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Review

Compressibility of protein transitions

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Abstract

We review the results of compressibility studies on proteins and low molecular weight compounds that model the hydration properties of these biopolymers. In particular, we present an analysis of compressibility changes accompanying conformational transitions of globular proteins. This analysis, in conjunction with experimental compressibility data on protein transitions, were used to define the changes in the hydration properties and intrinsic packing associated with native-to-molten globule, native-to-partially unfolded, and native-to-fully unfolded transitions of globular proteins. In addition, we discuss the molecular origins of predominantly positive changes in compressibility observed for pressure-induced denaturation transitions of globular proteins. Throughout this review, we emphasize the importance of compressibility data for characterizing protein transitions, while also describing how such data can be interpreted to gain insight into role that hydration and intrinsic packing play in modulating the stability of and recognition between proteins and other biologically important compounds.

Keywords: Globular protein; Conformational transition; Hydration; Intrinsic packing; Compressibility; Ultrasonics

1. Introduction

The problem of protein folding remains a fascinating and much debated challenge of molecular biophysics [1–12]. After Anfinsen demonstrated that proteins fold spontaneously and introduced the hypothesis that the native state of the protein corresponds to the global minimum of free energy [13,14], thermodynamic characterizations of proteins in their native and denatured states have emerged as a major and fruitful approach to tackling the problem of protein folding [15–20]. The native state of the protein is separated from the ensemble of its denatured states by highly cooperative transition of the all-or-none type [21–25]. Elucidation of the thermodynamic nature and relative importance of the forces that govern the cooperativity of the folding/unfolding transitions of proteins is a key to understanding and, ultimately, addressing the protein folding problem [26,27]. In recognition of this fact and to this end, the thermodynamics of protein transitions has been thoroughly studied based on temperature-dependent [15,17,20], pH-dependent [19,24,25], denaturant-dependent [16,28,29], and pressure-dependent [18,30–34] protocols. In these studies, the equilibrium between the native and denatured protein states is perturbed by changing solution conditions (temperature, pressure, pH, salt, co-solvent, and denaturant concentration) and the concomitant change in a thermodynamic state function is monitored.

The effect of pressure on protein stability is determined by changes in volume, expansibility (the first

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temperature derivative of volume), and compressibility (the first pressure derivative of volume) associated with the unfolding transition of the protein at the experimental temperature and pressure [31]. The volumetric properties (volume and its derivatives expansibility and compressibility) of proteins and their low molecular weight analogs have proven uniquely sensitive to solute–solvent interactions (hydration) and intrinsic packing of globular proteins [35–38]. Consequently, any transition-induced change in protein hydration and/or intrinsic packing should be reflected in the accompanying changes in volume, expansibility, and compressibility [38–41]. Therefore, volumetric parameters represent a useful tool for macroscopic characterizations of structural transitions of proteins. It should be noted that thermodynamic information on intra- and intermolecular interactions of proteins provided by pressure-related volumetric observables (such as volume and compressibility) is complementary to that provided by temperature-related calorimetric observables (such as heat capacity and enthalpy). Hence, the combined use of volumetric and calorimetric observables often leads to more complete thermodynamic characterizations of protein systems and their conformational transitions.

The current interest of the biophysical/biochemical community toward compressibility as a tool for protein studies was first triggered several decades ago by progress in development of high precision techniques of sound velocity measurements and high-pressure studies [42–45]. Subsequently, compressibility measurements have begun to find an increasing employment as an independent and effective means for characterizing protein systems in a number of research laboratories across the globe [46–55]. In this paper, we review recent progress in the field of compressibility-based characterizations of structural transitions of water-soluble globular proteins. This review is not meant to be comprehensive with respect to critically evaluating the existing literature data on protein compressibility and microscopic interpretations of these data in terms of protein hydration and intrinsic packing. Instead, we focus on presenting our own results while also describing selected studies from other research groups. We begin by defining the partial compressibility of a solute and describing how it can be interpreted microscopically. Then, we briefly review the data on the partial compressibility of amino acids, peptides, and native globular proteins. Next, we present some theoretical considerations on compressibility changes associated with protein transitions. Finally, we present a survey of published experimental data on changes in compressibility associated with temperature-, pressure-, denaturant-, and pH-induced structural transitions of globular proteins. Throughout the review, we emphasize the significance of compressibility measurements for gaining insight into the forces that govern conformational preferences of polypeptide chains at given solution conditions.

2. Partial compressibility of proteins

2.1. Definitions

The most comprehensive and thermodynamically rigorous review of definitions and calculations of solute compressibility and related state functions has been recently presented by Blandamer et al. [56]. The isothermal compressibility, K_T, of a system can be defined as the negative pressure derivative of its volume, V, taken at constant temperature:

$$K_T = \beta_T V = -(\partial V/\partial P)_T \quad (1)$$

where T is the absolute temperature; and $\beta_T = -V^{-1}(\partial V/\partial P)_T$ is the coefficient of isothermal compressibility.

Similarly, the adiabatic compressibility of a system is the negative pressure derivative of its volume, V, taken at constant entropy:

$$K_S = \beta_S V = -(\partial V/\partial P)_S \quad (2)$$

where S is the entropy; and $\beta_S = -V^{-1}(\partial V/\partial P)_S$ is the coefficient of adiabatic compressibility.

Coefficients of isothermal, β_T, and adiabatic, β_S, compressibility are related to each other as follows:

$$\beta_T = \beta_S + T\alpha^2/\rho c_P \quad (3)$$

where ρ is the density; $\alpha = V^{-1}(\partial V/\partial T)_P$ is the coefficient of thermal expansion; and c_P is the specific heat capacity at constant pressure.

The partial molar isothermal/adiabatic compressibility, $K°_{T/S}$, of a solute is defined as the partial derivative of the isothermal/adiabatic compressibility of

the solution with respect to the number of moles of the solute, N:

$$K^{\circ}_{T} = (\partial K_T/\partial N)_{T,P} \quad (4)$$

$$K^{\circ}_{S} = (\partial K_S/\partial N)_{T,P} \quad (5)$$

For proteins, when it is often needed to compare molecules with vastly different molecular weights, M, the values of partial specific isothermal, $k^{\circ}_{T} = K^{\circ}_{T}/M$, and adiabatic, $k^{\circ}_{S} = K^{\circ}_{S}/M$, compressibilities are more convenient for use than the partial molar values. The relationship between K°_{T} and K°_{S} is as follows [56]:

$$K^{\circ}_{T} = K^{\circ}_{S} + (T\alpha_0^2/\rho_0 c_{P0})(2E^{\circ}/\alpha_0 - C^{\circ}_{P}/\rho_0 c_{P0}) \quad (6)$$

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 a solute; and C°_{P} is the partial molar heat capacity of a solute.

Sometimes, the so-called adiabatic, $\kappa^{\circ}_{S} = K^{\circ}_{S}/V^{\circ}$, and isothermal, $\kappa^{\circ}_{T} = K^{\circ}_{T}/V^{\circ}$, compressibilities of a protein are used in volumetric studies. The use of these characteristics is justified as far as there is a clear understanding of the relationships between κ_S, κ_T, K°_{S}, and K_T. In this respect, it should be noted that the relationship between κ_S and κ_T is given by a modification of Eq. 6 [$\kappa^{\circ}_{T} = \kappa^{\circ}_{S} + (T\alpha_0^2/V^{\circ}\rho_0 c_{P0})(2E^{\circ}/\alpha_0 - C^{\circ}_{P}/\rho_0 c_{P0})$] and not by Eq. 3.

2.2. Measurements

Currently, the most accurate method of determining the partial molar adiabatic compressibility, K°_{S}, of a solute is based on the Newton-Laplace equation which relates the coefficient of adiabatic compressibility, β_S, of a medium with its density, ρ, and sound velocity, U [49]:

$$U^2 = (\beta_S \rho)^{-1} \quad (7)$$

Differentiating Eq. 7 with respect to the molar concentration of a solute, C, and performing simple arithmetical rearrangements one obtains the following relationship for infinitely diluted solutions [57,58]:

$$K^{\circ}_{S} = \beta_{S0}(2V^{\circ} - 2[U] - M/\rho_0) \quad (8)$$

where β_{S0} is the coefficient of adiabatic compressibility of the solvent; $V^{\circ} = (\partial V/\partial N)_{T,P}$ is the partial molar volume of a solute; $[U] = (U - U_0)/U_0 C$ is the relative molar sound velocity increment of a solute; and U and U_0 are the sound velocities in the solution and solvent, respectively.

The partial molar volume, V°, of a solute can be calculated based on differential solution-versus-solvent density measurements [59]:

$$V^{\circ} = M/\rho_0 - (\rho - \rho_0)/\rho_0 C \quad (9)$$

where ρ and ρ_0 are the densities of the solution and solvent, respectively.

Inspection of Eqs. 7, 8, 9 reveals that the partial molar adiabatic compressibility of a solute can be calculated from differential solution-versus-solvent measurements of density and sound velocity. Recent developments of highly sensitive densimetric and acoustic apparatuses have enabled differential measurements of solution density and sound velocity in liquid samples with the minimum required volume of less than 1 ml and relative precision on the order of 10^{-4}% [49,60–65]. This precision permits one to carry out reliable compressibility measurements in solutions with solute concentration of $\sim$1 mg/ml or less. The partial molar isothermal compressibility, K°_{T}, of a solute can be recalculated from K°_{S} using Eq. 6 provided that the partial molar expansibility, E°, and heat capacity, C°_{P}, have been determined independently. Significantly, by conducting densimetric and acoustic measurements in a protein solution as a function of temperature, pressure, pH, salt, or denaturant concentration (all of which may perturb the equilibrium between the native and denatured protein species), one can determine the change in compressibility associated with a temperature-, pressure-, pH-, salt-, or denaturant-induced protein transition.

