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How large are the volume changes accompanying protein transitions and binding?

Tigran V. Chalikian*, Rana Filfil

Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, Ontario M5S 2S2, Canada

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Abstract

We present a simple model to describe volume changes accompanying protein folding and binding events. The model enables one to resolve the changes in volume accompanying conformational transitions of proteins as well as association of proteins with other molecules in terms of the intrinsic, thermal and interaction (hydration) contributions. The thermal contribution to protein volume results from thermally activated mutual vibrational motions of contacting solute and solvent molecules. Our calculations suggest that near zero volume changes accompanying protein folding and binding events reflect compensation between significant changes in the intrinsic, thermal and interaction terms. We have quantitatively estimated these terms as a function of the protein's molecular weight and degree of its unfolding. Results described in this work lay foundation for more reliable and physically justified interpretations of volumetric data on protein folding and binding events. We also discuss potential ways of extending applications of our model to analyzing other macromolecular systems and events, including drug-DNA and protein-DNA interactions and helix-to-helix and helix-to-coil transitions of nucleic acids.

Keywords: Globular proteins; Conformational changes; Protein binding; Volume; Intrinsic packing; Hydration

1. Introduction

The volume changes, ΔV, accompanying protein binding and conformational transitions are of general thermodynamic importance for several reasons. Firstly, ΔV represents a fundamental thermodynamic property which determines pressure stability of proteins and protein complexes [1–4]. Secondly, volume changes accompanying protein binding and conformational transitions reflect the associated alterations in protein hydration and intrinsic packing [5–9]. Thirdly, dynamically fluctuating, nearly isoenergetic native-like protein conformations are distinct with respect to volume, a feature that can be used to discriminate between such conformations [10–13]. Finally, volume can be used as a thermodynamic means to identify and characterize transition state intermediates in protein folding/unfolding transitions [14–17]. For these and other reasons, volumetric properties of proteins have been under intensive

*Corresponding author. Tel.: +1-416-946-3715; fax: +1-416-978-8511.

E-mail address: chalikan@phm.utoronto.ca (T.V. Chalikian).

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scrutiny during the past several decades [1–9,17–19].

As a general rule, for all instances of protein recognition, including folding and binding, measured changes in volume, ΔV, are very small and do not exceed in magnitude 1 to 2% of the absolute value of the protein partial molar volume, V°. The sign of ΔV can be either positive or negative if measured at or extrapolated to atmospheric pressure [9,20]. Small magnitudes of ΔV accompanying protein denaturation are not consistent with the expectation of large negative changes in volume based on elimination of intraglobular voids and an increase in hydration of protein groups. This inconsistency was first noticed and emphasized more than 3 decades ago by Brandts who suggested that ''there must be some fairly large and positive contribution to ΔV of denaturation which is always nearly sufficient to cancel the negative hydrophobic contribution'' [21]. Furthermore, Brandts concluded that ''there is little that can be said at the present time to explain the very small magnitude of the volume changes which have been observed for certain denaturation reactions'' [21].

At the present, the molecular origins of ΔV of protein folding and binding still represent a controversial topic, while interpretation of ΔV in terms of microscopic events is far from being rigorous [9,20,22,23]. These deficiencies are unfortunate, since efficacy of the use of volumetric measurements for solving problems of biological relevance ultimately depends on our ability to rationalize measured volumetric observables in terms of various inter- and intramolecular interactions including, but not limited to, hydration and intrinsic packing. Several years ago, Chalikian and Breslauer proposed a new volumetric model of protein denaturation that accounted for the 'protein volume paradox' [20]. A distinctive feature of this model was the heretofore unappreciated thermal volume contribution which results from the thermally-induced mutual vibrational motions of contacting solute and solvent molecules [20]. More specifically, we proposed that volume decreases due to a change in protein hydration and elimination of intramolecular voids are compensated by an increase in thermal volume which accompanies enhanced contacts between water molecules and protein groups [20]. The increase in the thermal volume represents the missing positive and previously unappreciated contribution to the volume change accompanying protein denaturation, which was presaged by Brandts [21]. In this work, we further develop and quantify this model and extend its applications to investigating protein binding events and conformational transitions between the native and unfolded conformations. In particular, we explore the compensation between the different contributions to denaturation volume as a function of the degree of protein unfolding.

