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On empirical decomposition of volumetric data

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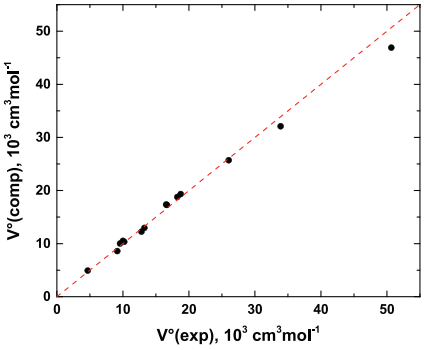
HIGHLIGHTS

- Empirical volumetric models treat solute and solvent as hard spheres.
- In such models, defining the dividing surface between solute and solvent is critical.
- We reanalyze the existing empirical schemes of interpretation of volumetric data.
- We offer improved parameters for characterizing the state of water of hydration.

GRAPHICAL ABSTRACT



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ABSTRACT

Volumetric characterization of proteins and their recognition events has been instrumental in providing information on the role of intra- and intermolecular interactions, including hydration, in stabilizing biomolecules. The credibility of molecular models and interpretation schemes used to rationalize experimental data are essential for the validity of microscopic insights derived from volumetric results. Current empirical schemes used to interpret volumetric data suffer from a lack of theoretical and computational substantiation. In this contribution, we take advantage age of recent MD simulations of proteins in solution coupled with Voronoi-Delaunay tessellation of simulated structures that have provided an exceptional level of structural detail on the nature of protein-water interfaces. We use these structural insights to re-evaluate empirical frameworks used for interpretation of volumetric data. An important issue in this respect is the actual dividing surface between water and protein atoms that is used in volumetric studies when the solute and solvent are treated as hard spheres enclosed within their respective van der Waals surfaces. In one development, using Voronoi tessellation of MD simulated protein-water systems the dividing surface has been defined as the points equidistant from the water and protein atoms. The interstitial void volume between the solute and the dividing surface corresponds to thermal volume envisaged by Scaled Particle Theory. In this communication, we explicitly account for the contributions of thermal volume to the partial molar volume, compressibility, and expansibility of proteins and re-examine and redefine the intrinsic and hydration volumetric contributions. We discuss the implications of our results for protein transitions and association events.

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1. Introduction

Volumetric properties of proteins contain information about intra- and intermolecular interactions and hydration and changes in these properties accompanying recognition events (e. g., folding, binding, oligomerization, aggregation) [1–5]. Experimental studies of the volumetric properties of proteins and other biologically relevant molecules have been instrumental in characterizing the role of hydration in biochemical reactions involving proteins, nucleic acids, and lipids [1,6–8]. Microscopic decomposition of experimental (macroscopic) volumetric data is not a trivial task; the same set of volumetric results may paint significantly differing molecular pictures depending on the models and interpretation schemes employed. The robustness and reliability of molecular models used to interpret volumetric data are as critical for the practical use and information content of volumetric experiments as the technical characteristics of the instrumentation, experimental protocols, and the purity of the compounds under investigation.

Molecular interpretation of volumetric data has been traditionally implemented within the framework of purely empirical considerations [1]. These considerations involve assumptions that often suffer a lack of independent experimental and theoretical support or verification. With this notion, recent molecular dynamics (MD) simulation studies published by the Halle group [9–12] and the Winter and Geiger groups [13–16] offer insights and minute structural, kinetic, and thermodynamic details of protein-water interfaces not accessible from experimental data. While contributing to our understanding of the phenomenon of protein hydration, the computational results do not readily offer a way to more reliably interpret experimental data. It is imperative to develop a robust framework for the valid microscopic interpretation of the volumetric properties of proteins and changes in these properties accompanying protein folding and binding reactions. In this contribution, we review the recent computational advances in characterization of proteins and protein-water interfaces to re-examine and re-evaluate the molecular models used to rationalize volumetric data. The goal of such models is not merely to reproduce volumetric results based on structural data (which is a phenomenological issue) but to yield quantitative information on the state of water of hydration in order to better understand its role in the energetics of biologically significant molecules and events.

2. Volume

2.1. Intrinsic volume

It was realized more than four decades ago that the partial molar volume of a small nonpolar solute can be approximated by $V^\circ = (4/3)\pi(r + \delta)^3$, where r is the van der Waals radius of the solute, and δ is the thickness of the void volume surrounding the solute [17]. The value of δ estimated to be ~0.5 Å reflects the relative position of the effective dividing surface between the solute and its hydration shell [17–21]. The void volume around a solute is equivalent to the thermal volume, V_T, envisaged by Scaled Particle Theory (SPT) which also includes the ideal term $\beta_{T0}RT$ (where β_{T0} is the coefficient of isothermal compressibility of solvent) to account for the volume contribution originating from the translational degrees of freedom of the solute [22,23]. According to SPT, the partial molar volume of a solute is given by the sum [18,22]:

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

where $V_C = V_M + V_T$ is the volume of the cavity in solvent that accommodates the solute; V_M is the molecular volume of solute; V_T is the thermal volume; and V_I is the change in volume due to solute-solvent interactions. The ideal term, $\beta_{T0}RT$, is on the order of ~1 $cm^3\,mol^{-1}$ and can be neglected for large molecules.

Under the assumption that, in the absence of partial or full electrostatic charges, the thickness of the void volume, δ, around a solute is constant and independent of the molecular geometry, the partial molar volumes of polar and nonpolar solutes have been analyzed, and the volumetric contributions of polar groups have been derived [18,24]. In a thorough analysis, Kharakoz has shown that each hydrogen bond formed between a polar solute and water at 25 °C causes a 2.2 $cm^3\,mol^{-1}$ decrease in volume [18]. The origin of the volume decrease has not been specified [18]. It may reflect a local diminution in the thickness of thermal volume, δ, an increase in the density of water of hydration, or both. In this respect, the distance between polar protein atoms (such as oxygen and nitrogen which are capable of hydrogen bonding) and water is roughly 1.5 Å less than that between nonpolar carbon and water [9].