An alternative way of calculating the change in isothermal compressibility, ΔK_T, accompanying the pressure-induced denaturation of a protein is based on a two-state analysis of the transition profile monitored by some physical (often spectroscopic) observable [31,44,45,66]. In this approach, a two-state analysis is used to describe the pressure dependence of the free energy, ΔG, of protein denaturation. The change in compressibility is determined from approx-

imation of the pressure dependence of ΔG by the function [31]:

$$\Delta G = \Delta G_0 + \Delta V(P-P_0) + 0.5\Delta K_T(P-P_0)^2 \qquad (10)$$

where ΔG_0 is the free energy of protein denaturation at reference pressure, P_0; and ΔV and ΔK_T are, respectively, the changes in volume and isothermal compressibility accompanying the pressure-induced denaturation of the protein.

Finally, in one recent study, the partial specific isothermal compressibility, $k°_T$, of the globular protein staphylococcal nuclease was determined from direct density measurements as a function of pressure over the range of 1 to 1000 bar [67]. Even though this promising approach represents the most straightforward way of determining $k°_T$, its relative precision is still low. Consequently, high protein concentrations are required for experiments. For example, Seemann et al. have used a protein concentration of $\sim$40 mg/ml for evaluating $k°_T$ of staphylococcal nuclease [67].

2.3. Interpretations

The partial molar adiabatic compressibility, $K°_S$, of a solute can be represented by the sum of intrinsic, K_{SM}, and hydration, ΔK_{Sh}, contributions [37,38,48,49,68]:

$$K°_S = K_{SM} + \Delta K_{Sh} = \beta_{SM} V_M + n_h(K_{Sh}-K_{S0}) \qquad (11)$$

where K_{SM} is the intrinsic adiabatic compressibility of a solute; V_M is the intrinsic volume of a solute; β_{SM} is the intrinsic coefficient of adiabatic compressibility of a solute; ΔK_{Sh} is the compressibility effect of hydration; K_{S0} and K_{Sh} are the partial molar adiabatic compressibilities of water in the bulk state and in the hydration shell of a solute, respectively; and n_h is the 'hydration number' which corresponds to the number of water molecules in the hydration shell of a solute.

The partial molar isothermal compressibility, $K°_T$, of a solute can be interpreted in terms of hydration and intrinsic contributions using a similar relationship:

$$K°_T = K_{TM} + \Delta K_{Th} = \beta_{TM} V_M + n_h(K_{Th}-K_{T0}) \qquad (12)$$

In Eqs. 11, 12, the intrinsic volume, V_M, represents the geometric volume of the solute that is not penetrable by surrounding water molecules. For a globular protein, V_M is equal to the sum of the van der Waals volumes, V_W, of all constituent atoms plus the volume of intraglobular voids, V_V, resulting from its imperfect internal packing. Consequently, V_M reflects the spatial architecture of protein interior. The intrinsic coefficient of adiabatic, β_{SM}, or isothermal, β_{TM}, compressibility of a globular protein represents a measure of intraglobular interactions and, consequently, reflects the tightness of intrinsic packing. In addition, β_{TM} reflects the dynamic properties of a protein since the mean square volume fluctuation of a solute molecule, $\langle V_M^2 \rangle$, is directly proportional to β_{TM} [69,70]:

$$\langle V_M^2 \rangle = k_B T \beta_{TM} V_M \qquad (13)$$

where k_B is Boltzmann constant.

The hydration terms, ΔK_{Sh} and ΔK_{Th}, in Eqs. 11, 12 represent the change in solvent compressibility induced by solute–solvent interactions. Being so, ΔK_{Sh} and ΔK_{Th} are sensitive to the amount (n_h) and strength (K_{Sh} and K_{Th}) of solute–solvent interactions in the vicinity of solute molecule. Consequently, the values of ΔK_{Sh} and ΔK_{Th} depend on the number and chemical nature (charged, polar, and nonpolar) of solvent-exposed protein groups.

Based on the foregoing discussion, it is clear that any transition-induced change in protein hydration, interior packing, or size of the solvent-inaccessible core should be reflected in the values of $K°_S$ and $K°_T$. Hence, compressibility measurements can be used as an effective means for detecting and characterizing protein transitions. Eqs. 11, 12, 13 in conjunction with the accumulated volumetric database on globular proteins and their low molecular weight model compounds form the basis for microscopic interpretations of compressibility data.

3. Partial compressibility of low molecular weight protein analogs and unfolded polypeptide chains

In an attempt to better understand and, ultimately, predict the hydration properties of proteins, their low molecular weight analogs, including amino acids and short oligopeptides, have been widely used as model

systems. To this end, the volumetric properties of these relatively simple compounds have been studied as a function of temperature, pressure, and pH [71–91]. One important advantage of small molecules for modeling protein hydration is that their intrinsic compressibility, K_M, which is determined mostly by the compressibility of covalent bonds and external electron shells, is small and can be neglected in Eqs. 11, 12 [37,38,49]. Consequently, as is seen from Eqs. 11, 12, the partial compressibility of a low molecular weight model compound is predominantly determined by its solute–solvent interactions as reflected in the values of ΔK_{Sh} and ΔK_{Th}.

Based on model compound studies, the compressibility contributions of charged, polar, and nonpolar groups have been determined as a function of temperature. Fig. 1A presents the temperature dependence of the adiabatic compressibility contribution of an independently hydrated pair of the charged amino $-NH_3^+$ and carboxyl $-COO^-$ (●) termini in long α,ω-aminocarboxylic acids [79]. Fig. 1B presents our data on the adiabatic compressibility contributions of an independently hydrated methylene $-CH_2-$ group in α,ω-aminocarboxylic acids [79] (○), peptide –CONH- group in oligoglycines [81] (■), hydroxyl –OH group in pentoses and hexoses [92] (□), and benzene $-C_6H_5$ ring in the GlyGlyPhe tripeptide [88] (◇) plotted against temperature. In addition, Fig. 1B presents the literature data on the temperature dependence of the adiabatic compressibility contribution of the hydroxyl –OH group in long α,ω-diols (◆) [77]. The data presented in Fig. 1A,B in conjunction with Eq. 11 enable one to quantitatively characterize the hydration properties of charged, polar, and nonpolar groups and evaluate the differential compressibility of water of hydration and bulk water. Within the entire temperature range studied, waters solvating charged groups are less compressible than bulk water [37,49,77,79]. Water molecules solvating aliphatic groups are less compressible than bulk water below 35°C while exhibiting a greater compressibility at higher temperatures [37,49,77,79]. Hydration of a polar group depends on its position relative to other polar or charged groups in the solute molecule, with the two extreme cases represented by 'closely located' and 'single' polar groups [77]. For closely located polar groups (such as the –OH groups in pentoses and hexoses), the compressibility

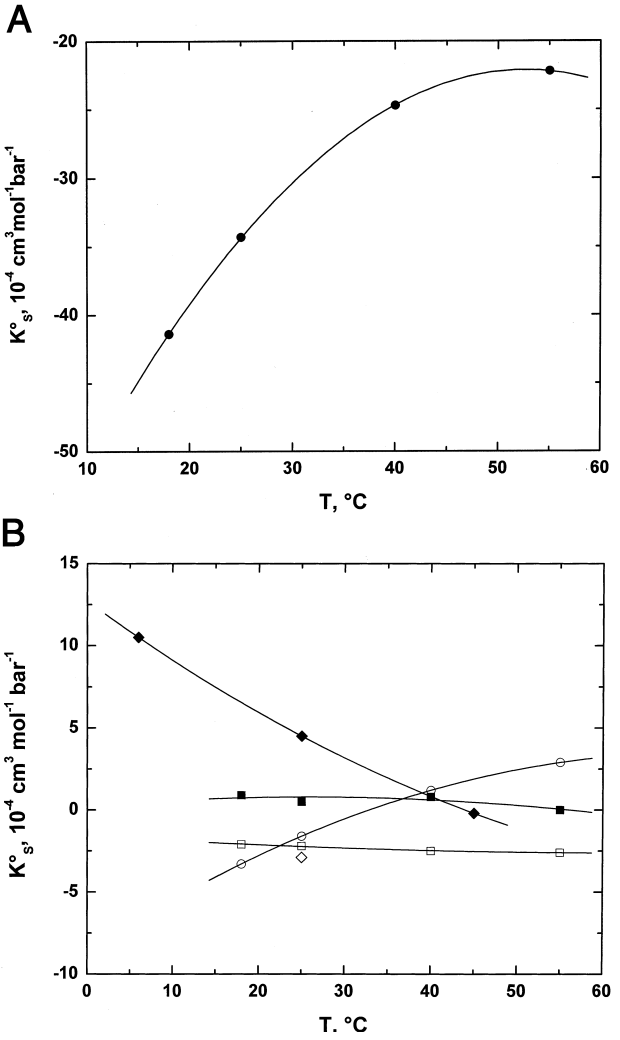

Fig. 1. The temperature dependences of compressibility contributions of different atomic groups: (a) a pair of the charged amino $-NH_3^+$ and carboxyl $-COO^-$ (●) termini in long α,ω-aminocarboxylic acids [79]; (b) an aliphatic $-CH_2-$ group in α,ω-aminocarboxylic acids [79] (○), peptide –CONH- group in oligoglycines [81] (■), hydroxyl –OH group in pentoses and hexoses [92] (□), aromatic benzene $-C_6H_5$ ring in the GlyGlyPhe tripeptide [88] (◇), and the hydroxyl –OH group in long α,ω-diols (◆) [77].

of water of hydration is reduced compared to that of bulk water and weakly depends on temperature. By contrast, for a single polar group (such as, the –OH groups in alcohols and long α,ω-diols), the compressibility of water of hydration is significantly larger than that of bulk water, but the difference steeply decreases with increasing temperature (see Fig. 1B). Recently, the thermodynamics of water solvating atomic groups of different chemical nature has been

analyzed and rationalized based on the two-state representation of liquid water [93].