2. Model

The dissolution of a solute can be considered to be a two-step process: creation of a cavity in the solvent large enough to enclose the solute molecule and placement of the solute molecule into the cavity so that it can interact with the solvent [24–26]. The volume of the cavity consists of two terms: the geometric volume occupied by the solute molecule itself; and thermal volume, the volume of the void space surrounding the solute molecule which is due to the thermal motion of contacting solute and solvent molecules. Simply speaking, thermally activated vibrational motions cause the solute molecule and its solvating waters to push each other away thereby effectively creating a layer of empty space around the solute. Consequently, the partial molar volume of any solute, including proteins, can be presented as a sum of the following terms [24–26]:

$$V^\circ = V_M + V_I + V_T + \beta_{T0}RT \quad (1)$$

where V_M is the intrinsic volume of a protein; V_I is the interaction volume that refers to solvent contraction in the vicinity of charged and polar groups of the solute (water molecules solvating polar and charged atomic groups exhibit reduced partial molar volumes compared to water molecules in the bulk state); V_T is the thermal volume, a void volume around the solute that results from thermally activated mutual vibrations of contacting solute and solvent molecules; and $\beta_{T0}RT$ is the ideal term that describes the volume effect related to the kinetic contribution to the pressure of a

solute molecule due to translational degrees of freedom. The ideal term, $\beta_{T0}RT$, is small. At 25 °C, it is 1.1 cm^3 mol^{-1}, which is 4 orders in magnitude smaller than the partial molar volume of an average globular protein. Therefore, in protein-related studies, this term can be safely neglected.

When a protein binds another molecule or undergoes a conformational transition, the accompanying change in volume, ΔV, is contributed by changes in the intrinsic, interaction and thermal contributions:

$$\Delta V = \Delta V_M + \Delta V_I + \Delta V_T \quad (2)$$

As shown below, the ΔV_M, ΔV_I and ΔV_T terms may often exhibit large magnitudes and opposite signs. Consequently, a small change in volume may be a consequence of compensation between large changes in V_M, V_I and V_T.

2.1. Protein binding

In protein binding events, the change in the intrinsic volume, ΔV_{Mb}, can be calculated from X-ray crystallographic structures of the end (free and bound) protein states using standard approaches for computation of Voronoi [5] or molecular [27,28] volumes. The binding-induced change in thermal volume, V_T, can be calculated based on the change in solvent-accessible surface area, ΔS_{Ab}. As a first approximation, the thermal volume, V_T, is proportional to the number of contacts between the solute molecule and surrounding water molecules. Therefore, V_T should be roughly proportional to the solvent accessible surface area of the solute. In turn, the change in the thermal volume, V_T, should be proportional to the binding-induced change in solvent accessible surface area, ΔS_{Ab}: $\Delta V_{Tb} = \delta_N \Delta S_{Ab}$, where δ_N is the effective thickness of the thermal volume around the protein in its native conformation. The value of ΔS_{Ab} can be computed from X-ray crystallographic structural data on free protein, its counterpart molecule (small ligand, protein, or another macromolecule) and the complex. For native globular proteins at 25 °C, δ_N, has been evaluated to be ~1 Å [29]. However, for small molecules, the effective thickness of thermal volume, δ, is smaller, with different estimates yielding the values between 0.50 and 0.56 Å [26,30,31]. The binding-induced change in the interaction volume, ΔV_I, can be determined by subtracting the sum $(\Delta V_M + \Delta V_T)$ from the measured value of ΔV [see Eq. (2)].