The problem of the dividing surface between the solute and solvent becomes essential only when the solute and solvent are treated as discreet entities, i. e. hard spheres. If the volumetric properties of a solute are treated as radial distribution functions (RDF), e. g., based on the Kirkwood-Buff formalism [25–29], the definition of the dividing surface and the related problems do not exist. On the other hand, empirical algorithms of microscopic rationalization of volumetric data all rely on discreet representation of the molecules of a solute and solvent. Failure to account correctly for the effective dividing surface between the protein proper and water may lead to incorrect inferences regarding the properties of the hydration shell. For example, if the molecular surface of a solute (which, for a small solute, coincides with its van der Waals surface) is taken as the dividing surface, the density of water solvating charged atomic groups appears to be reduced relative to bulk water, which is counterintuitive [30,31]. Such a representation, while not precluding a phenomenological description of partial molar volume data, adds little to our understanding of the state of water in the hydration shell.

Thus, if the solute and solvent are viewed as hard spheres, the thermal volume (void volume separating the solute and solvent molecules) needs to be accounted for explicitly and properly. One ambiguity related to Eq. (1) is the vagueness of the definition of thermal volume, V_T, and the empirical nature of the estimate of δ. In a new development, the cavity volume, V_C, of a protein in Eq. (1) has been explored in terms of the Voronoi volume [9,14]. Voronoi tessellation provides a rigorous geometric criterion for separating the solute proper (protein) from its hydration shell [14,32–35]. Compared to standard (primitive) Voronoi tessellation, additively weighted Voronoi tessellation yields a more realistic assignment of the void space between the protein and its hydration shell [9]. The difference between the primitive and additively weighted algorithms is that, in the latter algorithm, each point of the dividing surface is equidistant from the van der Waals surfaces of neighboring water and protein atoms. According to Persson et al. [9] and Voloshin et al. [15], the Voronoi volume of a protein, in addition to the molecular volume, also contains half of the interstitial (boundary) void space between the water and protein atoms. As first noticed and emphasized by Voloshin et al. [15], the interstitial volume around the protein contributing to its Voronoi volume corresponds to thermal volume envisaged by SPT.

For an accurate description, Voronoi tessellation requires temporally defined positions (coordinates) of protein atoms and waters of hydration. Such information cannot be gleaned from experimental (structural) studies; however, it can be provided by MD simulations. We take advantage of the published additively weighted Voronoi volumes of BPTI, truncated ubiquitin, and staphylococcal nuclease computed based on MD simulations to develop a more realistic way to identify and quantify the components of partial molar volume including thermal volume [9,15]. Since the thermal volume, V_T, of a solute can be approximated by the product of δ and solvent accessible surface area, S_A, the ratio of the difference between the Voronoi, V_{vor}, and molecular, V_M, volumes to S_A can be taken as a measure of the thickness of thermal volume, $\delta = (V_{vor} - V_M)/S_A$. The calculations yield values of δ equal to 0.51, 0.29, and 0.32 Å for staphylococcal nuclease, ubiquitine, and BPTI, respectively (see Table 1). These numbers are numerically similar to the empirical and SPT-based estimates of δ. The similarity is

Table 1
The solvent accessible surface areas, S_A, intrinsic V_M, Voronoi, V_{vor}, and thermal, V_I, volumes, and the thickness of thermal volume, δ, of proteins. The Voronoi volumes, V_{vor}, of BPTI and truncated ubiquitin are from ref. [9], while that of staphylococcal nuclease is from ref. [15].

Proteins	S_A, Å^2	V_M, Å^3	V_{vor}, Å^3	V_T, Å^3	$\delta = (V_{vor} - V_M)/S_A$, Å
BPTI[a]	4009	7114	8378.9	1264.9	0.32
Ubiquitin[b]	4495	9723	11,012.5	1289.5	0.29
Staphylococcal nuclease[b]	7888	18,253	22,300	4047	0.51

[a] The values of S_A and V_M for BPTI are from ref. [63].
[b] The values of S_A and V_M for ubiquitin and staphylococcal nuclease have been computed using 1UBQ (with removed residues R74, G75, and G76) and 1STN PDB entries, respectively, with the program MSP Version 3.9.3 (obtained from Dr. Michael Connolly).

significant and lends credence to the notion of thermal volume, V_T, and its applicability to empirical dissection of partial molar volume data.

As mentioned above, for small nonpolar solutes, empirical and SPT-based estimates of the thickness of thermal volume, δ, are on the order of ~0.5 Å [17,18]. Similar estimates have been made based on MD simulations of the partial molar volume of a number of small organic molecules computed with the Lennard-Jones potential in the absence of the Coulombic term [26]. The situation is more complicated for larger solutes such as proteins. A correlation analysis of the partial molar volume and X-ray crystallographic data on 12 globular proteins has revealed a value of δ of ~1 Å [36]. In that analysis, no limitation has been set on the number of hydration layers [36]. In a subsequent publication, in which waters of hydration have been restricted to those tightly bound to the protein, the analysis and assumptions used in ref. [36] have been reexamined, and a range of 0.6 to 0.65 Å has been proposed as an estimate of δ [19]. In retrospect, the larger estimate of δ (~1 Å) obtained from the correlation analysis [36] may have been compromised by the relatively small number of sampled proteins, a narrow range of partial specific volumes of globular proteins (all grouped around 0.72 $cm^3\,g^{-1}$), and the virtually constant solvent accessible surface area fractions of charged, polar, and nonpolar groups in globular proteins.