Volumetric studies on amino acids and systematically altered oligopeptides have been used to determine the compressibility contributions of glycyl unit (-CH_2CONH-) and different amino acid side chains [71,73,77,79–81,83,84,88,90]. These contributions are required to develope additive algorithms for calculating the partial molar (or specific) adiabatic compressibility of an extended polypeptide chain of a known primary sequence [77,83,94]. However, a survey of the relevant literature reveals that the magnitude and even the sign of the compressibility contribution of a given amino acid side chain strongly depends on the model compound used. For example, the compressibility contribution of the Phe side chain calculated as the difference between the partial molar adiabatic compressibilities of the amino acids Phe and Gly equals -7.54×10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$ [71]. The same contribution determined from di- and tripeptide studies is equal to -1.79×10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$ (GlyPhe/GlyGly) [75], 5.8×10^{-4} $cm^3 mol^{-1}\,bar^{-1}$ (GlyPheGly/GlyGlyGly) [83], 1.2×10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$ (PheGlyGly/GlyGlyGly), and 0.6×10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$ (GlyGlyPhe/GlyGlyGly) [88]. These disparities, which result from unaccounted intramolecular interactions between the side chain and the rest of the solute molecule, make it very difficult to choose a satisfactory set of data for modeling the compressibility contributions of amino acid residues in a polypeptide chain. In this respect, free amino acids are the least reliable models since, in these molecules, the closely located side chain and zwitterionic skeleton may strongly interact with each other via overlap of hydration shells. Hence, the side chain of a free amino acid can hardly be considered independently hydrated. Perhaps, amino acid side chains in Gly-X-Gly tripeptides and uncharged *N*-acetyl amino acid amides represent the best models for mimicking amino acid residues in proteins. Unfortunately, systematic volumetric databases on these promising substances are still lacking.

The compressibility contribution of glycyl unit (-CH_2CONH-) obtained on the basis of similar (but not identical) peptide systems also can be quite different. For example, in two recent studies on the homologous series of oligoglycines (glycine to pentaglycine), similar values for the compressibility contribution of glycyl unit equal to $-(1.1\pm0.5)\times10^{-4}$ $cm^3\,mol^{-1}\,bar^{-1}$ [81] and $-(1.08\pm0.08)\times10^{-4}$ $cm^3\,mol^{-1}\,bar^{-1}$ [90] have been obtained. By contrast, the same contribution obtained from the Ala, AlaGly, $AlaGly_2$, $AlaGly_3$, and $AlaGly_4$ series is significantly more negative and equals $-(2.75\pm0.03)\times10^{-4}$ $cm^3\,mol^{-1}\,bar^{-1}$ [81]. The reason(s) for the observed disparity is (are) yet to be uncovered.

In the aggregate, a wealth of information on the partial molar adiabatic compressibilities of amino acids and oligopeptides has been accumulated to date. Nevertheless, reliable derivation of the compressibilty contributions of amino acid residues required for calculating the partial compressibility of individual polypeptide chains of known primary sequences is not yet possible. It should be noted however that, in a recent publication, an additive method for calculating the partial compressibility of an unfolded polypeptide chain has been put forward [94]. Based on this method, the author calculated the compressibility effects of complete unfolding for a number of globular proteins [94]. In our opinion, the proposed additive method is of limited applicability for experimental analyses and practical predictions. In this method, amino acid residues of polypeptide chains have been somewhat arbitrarily modeled by zwitterionic amino acids. The use of free amino acids may be the source of large, unaccountable calculation errors since, as noted above, in these compounds, the side chain is not independently hydrated. Hence, free amino acids make a poor model for mimicking amino acid residues in polypeptide chains.

Another strategy for estimating the partial compressibility of an unfolded protein is to focus on an 'average' polypeptide chain rather than to attempt discriminating between individual polypeptides differing in primary sequences. The partial compressibility of an average unfolded protein can be obtained based on the following assumption: the average unit hydration (normalized per 1 $Å^2$) of an unfolded protein, independent of its exact amino acid sequence, is roughly similar to that of short, structureless oligopeptides of mixed composition, such as tripeptides. Based on this assumption, the average unit compressibility contribution (per 1 $Å^2$) of a polypeptide

surface, γ, should be roughly equal to the arithmetic mean of unit contributions of a large number of relatively short oligopeptides [95]. For example, for the X-Gly-Gly and Gly-Gly-X families of tripeptides (where X is an apolar amino acid), the values of γ range from -10×10^{-6} to -14×10^{-6} $cm^3\,mol^{-1}\,bar^{-1}$ $Å^{-2}$ as can be calculated by dividing the partial molar adiabatic compressibilities, $K°_S$, of a tripeptide by its solvent accessible surface area, S_A ($\gamma = K°_S/S_A$) [88]. In our analysis below, we will assume that the unit compressibility contribution of an unfolded protein, γ_U, is equal to $(-12\pm2)\times10^{-6}$ $cm^3\,mol^{-1}\,bar^{-1}$ $Å^{-2}$. This approach does not permit one to discriminate between specific polypeptides, but rather provides an estimate of the partial specific adiabatic compressibility of an 'average' fully extended polypeptide chain. An implicit assumption of this approach is that the partial *specific* adiabatic compressibility, $k°_S$, of an unfolded polypeptide chain is roughly similar to that of short oligopeptides. Based on our tripeptide data, we estimate the partial specific adiabatic compressibility, $k°_S$, of an 'average' unfolded protein to be $-(18\pm3)\times10^{-6}$ $cm^3\,g^{-1}\,bar^{-1}$ [88].

4. Partial compressibility of native globular proteins

The largest set of partial specific adiabatic compressibilities, $k°_S$, of native globular proteins at 25°C has been reported by Gekko and coworkers [46,47]. Subsequently, Gekko and Hasegawa [96] and Chalikian et al. [97] studied the effect of temperature on the partial compressibility of native globular proteins. Analysis of the available data on protein compressibility reveals that native globular proteins predominantly exhibit positive values of $k°_S$. Microscopic interpretation of data on the partial specific adiabatic compressibility of proteins conventionally relies on an expanded version of Eq. 11 [98]:

$$k^\circ_S = k_{SM} + \Delta k_{Sh} + k_{rel} \quad (14)$$

In this equation, $k_{SM} = K_{SM}/M$ is the specific intrinsic compressibility of a protein; M is the molecular weight of a protein; $\Delta k_{Sh} = M^{-1}\sum_i n_{Shi}(K_{Shi} - K_{S0})$ is the compressibility effect of hydration; K_{Shi} is the partial molar adiabatic compressibility of water solvating the ith solvent-exposed protein group; n_{hi} is the number of water molecules in the hydration shell of the ith solvent-exposed protein group; k_{rel} is the relaxation contribution to protein compressibility, which can be presented as the sum of the pH-dependent, $k_{rel}(pH)$, and pH-independent, k_{rel0}, terms:

$$k_{rel} = k_{rel0} + k_{rel}(pH) \quad (15)$$

The pH-independent term, k_{rel0}, results from fluctuations (subtransitions) within the ensemble of nearly isoenergetic, native-like conformational states of the protein, which are, nevertheless, distinct with respect to their partial volumes. Based on the theory of chemical relaxation [99], it can be shown that the pH-independent relaxation contribution to protein compressibility is given by the following expression:

$$k_{rel0} = M^{-1}\sum_j [\Delta V_j(\Delta V_j - \alpha\Delta H_j/\rho c_P)/RT]\,[K_j/(1+K_j)^2](1+\omega^2\tau_j^2)^{-1} \quad (16)$$

where ΔV_j is the differential volume of the two native-like conformational substates of the jth subtransition; ΔH_j is the enthalpy of the jth subtransition; α, ρ, and c_P are the coefficient of thermal expansion, density, and specific heat capacity of the protein solution, respectively; K_j is the equilibrium constant for the jth subtransition; ω is the angular frequency of ultrasonic waves used for sound velocity measurements; $\tau_j = k_{fj} + k_{rj}$ is the relaxation time of the jth subtransition; and k_{fj} and k_{rj} are the rate constants for the forward and reverse processes of the jth subtransition, respectively. The characteristic range of relaxation times for a native globular protein is on the order of 10^{-9} to 10^{-3} s [98].

The pH-dependent term, $k_{rel}(pH)$, becomes important only at acidic and alkaline pH and is related to proton transfer reactions accompanying protonation/deprotonation of acidic ($–COO^- + H^+ \leftrightharpoons$ –COOH) and basic ($–NH_3^+ + OH^- \leftrightharpoons –NH_2 + H_2O$) groups [72,78,98–108]:

$$k_{rel}(pH) = M^{-1}\sum_k [\Delta V_k(\Delta V_k - \alpha\Delta H_k/\rho c_P)/RT]\,\Gamma_k(1+\omega^2\tau_k^2)^{-1} \quad (17)$$

where ΔV_k and ΔH_k are the volume and enthalpy of the kth proton transfer reaction; τ_k is the relaxation

time for the kth proton transfer reaction. For acidic pH, the concentration-dependent coefficient Γ_k can be calculated from the relationship:

$$\Gamma_k^{-1} = [(2 + 10^{\mathrm{pH}-\mathrm{p}Kak} + 10^{pKak-\mathrm{pH}})/C_k] + 10^{\mathrm{pH}} \quad (18)$$

where C_k is the molar concentration of the kth atomic group being titrated; and $\mathrm{p}K_{ak}$ is the dissociation constant of the kth atomic group being titrated.

For alkaline pH, Γ_k is given by the relationship:

$$\Gamma_k^{-1} = [(2 + 10^{\mathrm{pH}-\mathrm{p}Kak} + 10^{\mathrm{p}Kak-\mathrm{pH}})/C_k] + 10^{14-\mathrm{pH}} \quad (19)$$

For acidic pH, the relaxation time, τ_k, can be calculated from the equation:

$$\tau_k^{-1} = k_{fk}[C_k/(1 + 10^{\mathrm{p}Kak-\mathrm{pH}}) + 10^{-\mathrm{pH}} + 10^{-\mathrm{p}Kak}] \quad (20)$$

where k_{fk} is the rate constant for the forward kth proton-transfer reaction.