2.2. Conformational transitions

Depending on solution conditions, denatured proteins may exist as heterogeneous ensembles of unfolded structures ranging from compact intermediates to the fully unfolded, random coil-like conformation [32–34]. There is a growing body of evidence suggesting that, even under strongly denaturing conditions, proteins are not random coils but may retain many features of the native state, including native-like topology and long-range interaction [35,36]. Due to conformational heterogeneity, structural characterization of the denatured state is not a simple matter. Consequently, changes in the intrinsic volume, V_M, and solvent-accessible surface area, S_A, accompanying protein denaturation cannot be readily computed. In addition, the hydration properties of the native and denatured states, if normalized per unit of solvent-accessible surface area, may be distinct. Therefore, the volumetric model of protein denaturation is more assumption-dependent than that of protein binding when three-dimensional structures of free and bound protein states are usually known (or can be determined).

2.2.1. The partial molar volume of the native protein state

The partial molar volume, V°_N, of a globular protein in its native state is given by a simplified version of Eq. (1):

$$V^\circ_N = V_{MN} + V_{IN} + V_{TN} \quad (3)$$

where subscript N denotes the native state.

The intrinsic (molecular) volume, V_{MN} (in $Å^3$), of a globular protein is proportional to its molecular weight, M (in Da), via $V_{MN} = 1200 + 1.04$ M [29], while the solvent-accessible surface area, S_{AN} (in $Å^2$), correlates to M via $S_{AN} = 4.7$ $M^{0.76}$ [7,19,37]. The intrinsic volume of a native globular

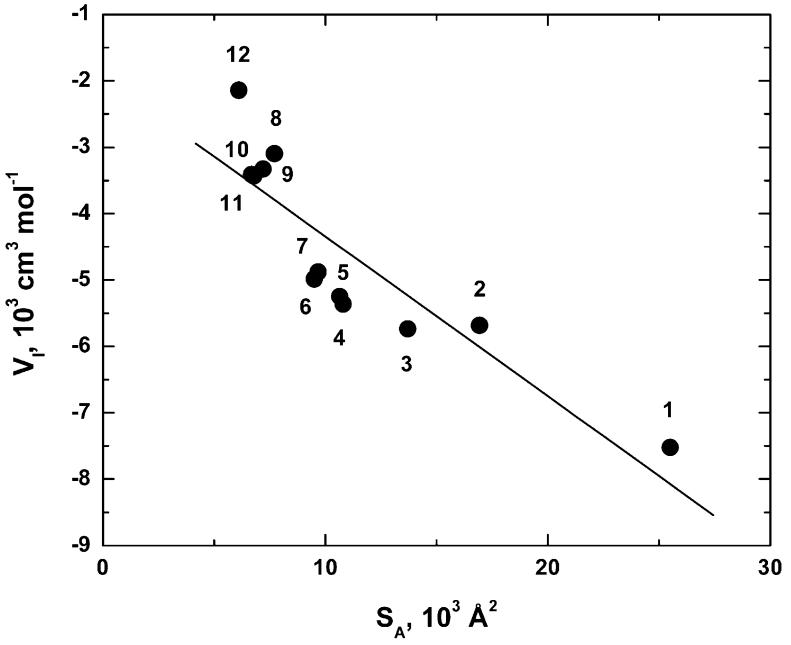

Fig. 1. Dependence of the interaction volume, V_I, upon the solvent accessible surface area, S_A, of 12 globular proteins at 25 °C: 1 hemoglobin; 2 ovalbumin; 3 pepsin; 4 α-chymotrypsinogen A; 5 α-chymotrypsin; 6 trypsin; 7 trypsinogen; 8 myoglobin; 9 α-lactalbumin; 10 lysozyme; 11 ribonuclease A; and 12 cytochrome *c*. The values of V_I are taken from Chalikian [39], while the values of S_A are presented in Chalikian et al. [29].

protein, V_{MN}, consists of the van der Waals volume, V_W, of constituent atoms and intraglobular voids arising from imperfect packing of the polypeptide chain(s) [38].