This said, the estimate of 1 Å is in qualitative agreement with the results of MD simulations of the partial molar volumes of organic compounds of various size that showed a sigmoidal increase in δ from ~0.5 Å for small solutes (with radii of ~2 Å) to ~1 Å for larger solutes with radii greater than 6 Å [37]. The sigmoidal shape of the size dependence of δ has been obtained under the spherical approximation of solute molecules [37]. In a subsequent publication, however, it was argued that if, instead of the spherical approximation, the geometric shape of the solutes is treated with individual atomic details, the thickness of the void space would become less dependent on the size of the molecules and the value of δ would range from 0.32 to 0.55 Å [20]. Simply speaking, the deviation from sphericity of an extended (non-spherical) molecule becomes more prominent as the size of the molecule increases making the "spherical" estimates of δ unrealistically large. While more studies are needed to clarify this issue, based on the evidence collected so far, 0.5 Å appears to be a plausible compromise for an average value of δ for both small molecules and biopolymers. It is supported by empirical and computational results. This value is hardly constant and may change from solute to solute. However, it represents a valid compromise and good starting point for microscopic interpretations of volumetric properties of solutes in terms of hydration.

2.2. Hydration shell

The MD simulations of four small proteins performed by Halle and colleagues showed that the solute-induced thermodynamic and kinetic changes are confined to the first hydration shell with the second and third hydration shells contributing insignificantly [9–12]. This notion is in agreement with empirical estimates which suggest that solute-induced volumetric changes in solvent do not extend beyond the first or, maximum, the second layer of hydration [38]. Thus, empirical dissection of volumetric data can be restricted to the first hydration layer. The mean cross-sectional surface area of a water molecule in the first hydration shell of a protein estimated by Persson et al. [9] is 11.11 ± 0.02 Å^2 which is ~10% larger than that of water in the bulk. Thus, the number of water molecules within the first hydration shell of a protein can be found by dividing its solvent accessible surface area, S_A, by 11.11 Å^2 ($n_h = S_A/11.11$) [9].

As mentioned above, for practical estimates, the thickness of thermal volume, δ, is ~0.5 Å. Although δ may decrease if the solute is hydrogen-bonded with solvating water molecules, any change in δ can be formally ascribed to a change in the density of water of hydration. MD simulations have revealed the average density of water within the first hydration shell of a globular protein to be 6.3 ± 0.3% greater than that in the bulk [9] which translates into the differential partial molar volume of water of hydration and bulk water $V_h - V_0 = -1.1\,cm^3\,mol^{-1}$. In polar and charged interfacial regions of the protein, the differential density is about 10% leading to $V_h - V_0 = -1.8\,cm^3\,mol^{-1}$ [9]. In nonpolar regions, the differential density of the bulk and hydration phases is 3.2% ($V_h - V_0 = -0.6\,cm^3\,mol^{-1}$) [9].

Assuming a single layer of hydration and not differentiating between polar and nonpolar regions, Eq. (1) can be modified as follows:

$$V^\circ = V_M + \delta S_A + (S_A/11.11)(V_h - V_0) \quad (2)$$

We use Eq. (2), to calculate the thermal, $V_T = \delta S_A$, and interaction volumes, $V_I = n_h(V_h - V_0) = V^\circ - V_M - V_T$, for 14 globular proteins with solved three-dimensional structures and measured partial molar volumes, V°. Table 2 shows the results of these calculations. By dividing V_I by hydration number, $n_h = S_A/11.11$, one obtains $(V_h - V_0)$, the average contribution to V_I of each water of hydration (see Table 2). The variability of $(V_h - V_0)$ in Table 2 reflects the individual structural properties and chemical nature of solvent-exposed atomic groups of the proteins. The average value of $(V_h - V_0)$ is $-1.6 \pm 0.8\,cm^3\,mol^{-1}$ which signifies a − 8.9 ± 4.4% increase in the density of water of hydration compared to bulk water in reasonable agreement with −6.3 ± 0.3% computed by Persson et al. [9].

Table 2
The partial molar volumes of proteins, V° ($cm^3\,mol^{-1}$), solvent accessible surface areas, S_A (Å^2), intrinsic V_M ($cm^3\,mol^{-1}$), thermal V_T ($cm^3\,mol^{-1}$), and interaction, V_I ($cm^3\,mol^{-1}$), volumes, hydration number, n_h, and the differential partial molar volume of water of hydration and bulk water, $(V_h - V_0)$ ($cm^3\,mol^{-1}$).

Proteins	V°	S_A	V_M	V_T	V_I	n_h	$V_h - V_0$
Hemoglobin[a]	50,662	25,508	42,824	7680	−158	2296	−0.1
Ovalbumin[a]	33,902	16,938	29,385	5100	−583	1525	−0.4
Pepsin[a]	26,022	13,715	23,500	4130	−1608	1234	−1.3
α-Chymo-trypsinogen A[a]	18,761	10,815	17,611	3257	−2106	973	−2.2
α-Chymo-trypsin[a]	18,241	10,655	17,074	3209	−2041	959	−2.1
Trypsin[a]	16,560	9505	15,831	2862	−2133	856	−2.5
Trypsinogen[a]	16,675	9675	15,729	2913	−1968	871	−2.3
Myoglobin[a]	13,261	7716	11,718	2323	−780	695	−1.1
α-Lactalbumin[a]	10,196	7216	9184	2173	−1160	650	−1.8
Lysozyme[a]	9996	6685	9430	2013	−1447	602	−2.4
Ribonuc-lease A[a]	9574	6790	8921	2044	−1391	611	−2.3
Cytochrome c[a]	9151	6115	7608	1842	−299	550	−0.9
BPTI[b]	4680	4009	4284	1207	−811	361	−3.7
Staphylococcal nuclease[c]	12,818	7888	10,992	2375	−549	710	−0.8

[a] From ref. [36].
[b] From ref. [63].
[c] From ref. [64].