For alkaline pH, τ_k is give by the following relationship:

$$\tau_k^{-1} = k_{fk}[C_k/(1 + 10^{\mathrm{pH}-\mathrm{p}Kak}) + 10^{\mathrm{pH}-14} + 10^{\mathrm{p}Kak-14}] \quad (21)$$

Experimentally, the net relaxation contribution to protein compressibility, k_{rel}, can be determined from measurements of the excess ultrasonic absorption per wavelength, $\alpha\lambda$, in the protein solution [72,78,98,99,108]:

$$k_{\mathrm{rel}} = -\beta_{S0}/(\pi\omega)\sum_{j,k}[\alpha\lambda]_{j,k}/\tau_{j,k} \quad (22)$$

where $[\alpha\lambda]_{j,k} = [(\alpha\lambda)_{j,k} - (\alpha\lambda)_0]/c$; $(\alpha\lambda)_{j,k}$ is the excess ultrasonic absorption per wavelength in the protein solution caused by the jth (subtransition between the native-like protein states) or kth (proton-transfer reaction) relaxation process; $(\alpha\lambda)_0$ is the ultrasonic absorption per wavelength in the neat solvent; and c is the specific protein concentration. Significantly, $[\alpha\lambda] = \Sigma_{j,k}[\alpha\lambda]_{j,k}$ is the net specific increment of ultrasonic absorption per wavelength which can be measured directly. Based on a careful analysis of ultrasonic data, Sarvazyan and Hemmes [98] have demonstrated that the relaxation contribution, k_{rel}, to the partial specific adiabatic compressibility, $k°_S$, of globular proteins does not exceed $\sim$10%. This contribution is well within experimental error of most $k°_S$ measurements. Therefore, as a first approximation, the relaxation term, k_{rel}, in Eq. 14 can be neglected, and the partial specific adiabatic compressibility, $k°_S$, of a globular protein can be viewed as the sum of the intrinsic, k_{SM}, and hydration, Δk_{Sh}, contributions.

Our ability to interpret protein compressibility data in terms of microscopic events ultimately depends on our ability to resolve $k°_S$ into its k_{SM} and Δk_{Sh} contributions. This separation is model-dependent and can be performed only based on some assumptions. Below, we discuss some current approaches to evaluating the intrinsic and hydration contributions to the partial specific adiabatic compressibility of a globular protein.

4.1. Intrinsic compressibility

The protein material is very tightly packed as can be judged by the value of the packing density of the protein interior [109,110]. From this point of view, the protein interior is solid-like with the mean packing density of $\sim$0.75 which is close to the maximum value for closely packed spheres (0.74) and corresponds to the upper limit of the packing densities of organic crystals [97,109,110]. The amount of the interior empty space is only on the order of 25% of the total protein volume although may vary somewhat from protein to protein [111]. As pointed out by Richards and Lim [110], the packing density is not uniform inside the protein. For example, packing of the backbone of the polypeptide chain is much denser than that of the side chains.

Analogous to packing density, the intrinsic compressibility of a globular protein falls within the range typical for organic crystals and characterizes the protein interior as a solid-like material [38,46,47,97,112–114]. Compressibility is not uniform inside the protein structure in which rigid (low compressibility) and soft (high compressibility) packing domains can be intermixed [115–122]. In particular, it has been suggested that helices and loops exhibit

larger microscopic compressibilities than β-strands [118]. There have been numerous attempts to evaluate the intrinsic compressibility of globular proteins [42,46,47,51,97,112,122–128]. Most of these evaluations yield the values of the coefficient of adiabatic (β_{SM}) or isothermal (β_{TM}) compressibility of the protein interior within the range of 10×10^{-6} to 30×10^{-6} bar^{-1}. In these estimates, the difference between β_{TM} and β_{SM} is thought to be smaller than the calculation uncertainties and, therefore, can be neglected. It is out of the scope of this paper to describe and/or critically analyse all methods and approaches that have been used to estimate the compressibility of the protein material. We believe that the most reliable estimate of the intrinsic compressibility of a globular protein reported to date is 25×10^{-6} bar^{-1}. This value was originally determined by Chalikian et al. [97] based on the first thermodynamic characterization of protein hydration and interior packing that does not depend on model compound data but rather is based exclusively on macroscopic (volumetric) and microscopic (X-ray) measurements on protein molecules themselves. Specifically, acoustic and densimetric techniques were used to measure $k°_S$ for 15 globular proteins over a temperature range of 18–55°C [97]. For the subset of the 12 proteins with known three-dimensional structures, the molecular volumes, V_M, and the solvent-accessible surfaces of the constituent charged, S_c, polar, S_p, and nonpolar, S_n, atomic groups were calculated. Assuming that the partial specific adiabatic compressibility, $k°_S$, of a protein is the sum of its intrinsic compressibility and hydration contributions of charged, polar, and nonpolar groups, one obtains the following linear relationship [97]:

$$k^\circ_S = (\beta_M V_M + B_c S_c + B_p S_p + B_n S_n)/M \qquad (23)$$

where B_c, B_p, and B_n are the unit compressibility contributions (per 1 Å^2) of the solvent accessible surfaces of charged, polar, and nonpolar atomic groups, respectively.

This regression analysis allows determination, as a function of temperature, of the intrinsic coefficient of adiabatic compressibility, β_M, and the average hydration contributions to $k°_S$ of 1 Å^2 of the charged, polar, and nonpolar solvent-accessible protein surfaces. The value of β_M was found to be $(25\pm1)\times10^{-6}$ bar^{-1} and practically independent of temperature between 18°C and 55°C [97].

Another way to estimate the intrinsic compressibility of globular proteins was proposed several years ago by Kharakoz and Sarvazyan [112]. It is based on an analysis of the correlation between the partial molar adiabatic compressibility, $K°_S$, and solvent accessible surface area, S_A, of a globular protein. Recall that $K°_S = K_M + \Delta K_{Sh} = \beta_M V_M + \gamma_N S_A$, where γ_N is the average unit contribution (per Å^2) of the surface of a native protein to its partial molar adiabatic compressibility. The partial specific adiabatic compressibility, $k°_S$, of a globular protein is then given by the relationship:

$$k^\circ_S = K^\circ_S/M = k_M + \Delta k_{Sh} = \beta_M v_M + \gamma_N S_A/M \qquad (24)$$

where $v_M = V_M/M$ is the specific intrinsic volume of a globular protein (in cm^3 g^{-1}).

Using this method and a spherical approximation of a globular protein (S_A was set to be proportional to $M^{2/3}$), Kharakoz and Sarvazyan [112] calculated the value of β_M equal to 14×10^{-6} bar^{-1} relative to our value of 25×10^{-6} bar^{-1}. However, as emphasized by Miller et al. [129], because of the roughness of the protein surface, the solvent accessible surface area, S_A, of a globular protein cannot be approximated as proportional to $M^{2/3}$. In fact, Miller et al. have shown that S_A is proportional to $M^{0.73}$ [129]. Consistent with this estimate, our own data (not shown) on the solvent accessible surface areas, S_A, for 20 monomeric globular proteins yield a slope of 0.76 ± 0.03 for the dependence of $\log(S_A)$ on $\log(M)$. Specifically, we found that S_A (Å^2) $= (4.7\pm0.2)$ $M^{0.76\pm0.03}$. Hence, based on Eq. 24, $k°_S$ should be proportional to $M^{-0.24}$ and not to $M^{-1/3}$ as proposed in [112]. Fig. 2 presents the values of $k°_S$ for native globular proteins obtained at 25°C as a function of $M^{-0.24}$. Note that the data presented in Fig. 2 can be approximated reasonably well by a straight line. The intercept of this line on the y-axis (at $M^{-0.24}=0$) yields the value of $\beta_M v_M$ (see Eq. 24) equal to 16×10^{-6} cm^3 g^{-1} bar^{-1}. The intrinsic volume, V_M (Å^3), of a globular protein is related to its molecular weight via V_M (Å^3) $= (1200\pm500)+(1.04\pm0.02)M$ [97]. Consequently, the specific intrinsic volume, v_M (cm^3 g^{-1}) $= N_A V_M/M$ (where N_A is Avogadro's num-

ber), of a globular protein is equal to $700/M+0.626$. For a very large protein, for which $M^{-0.24}$ is zero, v_M equals 0.626. Using this value, one estimates the intrinsic coefficient of adiabatic compressibility of a globular protein, β_M, of 25×10^{-6} bar^{-1} (16×10^{-6}/0.626). This value is significantly larger than the original value of 14×10^{-6} bar^{-1} calculated by Kharakoz and Sarvazyan [112] and coincides with our previous estimate of 25×10^{-6} bar^{-1} [97]. It should be noted however that, in his subsequent publications, Kharakoz emphasized that 25×10^{-6} bar^{-1} is the most reliable estimate of β_M [113,114].

Importantly, the value of 25×10^{-6} bar^{-1} is in good agreement with the pressure dependent X-ray crystallographic data presented by Katrusiak and Dauter [125], who determined the unit cell dimensions and the volume of hen egg-white lysozyme crystals (orthorhombic form) at pressures from 1 to 1000 bar. A nonlinear exponential approximation of the pressure dependence of the crystal volume yields at 1 bar a coefficient of isothermal compressibility, β_M, of 20×10^{-6} bar^{-1} [38].

In the aggregate, the most reliable estimate of the intrinsic coefficient of protein compressibility is 25×10^{-6} bar^{-1}. This value characterizes the protein interior as a rigid, tightly packed, solid-like substance. For comparison, the coefficients of adiabatic compressibility, β_S, of liquid water, benzene, and hexane are equal to 45×10^{-6}, 96×10^{-6} bar^{-1}, and 165×10^{-6} bar^{-1}, respectively. However, the protein interior is much more flexible (softer) than most of the crystalline solids: for example, β_S of ice is 13×10^{-6} bar^{-1}. In a way, this observation 'makes sense': certain level of dynamic flexibility of the polypeptide chain and amino acid residues is required for the protein molecule to 'breath' and perform its biological function [130].

