over a particular temperature range (T_o to T_f), then integrating a over this range yields the volume change for unfolding at the transition temperature.

$$\Delta V = \int_{T_o}^{T_f} \alpha dT \quad (2)$$

The measurements of Lin and co-workers [13••] on amino acids (Fig. 3a) revealed positive thermal expansivity for polar amino acids at low temperature that decreased significantly with increasing temperature. In contrast, they found that the expansivity of hydrophobic amino acids was large and negative at low temperature and increasing to values similar to those found for polar amino acids at high temperature. We note that these expansivity values for the amino acid side chains were calculated by subtracting the values obtained for solutions of glycine in order to eliminate the strong effect of the zwitterion. We have obtained similarly negative values for hydrophobic amino acids side chains by comparing in a host–guest experiment the results for a gly-gly-gly tri-peptide with those obtained for gly-X-gly tripeptides, where X is the amino acid tested (Fig. 3b) [14].

How to explain this complex temperature behavior of the thermal expansivity? We note that the thermal expansivity of water was also reported by Lin and coworkers [13••]. Expansivity arises from three major contributions; changes in bond length (negligible in the range of temperatures that interest us), changes in thermal volume or void volume due to the increased translational and rotational motions of the molecules owing to the kinetic energy afforded by heating, and finally changes in density due to changes in molecular interactions. In the case of water, we can understand that at low temperatures water eventually freezes, and the density of ice is lower (volume is larger) than for liquid water. Ice floats! As temperature increases and the ice melts, water molecules on average behave more and more like a liquid, i.e. water molecules are released from residual ice-like structures into the liquid phase, where they exhibit a higher density, or lower volume. Hence, the expansivity of water is negative at low temperature; heating leads to an increase in density at low temperature. This is what is observed for hydrophobic amino acid