As discussed above, V_{TN} should be approximately proportional to S_{AN}, $V_{TN}=\delta_N S_{AN}$, where δ_N is 1 Å or 0.6 cm^3 mol^{-1} Å^{-2} [29]. The interaction volume, V_{IN}, representing a decrease in the solvent volume due to hydration of polar and charged groups, also should be proportional to S_{AN}, since, on average, the fractions of solvent exposed charged, polar and non-polar groups on the protein surface are remarkably constant. Specifically, the average contributions of charged, polar and non-polar atomic groups to S_{AN} are 14 ± 4, 33 ± 7 and 53 ± 5%, respectively [29]. Fig. 1, shows the values of V_I (as determined by Chalikian [39]) for 12 globular proteins at 25 °C plotted against their solvent accessible surface area, S_{AN}. As a first approximation, the data depicted in Fig. 1 can be fitted by a straight line, $V_{IN}=-1900+\gamma_N S_{AN}$, where $\gamma_N=-0.24\pm0.04$ cm^3 mol^{-1} Å^{-2}.

Substituting the molecular weight dependences of V_{MN}, V_{TN} and V_{IN} into Eq. (3), one obtains the following relationship for the partial molar volume of an average globular protein in its native conformation (in cm^3 mol^{-1}):

$$V^{\circ}_N = N_A[1200+1.04\ \mathrm{M}]\times10^{-24} + 4.7\ \mathrm{M}^{0.76}(\delta_N+\gamma_N)-1900 \quad (4)$$

where N_A is Avogadro's number.

2.2.2. The partial molar volume of denatured protein states

One volumetric model of the denatured state is based on the assumption that denatured protein may be presented as consisting of the compact and unfolded domains [19]. The extent of protein denaturation can be quantified by the degree of unfolding, σ, which is defined as the ratio of the

number of amino acid residues in the unfolded domains to the total number of amino acids in the protein ($0<\sigma<1$). Consequently, the molecular weight of the unfolded domain is σM, while the molecular weight of the compact domain is $(1-\sigma)$M. The value of σ equals zero for the native conformation and unity for the fully unfolded state. Not fully unfolded conformations exhibit intermediate values of σ ranging from 0 to 1 depending on the fraction of solvent-exposed atomic groups. For example, in molten globules, 40–60% of previously buried protein groups become exposed to the solvent [34,40]. By extension, the value of σ for the molten globule state can be set to be $0.4 \div 0.6$. In mildly denaturing conditions, the solvent accessible surface area of a protein in its partially unfolded state is only approximately 70–80% of the value expected for the fully extended conformation [7,41–46]. Hence, the degree of unfolding, σ, for the partially unfolded state can be set to be $0.7 \div 0.8$.

In our model, we assume that the molecular weight dependence of the intrinsic volume of the compact domain, V_{MDC}, is similar to that of the native state. Hence, one obtains $V_{MDC}=1200+1.04\ \mathrm{M}(1-\sigma)$. The intrinsic volume, V_{MDU}, of the unfolded domain, lacking all of its intraglobular voids, is given only by the van der Waals volumes of the constituent amino acid residues, V_W. The van der Waals volume (in Å^3) of a globular protein is a linear function of its molecular weight: $V_W=1100+0.77$ M [29]. Hence, one obtains $V_{MDU}=1100+0.77\ \mathrm{M}\sigma$, while the intrinsic volume, V_{MD} (in cm^3 mol^{-1}), of the denatured state is given by the following expression:

$$\begin{aligned} V_{MD} &\approx N_A\left[0.5(1200+1100)\right. \\ &\quad \left.+1.04\ \mathrm{M}(1-\sigma)+0.77\sigma\ \mathrm{M}\right]\times 10^{-24} \\ &= N_A\left[1150+\mathrm{M}(1.04-0.27\sigma)\right]\times 10^{-24} \end{aligned} \quad (5)$$