4.2. Hydration contribution to compressibility

A major question related to protein solvation is whether low molecular weight model compounds can model the hydration properties of native globular proteins. Alternatively, this question can be reformulated as whether the hydration characteristics of atomic groups at the protein surface are identical to those of similar groups in small molecules. One way to answer this question is to compare the average compressibility contribution of 1 Å^2 of protein surface, γ_N, with that of small molecules. As is seen from Eq. 24, the value of γ_N can be obtained if the difference between $K°_S$ and $\beta_M V_M$ is plotted against the protein solvent accessible surface areas, S_A. Fig. 3 shows such plots for 12 globular proteins at 18, 25, 35, 45, and 55°C (the data are taken from our previous work [97]). The data presented in Fig. 3 have been approximated by straight lines with slopes equal to the average values of γ_N at specific temperatures studied.

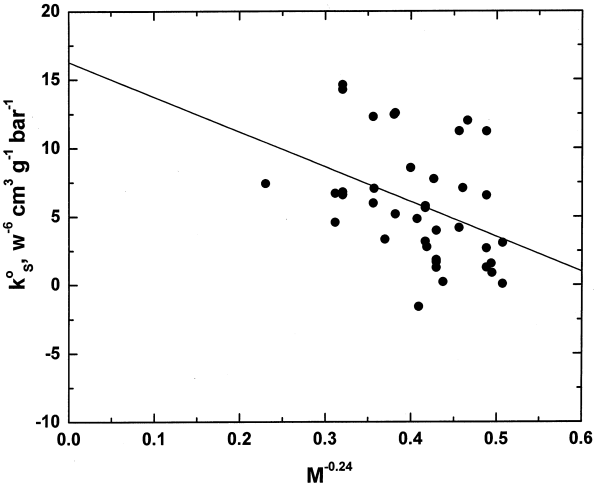

Fig. 2. The dependence of the partial specific adiabatic compressibility, $k°_S$, of globular proteins at 25°C [47,97] on $M^{-0.24}$ (where M is the molecular weight). The straight line represents the best-fit linear approximation of the data.

Fig. 4 shows the temperature dependence of γ_N between 18°C and 55°C. Inspection of Fig. 4 reveals that the average unit compressibility contribution of the protein surface, γ_N, is highly negative and slightly changes with temperature. The temperature dependence of γ_N can be approximated reasonably well by a second order polynomial. At 25°C, γ_N is equal to $(-23\pm3)\times10^{-6}$ cm^3 mol^{-1} bar^{-1} Å^{-2}. Note that this value is almost twice as small (more negative) as the average unit contribution of an unfolded polypeptide chain, γ_U, of $(-12\pm2)\times10^{-6}$ cm^3 mol^{-1} bar^{-1} Å^{-2} (see Section 3). For comparison, this value is even more negative than the unit compressibility contribution of glycine, the hydration shell of which is predominantly dictated by electrostatic solute–solvent interactions! At 25°C, the partial molar adiabatic compressibility, $K°_S$, of glycine is -26.6×10^{-4} cm^3 mol^{-1} bar^{-1} [71,77,79] while its sol-

vent accessible surface area, S_A, is 136 Å^2. Hence, the unit compressibility contribution for glycine is -20×10^{-6} cm^3 mol^{-1} bar^{-1} Å^{-2} (-26.6×10^{-4}/136).

The highly negative value of γ_N suggests that the native state of a globular protein is extensively hydrated with the pattern of hydration being distinct from that of low molecular weight model compounds. We propose that this distinction is related to the differential hydration of polar groups in proteins and small molecules. In fact, a polar group in a protein is characterized by a three times more negative unit compressibility contribution than a similar group in a small molecule [97]. This disparity may reflect the cooperative formation of networks of water molecules adjacent to the rigid matrix of closely located polar protein groups, with these networks involving waters from the second and third coordination spheres. Such cooperative networks have not been observed in small molecules.

Based on the foregoing discussion, the hydration properties of native globular proteins cannot be satisfactorily modeled based on additive calculations using small molecule data. In contrast to this notion, in two recent publications it has been suggested that

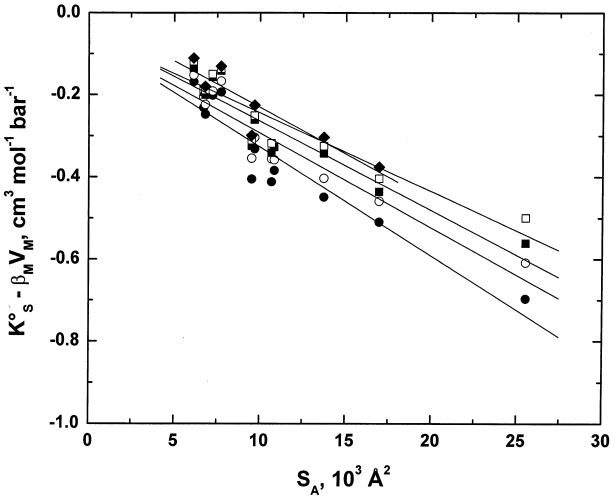

Fig. 3. The dependences of the difference ($K°_S-\beta_M V_M$) on the solvent accessible surface area, S_A, for globular proteins at 18°C (●), 25°C (○), 35°C (■), 45°C (□), and 55°C (♦). The data on the partial molar adiabatic compressibility, $K°_S$, intrinsic volume, V_M, and solvent accessible surface area, S_A, of globular proteins are from [97]. The value of the coefficient of intrinsic compressibility, β_M, of a globular protein is 25×10^{-6} bar^{-1}. The straight lines represent the best-fit linear approximations for each data set.

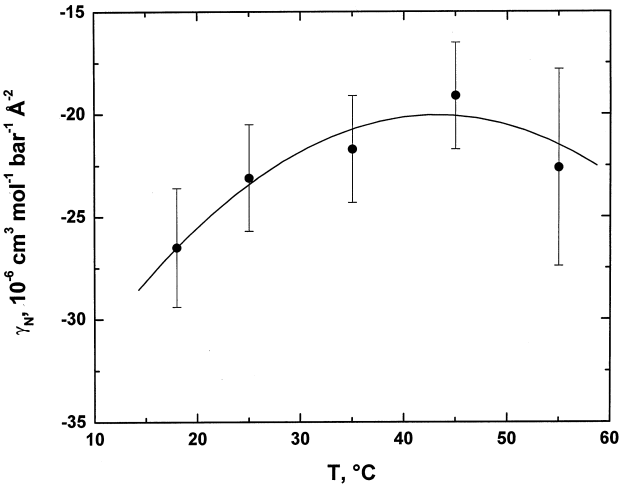

Fig. 4. The temperature dependence of the average unit compressibility contribution, γ_N, of the surface of a native globular protein.

hydration of protein groups is identical to that of similar groups in small molecules [94,112]. Based on this suggestion, additive calculations have been performed to determine the hydration contributions to compressibility, Δk_{Sh}, and intrinsic compressibilities, $k_M=\beta_M v_M$, for some globular proteins [112]. However, these calculations have produced significantly underestimated values of the intrinsic coefficient of protein compressibility, β_M, which undermines the validity of such additive analyses.

In the aggregate, the unit compressibility contribution of the surface of the native globular protein, γ_N, is 50% smaller (more negative) than the average unit contribution of an unfolded polypeptide chain, γ_U. At 25°C, γ_N is equal to $(-23\pm3)\times10^{-6}$ cm^3 mol^{-1} bar^{-1} Å^{-2}. This value is reflective of cooperative water networks adjacent to the polar protein surface and underscores the limitations of additive models of protein hydration based on small molecule data.

5. Compressibility changes associated with protein transitions: Theoretical considerations

5.1. The magnitude of compressibility changes

Some time ago, based on analysis of experimental data on compressibility changes associated with protein transitions, Δk_S, we proposed that a general relationship exists between the class of protein confor-

mational transitions and the sign and magnitude of Δk_S [40]. Specifically, we found that native-to-compact intermediate (such as native-to-molten globule) transitions are accompanied by small increases in $k°_S$ of $+(1–4)\times10^{-6}$ $cm^3\,mol^{-1}\,bar^{-1}$, native-to-partially unfolded transitions are accompanied by small decreases in $k°_S$ of $-(3–7)\times10^{-6}$ $cm^3\,mol^{-1}\,bar^{-1}$, and native-to-fully unfolded transitions are accompanied by large decreases in $k°_S$ of $-(18–20)\times10^{-6}$ $cm^3\,mol^{-1}\,bar^{-1}$ [40]. In other words, we proposed that for single-domain globular proteins, changes in $k°_S$ correlate with the type of transition being monitored, independent of the specific protein. To rationalize the observed correlation, we offered a qualitative explanation based on the interplay between the changes in intrinsic, k_M, and hydration, Δk_{Sh}, compressibilities associated with each transition type [40]. Below, we present a more quantitative analysis that shows that the observed correlation between Δk_S and the transition type can be obtained theoretically based on the interior packing, size of the solvent-inaccessible protein core, and solvent-accessible surface areas of each of the three protein states (R. Filfil, T.V. Chalikian, unpublished results).

From Eq. 24, the net change in compressibility, Δk_S, associated with a conformational transition of a globular protein can be presented as the sum of the changes in the intrinsic, Δk_M, and hydration, $\Delta\Delta k_{Sh}$, compressibilities:

$$\Delta k_S = \Delta k_M + \Delta\Delta k_h \tag{25}$$

where $\Delta k_M = k_{MD} - k_{MN}$; $k_{MN} = v_{MN}\beta_{MN}$ and $k_{MD} = v_{MD}\beta_{MD}$ are the intrinsic compressibilities of the native and denatured protein states, respectively; v_{MN} and v_{MD} are the intrinsic volumes of the native and denatured protein states, respectively; β_{MN} and β_{MD} are the intrinsic compressibilities of the native and denatured protein states, respectively; $\Delta\Delta k_{Sh} = \Delta k_{hD} - \Delta k_{hN}$; Δk_{hN} and Δk_{hD} are the hydration contributions to compressibility for the native and denatured protein states, respectively.