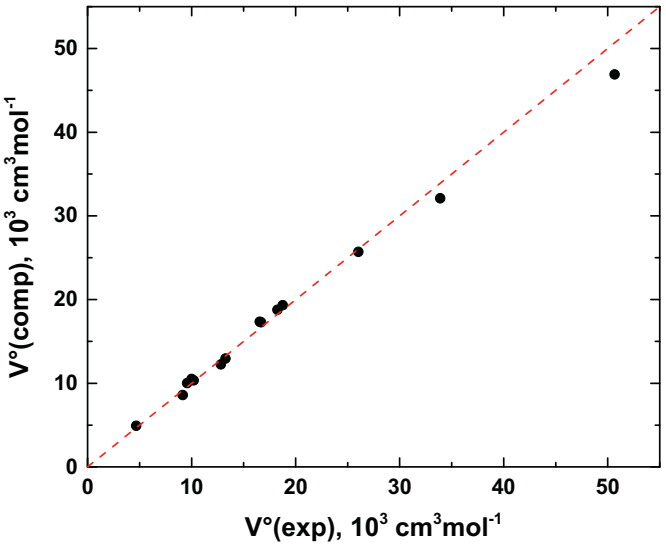

Fig. 1. The partial molar volumes of globular proteins from Table 2 computed with Eq. (2) plotted against their experimental values from refs. [36, 63, 64]. The slope of the dashed line is unity.

Note that, from the purely phenomenological point of view, the thermal (second) and hydration (third) terms can be algebraically combined into a single solvent term involving the accessible surface area. While not perturbing the analytical description of partial molar volume, such a simplification would prevent one from deriving microscopic information on the volumetric state of water of hydration from experimental data.

Fig. 1 shows a plot of the partial molar volumes, V°(comp), of "average" proteins from Table 1 computed with Eq. (2)*versus* their experimental values, V°(exp), reported by Chalikian et al. [36]. Inspection of Fig. 1 reveals a generally good agreement between the computed and experimental values, although the points corresponding to the larger proteins hemoglobin and ovalbumin deviate from the line of a slope of unity. The deviation suggests a weaker than average hydration of these proteins with less negative than the average hydration contributions to partial molar volume.

3. Compressibility

3.1. Intrinsic compressibility

Partial molar isothermal compressibility, K°, is defined as $K^\circ = -(\partial V^\circ/\partial P)_T$. Some researchers define partial compressibility as K° normalized per partial molar volume, V° ($\kappa^\circ = K^\circ/V^\circ$) [10,39,40]. Physically, the use of such a combined parameter does not add any practical advantage. Most importantly, the parameter is not additive and, therefore, cannot be used for comparative analyses (e. g., when comparing homologous compounds or oligomeric proteins and their monomeric counterparts). Another level of complication arises due to the fact that the contributions of V° and K° (or their changes) to κ° are interlinked and difficult to separate. Thus, below we discuss partial molar compressibility and not its ratio to partial molar volume.

The partial molar isothermal compressibility, K°, of a solute is, generally, evaluated from its partial molar adiabatic compressibility, K°_S, that can be derived from a combination of densimetric and ultrasonic velocimetric measurements [1,3,38,41–44]:

$$K^\circ = K^\circ_S + (T\alpha_0^2/\rho_0 c_{P0})(2E^\circ/\alpha_0 - C^\circ_P/\rho_0 c_{P0}) \quad (3)$$

where ρ_0 is the density of the solvent; α_0 is the coefficient of thermal expansion of the solvent; c_{P0} is the specific heat capacity at constant pressure of the solvent; E° is the partial molar expansibility of the solute; and C°_P is the partial molar heat capacity of the solute.

The partial molar isothermal compressibility, K°, of a protein is most often interpreted in terms of the intrinsic and hydration properties [38]:

$$K^\circ = K_{int} + \Delta K_h \quad (4)$$

where $K_M = \beta_{int} V_{int}$ is the intrinsic compressibility of the solute; and ΔK_h is the solute-induced change in the compressibility of solvent.

Comparison of Eqs. (1) and (4) reveals that intrinsic compressibility, K_{int}, refers to the compressibility of the cavity which includes the intramolecular compressibility of the protein $[-(\partial V_M/\partial P)_T]$ and the compressibility of its thermal volume $[-(\partial V_T/\partial P)_T]$. Estimates of β_{int} range from 10×10^{-6} to $25 \times 10^{-6}\,bar^{-1}$ [1,3,36,45–47]. Experimental methods to dissect measured values of K° into the K_{int} and ΔK_h terms in a model-independent way do not presently exist.

MD simulations performed by Persson et al. [10] and Voloshin et al. [16] have offered new avenues for determining β_{int} which can be taken equal to the compressibility of the Voronoi volume, V_{vor}. The value of $\beta_{vor} = -(1/V_{vor})(\partial V_{vor}/\partial P)_T$ ranges between $(19 \pm 2) \times 10^{-6}$ and $(26 \pm 2) \times 10^{-6}\,bar^{-1}$ for four small globular proteins with molecular masses of 6.2–8.4 kDa (a triple mutant of the immunoglobulin-binding domain B1 of protein G, bovine pancreatic trypsin inhibitor, mammalian ubiquitin without the three C-terminal residues, and isoform 2–14 of the β-helical antifreeze protein from *Tenebrio molitor*) [10] and equals $18.3 \times 10^{-6}\,bar^{-1}$ for staphylococcal nuclease [16]. These computed values are in reasonable agreement with an experimental estimate of $25 \times 10^{-6}\,bar^{-1}$ [36,47].

Note that $K_{vor} = \beta_{vor} V_{vor} = \beta_M V_M + \beta_T V_T$, where β_M is the coefficient of intramolecular compressibility of the protein, while β_T is the coefficient of compressibility of thermal volume. Voloshin et al. [16] have computed the pressure dependences of the intramolecular volume, V_M, and interstitial void volume (corresponding to thermal volume, V_T). From these dependences, the values of $\beta_M = -(1/V_M)(\partial V_M/\partial P)_T$ and $\beta_T = -(1/V_T)(\partial V_T/\partial P)_T$ can be estimated to be 11.4×10^{-6} and $40 \times 10^{-6}\,bar^{-1}$, respectively.