We assume that the thermal, V_{TDU}, and interaction, V_{IDU}, volumes of the unfolded domain are proportional to its solvent accessible surface area, S_{ADU}: $V_{TDU}=\delta_U S_{ADU}$ and $V_{IDU}=\gamma_U S_{ADU}$. For a fully unfolded polypeptide chain, the solvent-accessible surface area, S_{ADU} (in Å^2), is proportional to the molecular weight: $S_{ADU}=1.45\sigma$M [5]. Another assumption we use in developing this model is that the thermal and hydration properties of the unfolded domain are similar to those exhibited by low molecular weight model compounds. In this respect, recall that the thickness of the thermal volume, δ_U, for small molecules (and, by extension, for the unfolded domain) is 0.56 Å or 0.34 cm^3 mol^{-1} Å^{-2} [26,30,31]. The interaction volume normalized per unit area, γ_U, is assumed to be equal to the average value that can be calculated by dividing the interaction volumes of diglycyl tripeptides with aliphatic side chains by their solvent-accessible surface areas [47]. At 25 °C, the value of γ_U equals -0.12 ± 0.02 cm^3 mol^{-1} Å^{-2} [47]. Note that, in absolute magnitude, γ_U is twice as small as γ_N. This discrepancy is consistent with the notion that the hydration shells of native globular proteins consist of approximately two layers of hydration, while low molecular weight compounds and, presumably, unfolded polypeptide chains have only a single layer of hydration [19,29,39].

The solvent-accessible surface area of the compact domain of the denatured state, S_{ADC}, correlates to $(1-\sigma)$M via $S_{ADC}=4.7[(1-\sigma)\mathrm{M}]^{0.76}$. The thermal and interaction properties of the compact domain are intermediate between those corresponding to the native and fully unfolded conformations. Significantly, we assume that the more unfolded the protein (the larger the value of σ) the closer the thermal and interaction properties of the compact domain to those corresponding to the fully unfolded state: $V_{TDC}=[\delta_N+\sigma(\delta_U-\delta_N)]S_{ADC}$ and $V_{IDC}=-1900+[\gamma_N+\sigma(\gamma_U-\gamma_N)]S_{ADC}$. In the aggregate, one obtains the following expression for the partial molar volume of the denatured conformation:

$$\begin{aligned} V^\circ_D &= V_{MD}+V_{TDU}+V_{IDU}+V_{TDC}+V_{IDC} \\ &= N_A\left[1150+\mathrm{M}(1.04-0.27\sigma)\right]\times 10^{-24} \\ &\quad +1.45\sigma\mathrm{M}(\delta_U+\gamma_U)+4.7\left[(1\right. \\ &\quad \left.-\sigma)\mathrm{M}\right]^{0.76}\left[\delta_N+\gamma_N\right. \\ &\quad \left.+\sigma(\delta_U-\delta_N+\gamma_U-\gamma_N)\right]-1900 \end{aligned} \quad (6)$$

2.2.3. Change in volume accompanying protein denaturation

The change in volume accompanying protein denaturation, ΔV, equals the difference between the partial molar volumes of the denatured, V°_{D}, and native, V°_{N}, states. Combining Eq. (4) and Eq. (6), the molar change in volume, ΔV (in cm^3 mol^{-1}), accompanying protein denaturation is given by the relationship:

$$\begin{aligned}\Delta V = V^{\circ}_{D} - V^{\circ}_{N} = & -0.27 N_A M \sigma \times 10^{-24} \\ & + 1.45 \sigma M (\delta_U + \gamma_U) \\ & + 4.7 \left[(1-\sigma)M\right]^{0.76} \left[\delta_N \right. \\ & + \gamma_N \\ & \left. + \sigma(\delta_U - \delta_N + \gamma_U - \gamma_N)\right] \\ & - 4.7 M^{0.76} (\delta_N + \gamma_N) \end{aligned} \quad (7)$$

Finally, dividing Eq. (7) by the protein molecular weight, M, one derives the following expression for specific change in volume, Δv (in cm^3 g^{-1}), accompanying protein denaturation:

$$\begin{aligned}\Delta v = & -0.27 N_A \sigma \times 10^{-24} + 1.45 \sigma (\delta_U + \gamma_U) \\ & + 4.7 (1-\sigma)^{0.76} M^{-0.24} \left[\delta_N + \gamma_N \right. \\ & \left. + \sigma(\delta_U - \delta_N + \gamma_U - \gamma_N)\right] \\ & - 4.7 M^{-0.24} (\delta_N + \gamma_N) \end{aligned} \quad (8)$$