The denatured protein, while exposing to the solvent a substantial number of previously buried atomic groups, generally retains a water-inaccessible core of loosely packed amino acid residues. The water-inaccessible core may be sizeable, as in molten globules, or insignificant, as in random coil-like, fully unfolded polypeptide chains. Quantitatively, the water-inaccessible core can be described in terms of the degree of unfolding, σ, of a protein. The degree of unfolding, σ, can be defined as the ratio of the number of amino acid residues in the unfolded, random coil-like domains of the polypeptide chain to the total number of amino acids. Clearly, the value of σ equals zero for the native state and unity for the fully unfolded state.

Recall that the intrinsic volume, V_M ($Å^3$), of a native globular protein of a molecular weight, M, is equal to $1200+1.04M$ [97]. Consequently, the specific intrinsic volume, v_{MN} ($cm^3\ g^{-1}$), is equal to $(N_A/M)\,(1200+1.04M)$. Since, the molecular weight of the solvent-inaccessible core of the protein in its denatured state is equal to $M(1-\sigma)$, the value of v_{MD} is $(N_A/M)\,[1200+1.04M(1-\sigma)]$. As discussed above, the intrinsic coefficient of compressibility of the tightly packed, solid-like interior of the native protein state, β_{MN}, is 25×10^{-6} bar^{-1}. By contrast, the loosely packed solvent inaccessible core of a denatured protein is liquid-like. Its intrinsic compressibility should be close to that of organic liquids ($\sim100\times10^{-6}$ bar^{-1}). With this notion in mind, we assume that the intrinsic compressibility of the denatured state, β_{MD}, linearly changes with increasing σ from 25×10^{-6} bar^{-1} at $\sigma=0$ to 100×10^{-6} bar^{-1} at $\sigma=1$: $\beta_{MD} = 25\times10^{-6}+75\times10^{-6}\ \sigma$. Consequently, one obtains the following relationship for the denaturation-induced change in the intrinsic compressibility of a protein, Δk_M:

$$\Delta k_M = k_{MD} - k_{MN} = (N_A/M)\{[1200 + 1.04M(1-\sigma)]$$
$$(25\times10^{-6} + 75\times10^{-6}\sigma) - (1200 + 1.04M)25\times10^{-6}\} \tag{26}$$

Further, we assume that the hydration contributions to the partial compressibility of both the native, Δk_{hN}, and denatured, Δk_{hD}, protein states correlate to the solvent-exposed surface area, S_A. Recall that, for the native state, S_{AN} ($Å^2$) is equal to $4.7\ M^{0.76}$. Consequently, the hydration contribution to compressibility for the native state is given by the expression:

$$\Delta k_{hN} = M^{-1}S_{AN}\gamma_N = 4.7M^{-0.24}\gamma_N \tag{27}$$

where γ_N is equal to -23×10^{-6} $cm^3\,mol^{-1}\,bar^{-1}$ $Å^{-2}$.

For the denatured state, the total solvent accessi-

ble surface area, S_{AD}, can be broken down into two contributions. The first contribution comes from the unfolded part of the chain. The solvent accessible surface area (in Å^2) of an extended polypeptide chain is equal to 1.45 M [109]. Hence, the solvent-accessible area of the unfolded domain of the polypeptide chain can be set equal to 1.45 σM. The hydration contribution to compressibility of the unfolded domain is equal to 1.45 $\sigma M\gamma_U$ (where γ_U is equal to -12×10^{-6} cm^3 mol^{-1} bar^{-1} Å^{-2}). The second contribution to S_{AD} comes from the solvent accessible surface area of the compact domain of the polypeptide chain which can be set to be equal to 4.7 $[M(1-\sigma)]^{0.76}$. In this representation, we assume that the unit compressibility contribution of the compact domain linearly changes with increasing σ from γ_N at $\sigma=0$ to γ_U at $\sigma=1$. Consequently, for the denatured state, Δk_{hD} is given by the relationship:

$$\Delta k_{hD} = M^{-1}\{4.7[M(1-\sigma)]^{0.76}[\gamma_N + (\gamma_U-\gamma_N)\sigma]+ 1.45M\sigma\gamma_U\} = 4.7M^{-0.24}(1-\sigma)^{0.76}[\gamma_N + (\gamma_U-\gamma_N)\sigma] + 1.45\sigma\gamma_U \quad (28)$$

Combining Eqs. 27, 28, one derives the following relationship for the change in the hydration contribution to compressibility, $\Delta\Delta k_h$, associated with protein denaturation:

$$\Delta\Delta k_h = \Delta k_{hD}-\Delta k_{hN} = 4.7M^{-0.24}(1-\sigma)^{0.76}[\gamma_N + (\gamma_U-\gamma_N)\sigma] + 1.45\sigma\gamma_U-4.7M^{-0.24}\gamma_N \quad (29)$$

Finally, substituting Eqs. 26 and 29 into Eq. 25, one obtains the expression for calculating the change in adiabatic compressibility, Δk_S, associated with the protein transition. We used Eqs. 25, 26, 29 to calculate the change in compressibility, Δk_S, associated with protein denaturation as a function of the degree of unfolding, σ. Fig. 5 shows results of these calculations for proteins with molecular weights, M, of 10, 15, 20, 30, 50, and 80 kDa. Inspection of Fig. 5 reveals a number of significant observations.

Firstly, for any molecular weight, M, the σ-dependence of Δk_S is parabolic-like. At small values of σ (when σ is smaller than $0.5\div0.6$), Δk_S is positive while becoming negative at larger values of σ.

Secondly, recall that, in molten globules, 40–60% of previously solvent inaccessible protein groups become exposed to the solvent. This estimate was made by comparing the changes in hydrodynamic and partial specific volumes accompanying native-to-molten globule transitions as well as from differential scanning calorimetric measurements [131–133]. By extension, the value of σ for the molten globule state can be set to be $0.4\div0.6$. Hence, inspection of Fig. 5 reveals that the range of Δk_S values for native-to-molten globule transitions is -3×10^{-6} to 6×10^{-6} cm^3 g^{-1} bar^{-1}. These values are in excellent agreement with the experimentally observed range of Δk_S values for native-to-molten globule transitions of 1×10^{-6} to 4×10^{-6} cm^3 g^{-1} bar^{-1} [40]. Note that in this treatment, there is no need to assume that water penetrates the intrinsic core of the molten globule as proposed in ref. [134].

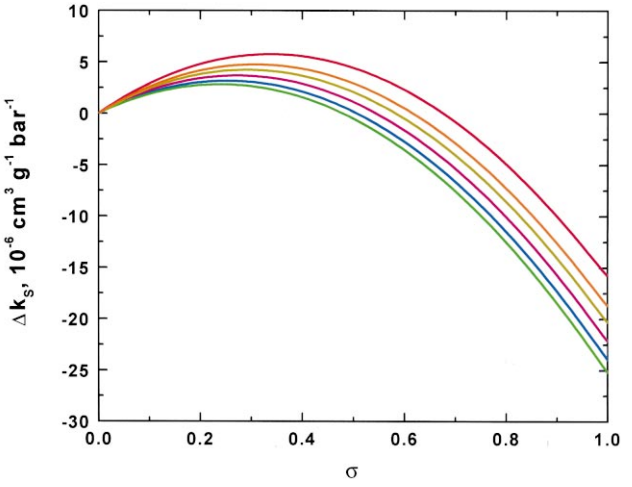

Fig. 5. The calculated dependences of the change in adiabatic compressibility, Δk_S, accompanying protein denaturation on the degree of unfolding, σ, for proteins with molecular weights, M, of 10 (red), 15 (orange), 20 (dark yellow), 30 (magenta), 50 (blue), and 80 kDa (green). Calculations have been performed using Eqs. 25, 26, 29.

Thirdly, the solvent accessible surface area of a protein in its partially unfolded state is about 70–80% of the value expected for the fully extended conformation [38,95,108,135–138]. Thus, the degree of unfolding, σ, for the partially unfolded state can be set to be $0.7\div0.8$. With this estimate, inspection of Fig. 5 reveals that, for native-to-partially unfolded transitions, the values of Δk_S are between -2×10^{-6} and -12×10^{-6} cm^3 g^{-1} bar^{-1}, in agreement with the experimentally observed range of -3×10^{-6} to -7×10^{-6} cm^3 g^{-1} bar^{-1} [40].

Fourthly, for native-to-fully unfolded transitions ($\sigma = 1$), the values of Δk_S are between -16×10^{-6} and -25×10^{-6} cm^3 g^{-1} bar^{-1}, in excellent agreement with experimental values of Δk_S on the order of -20×10^{-6} cm^3 g^{-1} bar^{-1} [40].

In the aggregate, the observed correlation between Δk_S and the transition type can be accounted for by simple theoretical calculations based on Eqs. 25, 26, 27, 28, 29. The excellent numerical agreement between our calculated values and the observed range of experimental values of Δk_S for each transition type lends credence to the assumptions and approximations employed in our analysis (including the specific choice of the values of the γ_N and γ_U).

5.2. Transition profiles

Recently, Rösgen and Hinz [139,140] presented a comprehensive theoretical analysis of the thermodynamics of conformational transitions of proteins. For the simplest case of a monomeric protein that may exist only in two conformational states (native, N, and denatured, D), the partial specific volume, $v°$, at any given solution condition can be presented as follows:

$$v^\circ = v_N + f_D \Delta v \tag{30}$$

where $\Delta v = v_D - v_N$; v_N and v_D are the partial specific volumes of the native and denatured states, respectively; $f_D = K/(1+K)$ is the fraction denatured; and $K = [D]/[N]$ is the equilibrium constant.