Along the same lines, Persson et al. [10] have determined the compressibility contributions of self-correlated and cross-correlated volume fluctuations of the Voronoi volume for the four small globular proteins mentioned above (including BPTI and truncated ubiquitin). We propose that the self-correlated and cross-correlated compressibility contributions determined by Persson et al. [10] correspond to the intramolecular compressibility of the protein, $\beta_M V_M$, and the compressibility of thermal volume, $\beta_T V_T$, normalized per Voronoi volume, respectively ($\beta_M V_M/V_{vor}$ and $\beta_T V_T/V_{vor}$, respectively). For BPTI and truncated ubiquitin, we computed the molecular volumes, V_M, from which the thermal volumes, V_T, can be estimated as the difference between V_{vor} and V_M (see Table 1). We use these values and the compressibility contributions of self-correlated, $\beta_M V_M/V_{vor}$, and cross-correlated, $\beta_T V_T/V_{vor}$, volume fluctuations of BPTI and truncated ubiquitin reported by Persson et al. [10] to determine the coefficients of intramolecular compressibility, β_M, and thermal volume compressibility, β_T. The values of β_M equal 12×10^{-6} and $13 \times 10^{-6}\,bar^{-1}$ for BPTI and ubiquitin, respectively, while the values of β_T for the two proteins are of 86×10^{-6} and $85 \times 10^{-6}\,bar^{-1}$, respectively. While the estimated values of β_M are close to that of staphylococcal nuclease [16], the values of β_T are nearly double that of the nuclease. The discrepancy in β_T may reflect that the interstitial void around the protein computed for BPTI and ubiquitin (with δ equal to 0.32 and 0.29 Å, respectively) in ref. [10] is ~40% thinner than that computed for staphylococcal nuclease (with a δ of 0.51 Å) in ref. [16] (see Table 1).

An independent estimate of the compressibility of the void space in water and aqueous solutions can be made by considering the coefficient of isothermal compressibility of pure water, $45 \times 10^{-6}\,bar^{-1}$. Only a third of the volume of water is occupied by water molecules with the remaining two thirds being void. Thus, the compressibility of the void volume in water is $67.5 \times 10^{-6}\,bar^{-1}$ ($= 1.5 \times 45 \times 10^{-6}$) which is, roughly, the average of the two estimates of β_T (from refs. [10,16]). We

propose that the rounded number $\sim 65 \times 10^{-6}\,bar^{-1}$ is a reasonable measure of β_T in empirical analyses of protein compressibility.

As the protein unfolds, the solvent accessible surface area, S_A, may increase by up to a factor of three (depending on solution conditions and the degree of protein unfolding) with thermal volume increasing correspondingly. Thus, the intramolecular compressibility, $\beta_M V_M$, of an unfolded protein may be nearly zero because of the elimination of intraglobular voids, while its thermal compressibility, $\beta_T V_T$, would increase proportionally to the increase in S_A.

3.2. Hydration compressibility

The hydration contribution, ΔK_h, in Eq. (4) is given by $n_h(K_h - K_0)$, where $(K_h - K_0)$ is the differential partial molar compressibility of water of hydration and bulk water. The value of $(K_h - K_0) \approx -2 \times 10^{-4}\,cm^3\,mol^{-1}\,bar^{-1}$ has been estimated for proteins from experimental data in a model-based manner [48]. This empirical result is numerically similar to the estimate of the average compressibility of water within the first hydration shell of a protein which is 25 to 30% lower than that in the bulk [10].

Another way to estimate $(K_h - K_0)$ for proteins relies on the use of the relationship:

$$K^\circ = \beta_M V_M + \beta_T \delta S_A + (S_A/11.11)(K_h - K_0) \quad (5)$$

We used Eq. (5) to analyze the published set of partial molar adiabatic compressibilities of globular proteins [36]. The difference between the partial adiabatic and isothermal compressibilities is neglected here. The values of β_M and β_T in Eq. (5) are set to 12×10^{-6} and $65 \times 10^{-6}\,bar^{-1}$, respectively. Note that a β_M of $12 \times 10^{-6}\,bar^{-1}$ is on the order of intraglobular compressibility of proteins measured by local structural probes, such as hole burning spectroscopy or X-ray crystallography at elevated pressures [49–51]. Table 3 lists results of the computations. Inspection of Table 3 reveals that, for each protein, the intramolecular compressibility, K_M, and the compressibility of thermal volume, K_T, are numerically similar. The average differential partial molar compressibility of hydration and bulk water, $(K_h - K_0)$, is $-(3.3 \pm 0.5) \times 10^{-4}\,cm^3\,mol^{-1}\,bar^{-1}$. This corresponds to ~40% of the partial molar compressibility of bulk water, which is somewhat larger than the 25 to 30% change computed by Persson et al. [10].

Fig. 2 plots the partial molar compressibilities, K°(comp), of "average" proteins from Table 3 computed with Eq. (5) against their experimental values, K°(exp), from ref. [36]. Inspection of Fig. 2 reveals that, while a good agreement between the computed and experimental values is observed for smaller proteins, the points corresponding to larger proteins deviate from the line of a slope of unity. The deviation suggests a weaker than average hydration of the larger proteins with less negative than average hydration contributions to compressibility.

Table 3
The partial molar adiabatic compressibilities of proteins, K°_S ($cm^3\,mol^{-1}\,bar^{-1}$), intrinsic K_M ($cm^3\,mol^{-1}\,bar^{-1}$), thermal K_T ($cm^3\,mol^{-1}\,bar^{-1}$), and hydration, ΔK_h ($cm^3\,mol^{-1}\,bar^{-1}$), compressibilities, and the differential partial molar isothermal compressibilities of water of hydration and bulk water, $(K_h - K_0)$ ($10^{-4}\,cm^3\,mol^{-1}\,bar^{-1}$).