3. Discussion

3.1. Protein binding

In two previous studies, we evaluated the ΔV_M, ΔV_T and ΔV_I contributions to our measured ΔV values for two protein binding events: specifically, the binding of 2′-CMP and 3′-CMP to ribonuclease A [48] and the binding of turkey ovomucoid third domain (OMTKY3) to α-chymotrypsin [49]. At 25 °C, the volume changes associated with the binding of 2′-CMP and 3′-CMP to ribonuclease A are very small and equal to 22 ± 15 and 17 ± 15 cm^3 mol^{-1}, respectively [48]. We used these data in conjunction with the approach described above and X-ray crystallographic structures of the ligand-protein complexes to evaluate the values of ΔV_M, ΔV_T and ΔV_I for the binding of 2′-CMP and 3′-CMP to ribonuclease A [48]. For 2′-CMP association with ribonuclease A, our calculated values of ΔV_M, ΔV_T and ΔV_I are -141, -137 and 300 cm^3 mol^{-1}, respectively [48]. For 3′-CMP association with ribonuclease A, the values of ΔV_M, ΔV_T and ΔV_I are -207, -132 and 356 cm^3 mol^{-1}, respectively [48]. The decreases in the thermal volume, V_T, reflect the burial of large surface areas within the ligand-protein interfaces, while the increases in the interaction volume, V_I, reflect dehydration of the interacting surfaces with the release to the bulk of water molecules that were previously solvating polar and charged protein groups. These estimates reveal that the near zero values of ΔV observed for the binding of 2′-CMP and 3′-CMP to ribonuclease A result from compensation between very large intrinsic, thermal and interaction contributions.

The binding of OMTKY3 to α-chymotrypsin at 25 °C is accompanied by a volume increase of 100 ± 40 cm^3 mol^{-1} [49]. Our estimated values of ΔV_M, ΔV_T and ΔV_I for this protein–protein binding event are 210, -927 and 817 cm^3 mol^{-1}, respectively [49]. Again, note that a large decrease in V_T nearly offsets increases in V_M and V_I thereby bringing about a small ΔV. Significantly, in absolute magnitude, the values of ΔV_T and ΔV_I are roughly an order of magnitude larger than ΔV.

3.2. Conformational transitions of proteins

We have used Eq. (8) to simulate the dependences of changes in the partial specific volume, Δv, upon the degree of unfolding, σ, for globular proteins with molecular weights of 15, 20, 35 and 50 kDa. Results of these calculations are presented in Fig. 2. Inspection of Fig. 2 reveals a number of important observations. First, the calculated values of Δv are small in absolute magnitude (ranging from -0.02 to 0.03 cm^3 g^{-1}), an observation that agrees with experimental data. This agreement lends credence to the assumptions and derivations that have been used in developing the above described volumetric model of protein denaturation. Second, Δv non-linearly depends on the degree of unfolding, σ. For example, for a protein with a molecular weight of 20 kDa, Δv first

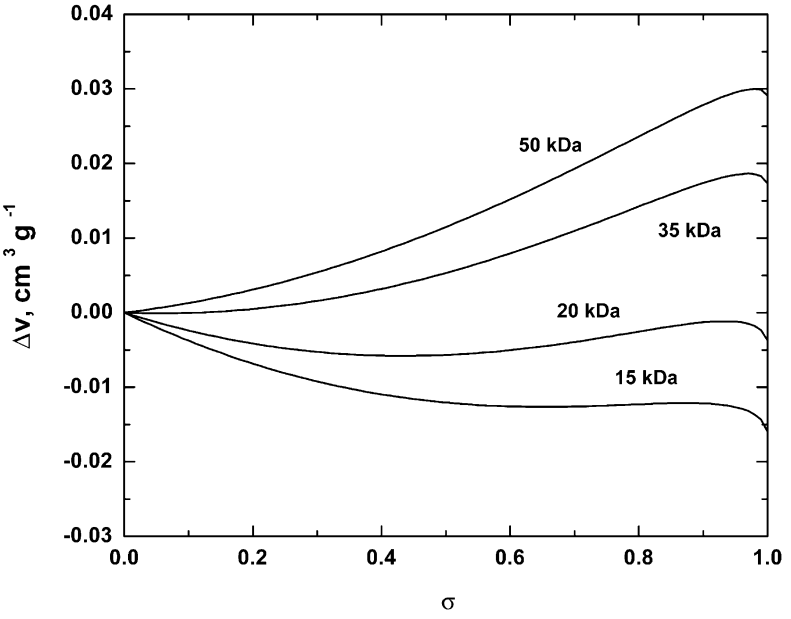

Fig. 2. Dependences of the specific change in volume, Δv, accompanying protein denaturation upon the degree of unfolding, σ, calculated using Eq. (8) for average globular proteins with molecular weights of 15, 20, 35 and 50 kDa.