The partial specific isothermal compressibility, $k°_T$, of the protein can be found by differentiating Eq. 30 with respect to pressure:

$$k^\circ_T = -(\partial v^\circ/\partial P)_T = k_{TN} + f_D \Delta k_T - (\partial f_D/\partial P)_T \Delta v \tag{31}$$

where $k_{TN} = -(\partial v_N/\partial P)_T$ is the partial specific isothermal compressibility of the native state; $\Delta k_T = k_{TD} - k_{TN}$; $k_{TD} = -(\partial v_D/\partial P)_T$ is the partial specific isothermal compressibility of the denatured state.

It can be shown that the pressure slope, $(\partial f_D/\partial P)_T$, is given by the relationship:

$$(\partial f_D/\partial P)_T = -(\Delta v/RT) f_D (1 - f_D) \tag{32}$$

Substituting Eq. 32 into Eq. 31 and taking into account that $f_D = K/(1+K)$, one obtains the following relationship for $k°_T$:

$$k^\circ_T = -(\partial v^\circ/\partial P)_T = k_{TN} + [K/(1+K)]\Delta k_T + (\Delta v^2/RT)[K/(1+K)^2] \tag{33}$$

Using a similar line of reasoning, it can be shown that the partial specific adiabatic compressibility, $k°_S$, of a protein determined from ultrasonic velocity measurements is given by the equation:

$$k^\circ_S = k_{SN} + [K/(1+K)]\Delta k_S + [\Delta v(\Delta v - \alpha \Delta h/\rho c_P)/RT][K/(1+K)^2](1+\omega^2\tau^2)^{-1} \tag{34}$$

where $\Delta k_S = k_{SD} - k_{SN}$; k_{SN} and k_{SD} are the partial specific adiabatic compressibilities of the native and denatured states, respectively; Δh is the differential specific enthalpy (per gram of protein) of the denatured and native states; ω is the angular frequency of ultrasonic waves; and τ is the relaxation time of the native-to-denatured protein transition.

Since the coefficient of thermal expansibility, α, of an aqueous solution is small while its specific heat capacity, c_P, is large, Eq. 34 can be simplified to the form:

$$k^\circ_S = k_{SN} + [K/(1+K)]\Delta k_S + (\Delta v^2/RT)[K/(1+K)^2](1+\omega^2\tau^2)^{-1} \tag{35}$$

Inspection of Eq. 35 reveals that the transition-induced change in protein compressibility, $k°_S - k_{SN}$, consists of the two terms. The structural term, $[K/(1+K)]\Delta k_S$, is frequency-independent and reflects the transition-induced change in the equilibrium distribution between the native and denatured protein species. The relaxation term, $(\Delta v^2/RT)[K/(1+K)^2](1+\omega^2\tau^2)^{-1}$, is frequency-dependent and reflects the pressure-induced change in the distribution between the two species. It should be noted that, the structural term in Eq. 35 can be either positive or negative (depending on the sign of Δk_S), while the relaxation term is always positive. If the ultrasonic velocity measurements were performed at very low frequencies ($\omega \approx 0$), the relaxation term in Eq. 35 would exhibit its maximum value. An increase in frequency, however, will bring about a decrease in the relaxation term, which, at very high frequencies ($\omega\tau \gg 1$), subsides to zero.

Relaxation times, τ, for protein folding/unfolding transitions are, typically, between milliseconds and days. On the other hand, due to technical difficulties, frequencies of ultrasonic velocimetric measurements are usually limited to the range of 1 to 10 MHz (i.e., $\omega\tau \gg 1$). Consequently, as emphasized by Rösgen and Hinz [140], in protein compressibility studies performed by means of ultrasonic measurements, the relaxation term in Eq. 35 is negligible and cannot be used for gaining practical information about the kinetics of protein folding/unfolding transitions. However, should a high precision method for direct measurements of isothermal compressibility in protein solutions be developed, the relaxation term can be determined based on Eq. 33.

Fig. 6 presents our calculated high-frequency and low-frequency profiles for the heat-induced denaturation of a hypothetical protein which exhibits either a positive (Fig. 6A) or a negative (Fig. 6B) value of Δk_S. Inspection of Fig. 6 reveals that the high-frequency transition profiles have a classical sigmoidal shape expected for a cooperative, two-state transition. In contrast, the low-frequency profiles deviate from the sigmoidal shape with the deviation being proportional to Δv^2. Thus, in principle, the difference between the low-frequency and high-frequency profiles can be used to evaluate the transition volume. In addition, inspection of Eq. 35 reveals that frequency-dependent studies may yield kinetic information about the relaxation time of the transition, τ. However, such low-frequency ultrasonic studies are not yet possible due to unresolved technical difficulties. Therefore, all reported ultrasonically detected transition profiles for temperature-, pH-, denaturant-, salt-, and cosolvent-induced conformational changes of proteins are of the high frequency, sigmoidal type.

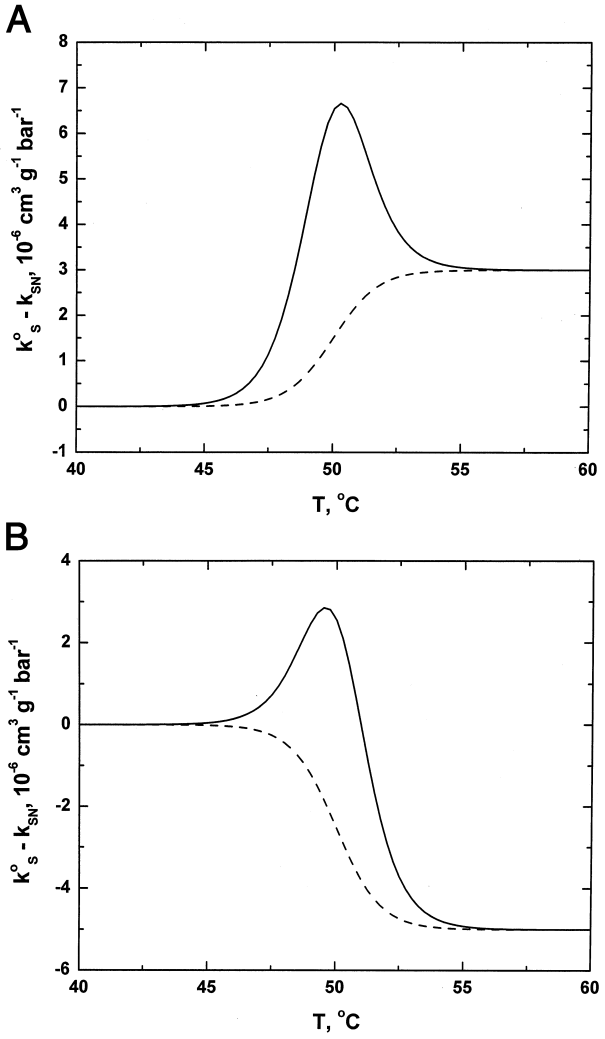

Fig. 6. The temperature dependence of the partial specific adiabatic compressibility, k°_S, of a hypothetical globular protein as calculated from Eq. 35: (a) $\Delta k_S = 3\times10^{-6}$ cm^3 g^{-1} bar^{-1}; (b) $\Delta k_S = -5\times10^{-6}$ cm^3 g^{-1} bar^{-1}. The transition volume, Δv, is 0.005 cm^3 g^{-1}. The equilibrium constant, K, is given by $(T/T_M)^{\Delta C_P/R}\exp[(T_M\Delta C_P - \Delta H^\circ)(T^{-1}-T_M^{-1})/R]$, where the denaturation temperature, T_M, is 50°C; the heat capacity change, ΔC_P, associated with protein denaturation is 1 kcal mol^{-1} K^{-1}; and the transition enthalpy, ΔH, is 100 kcal mol^{-1}. The solid and dashed lines represent the calculations performed for low ($\omega\tau \approx 0$) and high ($\omega\tau \approx 1$) frequencies of ultrasonic waves, respectively.

6. Compressibility changes associated with protein transitions: Experimental observations

Proteins may exist in their native, molten globule (compact intermediate), partially unfolded, or fully unfolded states. Transitions between these states can be induced by altering temperature, pressure, pH, salt, denaturant, and cosolvent concentration. Compressibility measurements have been applied to characterizing transition profiles, although such measurements are still relatively scarce. For the simplest case of two-state, sigmoidal transitions (with the exception of pH-induced transitions), the change in compressibility can be determined by extrapolating the 'native' and 'denatured' baselines to the transition midpoint and calculating the difference.

For a pH-induced transition, the situation is more complex since an alteration in the solution pH results in a change in the state of ionization of protein titrable groups with a concomitant change in hydration. By itself, a change in the state of ionization of titrable groups may or may not result in a global change in protein conformation. However, independent of the conformational state of the protein, the change in protein hydration due to protonation/deprotonation of ionizable groups will bring about a change in compressibility. The overall pH-induced change in protein compressibility can be presented as the sum of the three terms:

$$\Delta k_S(\mathrm{pH}) = \Delta k_{ion} + \Delta k_{conf} + \Delta k_{rel} \quad (36)$$

where the relaxation term, Δk_{rel}, is mostly related to proton-transfer reactions as discussed above in Section 4.

The ionization term, Δk_{ion}, in Eq. 36 is due to the change in hydration of ionizable groups upon their protonation/deprotonation. For protonation reactions, which predominantly occur at acidic pH, Δk_{ion} is given by the expression:

$$\Delta k_{ion} = M^{-1}\sum_i \Delta K_{si}(1 + 10^{\mathrm{pH}-\mathrm{p}Ka i})^{-1} \quad (37a)$$

For deprotonation reactions, which predominantly occur at alkaline pH, Δk_{ion} is given by the expression:

$$\Delta k_{ion} = M^{-1}\sum_i \Delta K_{Si}(1 + 10^{\mathrm{p}Ka i-\mathrm{pH}})^{-1} \quad (37b)$$

where pK_{ai} is the dissociation constant of the ith titrable group; ΔK_{Si} is the molar change in adiabatic compressibility upon complete protonation/deprotonation of the ith group; and M is the molecular weight of the protein.