Proteins	K°_S	K_M	K_T	ΔK_h	$K_h - K_0$
Hemoglobin[a]	0.4624	0.5139	0.4992	−0.5507	−2.4
Ovalbumin[a]	0.2760	0.3526	0.3315	−0.4081	−2.7
Pepsin[a]	0.1846	0.2820	0.2684	−0.3658	3.0
α-Chymo-trypsinogen A[a]	0.0822	0.2113	0.2117	−0.3408	−3.5
α-Chymo-trypsin[a]	0.0708	0.2049	0.2085	−0.3426	−3.6
Trypsin[a]	0.0391	0.1900	0.1860	−0.3369	−3.9
Trypsinogen[a]	0.0920	0.1888	0.1894	−0.2861	−3.3
Myoglobin[a]	0.1264	0.1406	0.1510	−0.1652	−2.4
α-Lactalbumin[a]	0.0386	0.1102	0.1412	−0.2476	−3.8
Lysozyme[a]	0.0186	0.1132	0.1308	−0.2254	−3.7
Ribonuc-lease A[a]	0.0122	0.1071	0.1329	−0.2277	−3.7
Cytochrome c[a]	0.0384	0.0913	0.1197	−0.1726	−3.1
BPTI[b]	0.0215	0.0514	0.0785	−0.1084	−3.0
Staphylococcal nuclease[c]	0.0370	0.1319	0.1544	−0.2493	−3.5

[a] From ref. [36].
[b] From ref. [63].
[c] From ref. [64].

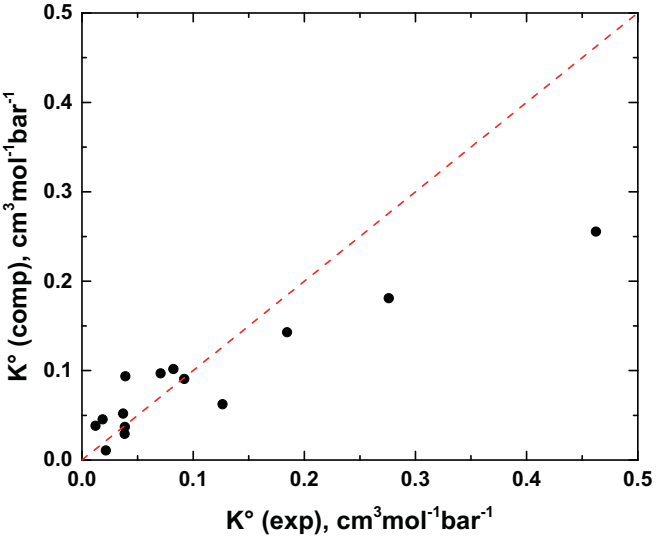

Fig. 2. The partial molar isothermal compressibilities of globular proteins from Table 3 computed with Eq. (5) plotted against their experimental values from refs. [36, 63, 64]. The slope of the dashed line is unity.

4. Expansibility

4.1. Intrinsic expansibility

The partial molar expansibility, E°, of a solute is the temperature derivative of its partial molar volume, V°; $E^\circ = (\partial V^\circ/\partial T)_P$. As in the case of compressibility, the use of normalized expansibility, $\alpha^\circ = E^\circ/V^\circ = (1/V^\circ)(\partial V^\circ/\partial T)_P$, does not offer any practical advantage (its thermal analog would be partial molar heat capacity $C^\circ_P = (\partial H^\circ/\partial T)_P$ normalized per partial molar enthalpy, H°). This parameter is not additive and, therefore, cannot be used for comparative analyses. In addition, any change in α may involve changes in E° and V° in an intricate, even confusing, way. In fact, relative changes in α° and E° may differ in both magnitude and sign.

By analogy with compressibility, the partial molar expansibility of a protein is the sum of the intrinsic and hydration contributions:

$$E^\circ = E_{int} + \Delta E_h \quad (6)$$

The intrinsic expansibility, E_{int}, given by the sum of intramolecular, $E_M = \alpha_M V_M$, and thermal, $E_T = \alpha_T V_T$, expansibilities, can be taken equal to that of the Voronoi volume: $E_{int} = \alpha_{vor} V_{vor} = \alpha_M V_M + \alpha_T V_T$. Temperature-dependent X-ray crystallographic studies of proteins have revealed an average intramolecular coefficient of thermal expansion, α_M, of $\sim 1 \times 10^{-4}\,K^{-1}$ [52–54]. Voloshin et al. [15] have used MD simulations to calculate the coefficient of thermal expansibility of the Voronoi volume for staphylococcal nuclease and found $\alpha_{vor} = V_{vor}^{-1}(\partial V_{vor}/\partial T)_P$ of $3.27 \times 10^{-4}\,K^{-1}$ [15]. These authors computed the coefficient of intramolecular expansibility, $\alpha_M = V_M^{-1}(\partial V_M/\partial T)_P$, to be $1.46 \times 10^{-4}\,K^{-1}$ [15] in good agreement with crystallographic studies. Thus, the rounded number $\sim 1.5 \times 10^{-4}\,K^{-1}$ can be taken as the coefficient of expansibility of protein interior, α_M.

As noted above, thermal volume corresponds to the boundary empty space allocated to the protein in Voronoi tessellation (defined as V_B^M in ref. [15]). For staphylococcal nuclease, the coefficient of thermal

expansibility of thermal volume $\alpha_T = V_T^{-1}(\partial V_T/\partial T)_P$ is $9.94 \times 10^{-4}\,K^{-1}$ [15]. It is plausible to expect this value to be comparable to the expansibility of the void space in liquid water. At 25 °C, the coefficient of thermal expansibility of water is $2.57 \times 10^{-4}\,K^{-1}$. Considering that, roughly, one third of the volume of water is occupied by non-expandable water molecules, the coefficient of expansibility of the void space in water is $\sim 3.86 \times 10^{-4}\,K^{-1}$ ($= 1.5 \times 2.57 \times 10^{-4}\,K^{-1}$) which is less than $9.94 \times 10^{-4}\,K^{-1}$ reported by Voloshin et al. [15]. We propose that $\alpha_T \approx 7 \times 10^{-4}\,K^{-1}$, the rounded average of the two numbers, is a valid estimate of the expansibility of the thermal volume around a globular protein.