decreases to -0.006 cm^3 g^{-1} upon σ increasing from 0 to 0.45, then increases to -0.001 cm^3 g^{-1} when σ increases to 0.93 and, finally, decreases to -0.004 cm^3 g^{-1} upon the full unfolding of the protein (at $\sigma=1$). Hence, for this protein, the native-to-molten globule ($\sigma\sim0.4$–0.6), native-to-partially unfolded ($\sigma\sim0.7$–0.8), and native-to-fully unfolded ($\sigma\sim1$) transitions will be accompanied by volume changes, Δv, of -0.006, -0.003 and -0.004 cm^3 g^{-1}, respectively. Accordingly, the molten globule-to-partially unfolded transition of this protein will result in a volume increase of ~0.003 cm^3 g^{-1}. Thirdly, the larger the molecular weight of the protein the more positive the volume change accompanying its denaturation. In this respect, it should be noted that the majority of the proteins, for which denaturation volumes have been determined, are of molecular weights between ~10 and 30 kDa.

To further explore the extent of compensation between Δv_M, Δv_T and Δv_I in protein denaturation, we have simulated the σ-dependences of these terms for a globular protein with a molecular weight of 20 kDa. Fig. 3 presents these dependences. Inspection of Fig. 3 reveals that the near zero values of Δv (which do not exceed in absolute magnitude 0.006 cm^3 g^{-1}) reflect a striking interplay between very large changes in v_M, v_T and v_I. Specifically, when σ increases from 0 to 1, v_M decreases by 0.17 cm^3 g^{-1}, v_I decreases by 0.07 cm^3 g^{-1}, while v_T increases by 0.23 cm^3 g^{-1} nearly compensating decreases in v_T and v_I. Note that, in absolute value, the changes in v_M, v_T and v_I can be 10–40 times larger than Δv itself. The decrease in v_M is due to elimination of intraglobular voids upon protein denaturation. The increase in v_T reflects the fact that almost a threefold increase in solvent accessible surface area, S_A, upon full unfolding of the protein prevails over a roughly twofold decrease in the effective thickness of the thermal layer, δ (from 0.6 cm^3 mol^{-1} Å^{-2} for the native state to 0.34 cm^3 mol^{-1} Å^{-2} for the fully unfolded state). Similarly, the decrease in v_I (the negative value of v_I becomes more negative) is related to that a threefold increase in S_A prevails over a twofold increase in the unit contribution of the interaction volume, γ (from -0.24 cm^3