Assuming a two-state transition for protein unfolding, the pH-dependence of the structural term, Δk_{conf}, in Eq. 36 can be expressed by a sigmoidal function:

$$\Delta k_{conf} = \Delta k_S K/(1 + K) \quad (38)$$

where K is the equilibrium constant that can be calculated if the pK_a values of all ionizable groups are known for the native and denatured protein states [24,25]; Δk_S is the pH-independent 'conformational' change in adiabatic compressibility equal to the difference in the partial specific adiabatic compressibility, $k°_S$, between the denatured and native protein states determined at the same pH.

6.1. Atmospheric pressure experiments

6.1.1. Native-to-molten globule transitions

Table 1 presents compilation of literature data on changes in adiabatic compressibility, Δk_S, accompanying native-to-molten globule (compact intermediate) transitions of globular proteins. There are only five proteins, for which the changes in compressibility upon the native-to-molten globule (N-to-MG) transition have been determined. Specifically, the following transitions have been studied: the acid-induced N-to-MG transitions of cytochrome *c* [54,95], human [134] and bovine [141] α-lactalbumin; the sorbitol-induced N-to-MG transition of cytochrome *c* [142]; the heat-induced N-to-MG transitions of ribonuclease A [143] and α-chymotrypsinogen A [136]; and the base-induced N-to-MG of bovine α-lactalbumin [141].

Table 1
Changes in adiabatic compressibility associated with native-to-molten globule transitions of globular proteins

Protein/transition	Δk_S, 10^{-6} cm^3 g^{-1} bar^{-1}	Ref.
Cytochrome *c* (acid-induced in 200 mM NaCl at 25°C)	0.3[a]	[54]
Cytochrome *c* (acid-induced in 200 mM CsCl at 25°C)	1.7[a]	[95]
Human α-lactalbumin (acid-induced in 50 mM KCl at 25°C)	1.8[a]	[134]
Bovine α-lactalbumin (acid-induced in 2 mM $CaCl_2$ at 25°C)	−0.6[a]	[141]
Bovine α-lactalbumin (base-induced in 2 mM $CaCl_2$ at 25°C)	1.6[a]	[141]
Cytochrome *c* (sorbitol-induced in water at 20°C)	11.2	[142]
Ribonuclease A (heat-induced in ~10 mM HCl at pH 1.9; T_M = 26°C)	4.0	[143]
α-Chymotrypsinogen A (heat-induced in 10 mM glycine buffer at pH 2.0; T_M = 41°C)	3.6	[136]

[a]Ionization effects have been taken into account according to Eqs. 36, 37a, 37b, 38.

Table 2
Changes in adiabatic compressibility associated with native-to-partially unfolded transitions of globular proteins

Protein/transition	Δk_S, 10^{-6} cm^3 g^{-1} bar^{-1}	Ref.
Cytochrome *c* (acid-induced in water at 25°C)	−3.8[a]	[95]
Cytochrome *c* (base-induced in water at 25°C)	−3.9[a]	[108]
Staphylococcal nuclease (acid-induced in 20 mM NaCl at 25°C)	−6.3[a]	[137]
β-Lactoglobulin (base-induced in 10 mM NaCl at 25°C)	−7.0[a]	[144]
Myoglobin (acid-induced in 100 mM acetic buffer at 20°C)	−4.8	[145]
Equinatoxin II (acid-induced in water at 25°C)	−9.5[a]	[138]

[a]Ionization effects have been taken into account according to Eqs. 36, 37a, 37b, 38.

Inspection of the data presented in Table 1 reveals that compressibility changes associated with N-to-MG transitions of globular proteins are generally positive (or slightly negative) ranging from -0.6×10^{-6} to 11.2×10^{-6} cm^3 g^{-1} bar^{-1}. It should be noted that that the outstanding value of 11.2×10^{-6} cm^3 g^{-1} bar^{-1} obtained for the sorbitol-induced N-to-MG transition of cytochrome *c* [142] may be an overestimate, since this value was determined by a linear extrapolation to zero of very 'noisy' compressibility data obtained at high concentrations of sorbitol. As discussed above (see Section 5.1), experimental values of Δk_S accompanying N-to-MG transitions of globular proteins can be accounted for by a relatively simple analysis which takes into account transition-induced changes in the intrinsic and hydration contributions to protein compressibility. The mostly positive values of Δk_S for N-to-MG transitions reflect the increase in the intrinsic compressibility, k_M, which prevails over the decrease in the hydration compressibility, Δk_h.

6.1.2. Native-to-partially unfolded transitions

Table 2 presents compilation of literature data on changes in adiabatic compressibility, Δk_S, accompanying native-to-partially unfolded (N-to-PU) transitions of globular proteins. Specifically, Table 2 presents the data for the base-induced N-to-PU transition of cytochrome *c* [108] and β-lactoglobulin [144] and the acid-induced N-to-PU transitions of cytochrome *c* [95], staphylococcal nuclease [137], equinatoxin II [138], and myoglobin [145].

Inspection of the data presented in Table 2 reveals that the compressibility changes associated with N-to-PU transitions of globular proteins are all negative ranging from -3.8×10^{-6} to -9.5×10^{-6} cm^3 g^{-1} bar^{-1}. As discussed above (see Section 5.1), the observed values of Δk_S for N-to-PU transitions of globular proteins can be accounted for by decreases in the intrinsic and hydration contributions to protein compressibility.

6.1.2. Native-to-fully unfolded transitions

Table 3 presents the data on changes in adiabatic compressibility, Δk_S, accompanying the GuHCl-induced native-to-fully unfolded (N-to-FU) transitions of ribonuclease A [143] and lysozyme [146]. These changes are equal to -11×10^{-6} to -18×10^{-6} cm^3 g^{-1} bar^{-1}, respectively. As discussed above (see Section 5.1) such large decreases in compressibility are anticipated for N-to-FU transitions of globular proteins when the intrinsic compressibility diminishes practically to zero and the majority of protein groups becomes exposed to solvent.

6.2. Pressure-induced transitions

Table 4 presents compilation of literature data on changes in isothermal compressibility, Δk_T, accompanying pressure-induced denaturation of chymotrypsi-

Table 3
Changes in adiabatic compressibility associated with native-to-fully unfolded transitions of globular proteins

Protein/transition	Δk_S, 10^{-6} cm^3 g^{-1} bar^{-1}	Ref.
Ribonuclease A (GuHCl-induced at pH 2.0 and 15°C)	−18	[143]
Lysozyme (GuHCl-induced at pH 4.0 and 25°C)	−11	[146]

Table 4
Changes in isothermal compressibility associated with pressure-induced denaturation of globular proteins

Protein/transition	Δk_T, 10^{-6} cm^3 g^{-1} bar^{-1}	Ref.
Ribonuclease A (pressure-induced at pH 2.0–4.0 and temperatures of 20–50°C)	0.7–1.5	[45]
Chymotrypsinogen (pressure-induced at pH 2.07 and 0°C)	1.2	[44]
Metmyoglobin (pressure-induced at pH 4.0–6.0 and 20°C)	−0.2	[147]
Ribonuclease A (pressure-induced at pH 2.0 and 22°C)	1.1	[66]
Staphylococcal nuclease (pressure-induced at pH 7.0 and 45°C)	2.4	[67]
Staphylococcal nuclease (pressure-induced at pH 5.3 and 24°C)	0.9	[148]

nogen [44], ribonuclease A [45,66], metmyoglobin [147], and staphylococcal nuclease [67,148]. Inspection of the data presented in Table 4 reveals that pressure-induced conformational transitions of globular proteins are generally characterized by positive values of Δk_S. In other words, the partial compressibility of the pressure-induced denatured state of a protein is generally larger than that of the native state. It is difficult to unequivocally rationalize this observation. Four explanations can be hypothesized. Firstly, in all of these studies (except [67]), the values of Δk_T have been calculated based on a two-state analysis of the denaturation profiles monitored by spectroscopic observables. Hence, should the transitions deviate from the two-state behavior (in other words, if intermediates are accumulated at some stage of the transition), calculations based on a two-state analysis may yield erroneous results.

Secondly, the pressure-induced denatured state of a globular protein may retain a sizeable core of loosely packed, water-inaccessible amino acid residues. In this respect, the pressure-induced denatured state of a protein may resemble a molten globule thereby exhibiting an enhanced compressibility.

Thirdly, as proposed by Kharakoz [94], the partial compressibility of the unfolded state may increase with increasing pressure more steeply than that of the native state. Consequently, at some elevated pressure, the compressibility difference between the denatured and native states will become positive even if the initial difference at atmospheric pressure was negative. In this scenario, the pressure-induced denatured state of a protein may be either unfolded or molten globule-like, in both cases exhibiting a partial compressibility greater than that of the native state.

Finally, it would not be unexpected if, in future studies (especially when Δk_T is measured directly), some proteins will be found to exhibit negative values of Δk_T for their pressure-induced conformational transitions. Clearly, further pressure-related studies on a larger set of globular proteins are required to determine the validity of each of the above outlined possibilities/explanations.

7. Concluding remarks

Recent advances in the development of highly sensitive, small volume densimetric, acoustic, and high-pressure spectroscopic instrumentation have facilitated compressibility studies on protein systems. This experimental capability is significant since compressibility data are uniquely sensitive to the hydration properties and intrinsic packing of proteins, while providing complementary information to other techniques for defining the nature of the forces that control stability and conformational transitions of proteins. In this article, we have reviewed recent progress in volumetric investigations of conformational transitions of proteins, with particular attention given to the partial compressibility of the native and denatured protein states.

Despite the limited number of compressibility measurements that have been conducted on proteins and their low molecular weight analogs, it already is clear that this characteristics is exquisitely sensitive to intrinsic packing and hydration properties. This sensitivity is particularly important considering the role of hydration and intraglobular interactions in stabilizing protein structure and in modulating protein recognition events. We believe that compressibility measurements represent a relatively untapped yet powerful means of probing and characterizing all interactions stabilizing/destabilizing the native and denatured protein states, particularly, as they relate to the problem of protein folding.

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