4.2. Hydration expansibility

Regarding the hydration term ΔE_h in Eq. (6), all solutes and atomic groups of solutes (ions, charged, polar, and nonpolar) contribute positively to expansibility at room temperature [55,56]. In other words, for all organic and inorganic solutes studied to date, the partial molar volume increases with temperature rising [55–57]. This notion may seem to contrast the negative "contribution" of nonpolar groups to normalized expansibility ($\alpha = E^\circ/V^\circ$) determined by comparing α-amino acids with nonpolar side chains [58]. However, as mentioned above, α is not an additive parameter and, therefore, not suitable for additive calculations and comparisons. A reduced value of α observed for larger amino acids with bulkier aliphatic side chains relative to smaller ones (e. g., glycine and alanine) [58] reflects the larger volumes, V°, and not the smaller expansibilities, E°, of the larger solutes.

In agreement with this notion, the coefficient of expansibility of water within the first hydration shell of staphylococcal nuclease has been computed to be ~20% greater than that of bulk water ($3.07 \times 10^{-4}\,K^{-1}$ compared to $2.57 \times 10^{-4}\,K^{-1}$) [15]. The hydration term in Eq. (4) can be found as $\Delta E_h = n_h(E_h - E_0) = n_h(\alpha_h V_h - \alpha_0 V_0)$, where α_0 and α_h are the coefficients of thermal expansion of bulk water and water of protein hydration, respectively.

Empirically, the differential expansibility of water of protein hydration and bulk water can be determined from the relationship:

$$E^\circ = \alpha_M V_M + \alpha_T \delta S_A + (S_A/11.11)(E_h - E_0) \quad (7)$$

We used Eq. (7) to analyze the partial molar expansibilities of a set of globular proteins that have been volumetrically characterized in ref. [36]. As explained above, the values of α_M and α_T in Eq. (7) are set to 1.5×10^{-4} and $7 \times 10^{-4}\,K^{-1}$, respectively. Table 4 presents the results of our computations. Analogous to compressibility, the expansibility contributions of the intramolecular volume, E_M, and thermal volume, E_T, are numerically similar for each protein. The average differential partial molar expansibility of hydration and bulk water, $(E_h - E_0) = (\alpha_h V_h - \alpha_0 V_0)$, is $(3.7 \pm 1.6) \times 10^{-3}\,cm^3\,mol^{-1}\,K^{-1}$, which translates into α_h of $(4.5 \pm 0.9) \times 10^{-4}\,K^{-1}$. The latter value is somewhat greater than $3.07 \times 10^{-4}\,K^{-1}$, the value of α_h computed for staphylococcal nuclease by Voloshin et al. [15].

Table 4
The partial molar expansibilities of proteins, E° ($cm^3\,mol^{-1}\,K^{-1}$), intrinsic E_M ($cm^3\,mol^{-1}\,K^{-1}$), thermal E_T ($cm^3\,mol^{-1}\,K^{-1}$), and hydration, ΔE_h ($cm^3\,mol^{-1}\,K^{-1}$), expansibilities, and the differential partial molar expansibilities of water of hydration and bulk water, $(E_h - E_0)$ ($10^{-3}\,cm^3\,mol^{-1}\,K^{-1}$).

Proteins	E°	E_M	E_T	ΔE_h	$E_h - E_0$
Hemoglobin[a]	17.17	6.42	5.38	5.37	2.34
Ovalbumin[a]	13.71	4.41	3.57	5.73	3.76
Pepsin[a]	10.12	3.53	2.89	3.70	3.00
α-Chymo-trypsinogen A[a]	8.97	2.64	2.28	4.05	4.16
α-Chymo-trypsin[a]	9.06	2.56	2.25	4.25	4.43
Trypsin[a]	8.10	2.37	2.00	3.72	4.35
Trypsinogen[a]	10.99	2.36	2.04	6.59	7.57
Myoglobin[a]	5.07	1.76	1.63	1.69	2.43
α-Lactalbumin[a]	4.88	1.38	1.52	1.98	3.05
Lysozyme[a]	4.09	1.41	1.41	1.27	2.10
Ribonuc-lease A[a]	4.04	1.34	1.43	1.27	2.08
Cytochrome c[a]	5.04	1.14	1.29	2.61	4.74

[a] Calculated from temperature-dependent partial molar volume data presented in from ref. [36].

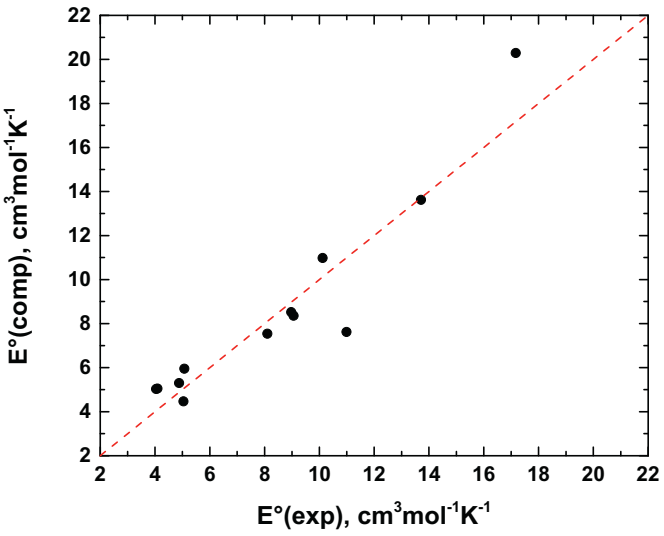

Fig. 3. The partial molar expansibilities of globular proteins from Table 4 computed with Eq. (7) plotted against their experimental values from ref. [36]. The slope of the dashed line is unity.

Fig. 3 presents the partial molar expansibilities, E°(comp), of "average" proteins from Table 4 computed with Eq. (3) plotted against their experimental values, E°(exp), from ref. [36]. Inspection of Fig. 3 reveals a satisfactory agreement between the computed and experimental partial molar expansibilities, although the point corresponding to the largest protein hemoglobin deviates from the slope-of-unity line in the direction of weaker hydration (analogous to volume and compressibility).