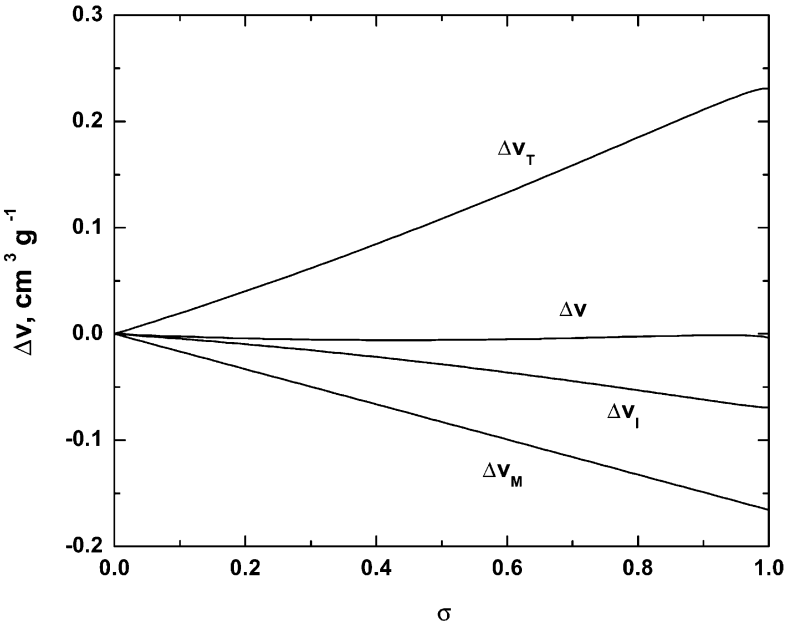

Fig. 3. Dependences of the intrinsic, Δv_M, thermal, Δv_T and interaction, Δv_I, contributions to Δv for an average globular protein with a molecular weight of 20 kDa. The dependences have been calculated as explained in text.

mol^{-1} $Å^{-2}$ for the native state to -0.12 cm^3 mol^{-1} $Å^{-2}$ for the fully unfolded state).

It should be noted that individual proteins can deviate from the average with respect to the volume of intraglobular voids and distribution and proportions of charged, polar and non-polar atomic groups in the native and denatured states. The latter will affect the hydration properties of the native and denatured protein states. Such proteins may exhibit transition volumes that are different, in both magnitude and sign, from the average values calculated using Eq. (8). Therefore, care should be exercised and individual properties of proteins should be properly considered before generalizations can be made regarding the sign and magnitude of Δv as a function of the molecular weight of a protein and the nature of its transition. In general, the difference between our calculated volumetric properties of an 'average' globular protein and the actual properties of a specific one can be as large as 0.02–0.03 cm^3 g^{-1}. This rough estimate is based on and corresponds to the range of observed deviations of experimental V_I values from the regression line (normalized per gram rather than per mole of protein) shown in Fig. 1.

3.3. Other macromolecular systems

The main concepts and formalisms of this work can be extended to other macromolecular systems and events, such as drug-DNA and protein-DNA interactions as well as helix-to-coil and helix-to-helix transitions of nucleic acids. However, before such applications can be realized, the unit contributions (per 1 $Å^2$ of surface area) of thermal δ, and interaction γ, volumes for nucleic acid structures in their different conformational states (A, B, Z-duplexes, triplexes and single stranded conformations) should be determined as a function of nucleotide composition and sequence. In this respect, our recent volumetric data on genomic and synthetic DNA duplexes suggested that some synthetic polymeric duplexes [such as poly(dAdT)poly(dAdT), poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC)] may influence water molecules beyond the first hydration layer, while

the hydration shell of genomic DNA is primarily confined within the first hydration layer [50,51]. However, to obtain the requisite sequence- and conformation-dependent values of δ and γ for nucleic acids, volumetric studies should be expanded to larger sets of DNA structures including duplexes, triplexes and single strands. Additional experimental data, coupled with the analysis described above, will ultimately facilitate development of more quantitative and robust ways of interpreting nucleic acid volume changes. Such work is underway in our group.

4. Conclusion

We have presented a simple yet realistic volumetric model of protein folding and binding. The model enables one to resolve changes in volume, ΔV, accompanying protein binding and folding events in terms of intrinsic, thermal and interaction (hydration) contributions. Our results reveal that near zero values of ΔV of protein folding and binding reflect compensation between significant changes in the intrinsic, thermal and interaction contributions. In fact, we have quantitatively estimated these contributions as a function of the protein's molecular weight and degree of its unfolding. Given the interplay between the changes in intrinsic, thermal and interaction volumes, one must pay careful attention when interpreting measured values of ΔV or $\Delta\Delta V$ in terms of various microscopic events (e.g. creation of a cavity inside the protein when a bulky amino acid is substituted by a smaller one). The results described in this work lay foundation for more robust and physically justified interpretations of volumetric data on protein folding and binding events. Finally, the basic assumptions and formalisms used in developing our model are not restricted to proteins only. Applications of this model can, in principle, be extended to analysis of other macromolecular systems and events, including drug-DNA and protein-DNA interactions and helix-to-helix and helix-to-coil transitions of nucleic acids.

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