5. Inferences and recommendations

Recent MD simulations of proteins in solution coupled with Voronoi-Delaunay tessellation of simulated structures have provided an exceptional level of structural information on protein-water interfaces [9–16]. We took advantage of these structural insights to redefine and reevaluate the empirical framework used to interpret volumetric data on proteins. Volumetric data are accessible in two ways. In the first, they are measured experimentally and subsequently interpreted based on our understanding of the nature of intermolecular interactions. In the second, the volumes result from analyses of computational simulations of molecules in solution (e. g., using Kirkwood-Buff integrals). In both cases, a robust interpretation framework is needed for reliable microscopic rationalization of the volumetric properties of proteins. An important issue in this respect is to identify the effective dividing surface between water and protein atoms when they are treated as hard spheres enclosed within their respective van der Waals surfaces. This will also prove useful in dissecting the Kirkwood-Buff integrals into the excluded volume and solvation shell contributions in many applications involving proteins [59–61].

In the simplest assumption, the dividing surface coincides with the molecular surface of the protein. While such an assumption is sufficient for a purely phenomenological description of volumetric data, it distorts the description of the thermodynamic state of water of hydration, in particular, leading to an underestimate of the density of water of hydration by assigning all the boundary void between solute and solvent exclusively to water. In an additively weighted Voronoi volume-

based analysis of MD simulated protein structures, the dividing surface is drawn equally distant from the van der Waals surfaces of neighboring water and protein atoms [9,15]. The distance between the molecular surface of a protein and the dividing surface has been found to be between 0.3 and 0.5 Å which is numerically similar to the thickness of thermal volume, δ, of ~0.5 Å derived from empirical estimates and SPT-based computations [17,18,20,24,37]. Thermal volume is a necessary component that needs to be carefully considered in hard sphere-based schemes of microscopic interpretation of volumetric properties of solutes, in general, and proteins, in particular.

Despite our previous estimates of the thickness of thermal volume of proteins and other large solutes of 1 Å [36,37], we now suggest that 0.5 Å is a better estimate of δ for both small molecules and proteins. The thermal volume, V_T, of a protein can be calculated as the product of δ and solvent accessible surface area, S_A; $V_T = \delta S_A$.

The contributions of thermal volume to compressibility and expansibility have never been considered explicitly but been treated as part of the intrinsic compressibility or expansibility of a protein. Here, we do it explicitly which is essential for rationalizing changes in volumetric parameters accompanying protein transitions and binding. The average intramolecular coefficients of isothermal compressibility, β_M, and thermal expansibility, α_M, of proteins are $12 \times 10^{-6}\,bar^{-1}$ and $1.5 \times 10^{-4}\,K^{-1}$, respectively. The coefficients of isothermal compressibility, β_T, and thermal expansibility, α_T, of the thermal volume of proteins are $65 \times 10^{-6}\,bar^{-1}$ and $7 \times 10^{-4}\,K^{-1}$, respectively.

The question of the number of layers of thermodynamically and kinetically altered water molecules around a protein is important for the volumetric description of proteins in solution. Results of highly detailed MD simulation studies are consistent with the picture in which solute-induced thermodynamic and kinetic changes are overwhelmingly confined to the first layer of water molecules around the protein [9–12]. It should be noted, however, that, for small nonpolar solutes (such as methane), the cage-like hydration shell may involve water molecules beyond the first layer. Assuming a single layer of hydration around a protein, the average differential partial molar volumes, $(V_h - V_0)$, compressibility, $(K_h - K_0)$, and expansibility, $(E_h - E_0)$, of water of protein hydration and bulk water are $-1.6 \pm 0.8\,cm^3\,mol^{-1}$, $-(3.3 \pm 0.5) \times 10^{-4}\,cm^3\,mol^{-1}\,bar^{-1}$, and $(3.7 \pm 1.6) \times 10^{-3}\,cm^3\,mol^{-1}\,K^{-1}$, respectively. Eqs. (2), (5), and (7) can be used to evaluate the partial molar volume, V°, compressibility, $K°_T$, and expansibility, E°, of an "average" globular protein based on its intrinsic volume, V_M, and solvent accessible surface area, S_A.

Our results have implications for understanding volumetric changes associated with protein folding and binding reactions. Folded-unfolded conformational transitions and binding events of proteins are, generally, accompanied by alterations in the volume of water-inaccessible protein core, V_M, and solvent accessible surface areas, S_A. An increase or decrease in S_A would alter the thermal volume, V_T, and its contributions to compressibility, K_T, and expansibility, E_T. If the thermal contributions are not properly analyzed and accounted for, the microscopic picture painted by volumetric data may well be skewed.

Regarding protein transitions, our recent work has revealed that the urea-induced denaturation of ribonuclease A and α-chymotrypsinogen A at 25 °C is accompanied by a two-fold increase in S_A [62]. A simple estimate based on Eqs. (5) and (7) suggests that a two-fold increase in S_A of a protein would cause increases in K_T and E_T comparable in magnitude to changes in the hydration contributions ΔK_h and ΔE_h. Hence, the volumetric contributions of thermal volume need to be considered and accounted for when interpreting experimental data on protein transition and binding events.

In the aggregate, all empirical schemes of interpretation of volumetric data implicitly treat solute and solvent as hard spheres enclosed within their respective van der Waals surfaces. In such a treatment, the problem of defining the effective dividing surface between solute and solvent becomes of critical importance; the contribution of thermal volume (void volume around the solute) to partial molar volume, compressibility, and expansibility needs to be carefully considered for accurate thermodynamic description of the state of water of hydration. Based on recent MD simulations of protein-water systems with the subsequent additively weighted Voronoi tessellation of the simulated systems, we have reevaluated the volumetric properties of proteins and their thermal volumes. The intrinsic volume, compressibility, and expansibility are explicitly considered to involve the intramolecular component and the thermal contribution. In order to analyze the volumetric changes accompanying protein folding and binding event, it is essential to separate the intramolecular and thermal components of the volumetric properties.

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