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Thermodynamic properties of peptide solutions 20. Partial molar volumes and isothermal compressions for some tripeptides of sequence gly-X-gly (X = gly, ala, leu, asn, thr, and tyr) in aqueous solution at T = 298.15 K and p = (10–120) MPa

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ABSTRACT

Sound speeds have been measured for aqueous solutions of six tripeptides of sequence glycyl-X-glycine, where X is one of the amino acids glycine, alanine, leucine, asparagine, threonine, and tyrosine at T = 298.15 K and at the pressures p = (10, 20, 40, 60, 80, 100, and 120) MPa. Using methods described in previous work, these sound speeds were used to derive the partial molar volumes at infinite dilution, V_2^o, the partial molar isentropic compressions at infinite dilution, $K_{S,2}^o$, and the partial molar isothermal compressions at infinite dilution, $K_{T,2}^o$ {$K_{T,2}^o = -(\partial V_2^o/\partial p)_T$}, for the tripeptides in aqueous solution at the elevated pressures. The results were used to calculate the partial molar volumes and partial molar isothermal compressions for the various amino acid side-chains over the pressure range p = (10–120) MPa.

1. Introduction

A complete understanding of the physical stability of a protein in aqueous solution requires knowledge of its dependence on both the fundamental thermodynamic variables of temperature and pressure. Although the perturbation by temperature of a protein's native state to give the unfolded or denatured state has been widely studied [1–4], pressure perturbation of the two-state process of protein unfolding has, until recently, received far less attention. This has been due, in part, to the difficulties associated with conducting experimental work at high pressures [5,6]. However, technical advances have now enabled many standard experimental methods to be adapted for use at high pressures [7–11] and, as such, there has been a growing interest in the study of proteins at high pressures [5,10,12–17].

As a consequence of the thermodynamic relation $(\partial G/\partial p)_T = V$, the change in the partial molar volume of a protein upon unfolding, ΔV_2, is paramount in the quantitative assessment of pressure-induced protein unfolding. Moreover, since ΔV_2 is itself pressure dependent, it is also necessary to determine the difference between the partial molar isothermal compressions, $K_{T,2}$ {$K_{T,2} = -(\partial V_2/\partial p)_T$}, of the native and unfolded states of a protein. Thus, a thorough quantitative analysis of the effect of pressure on proteins in aqueous solution requires methods to obtain over a wide pressure range these volumetric properties for both native and unfolded proteins. Several years ago, we reported [18] a procedure that enables the partial molar volume at infinite dilution, V_2^o, the partial molar isentropic compression at infinite dilution, $K_{S,2}^o$, and the partial molar isothermal compression at infinite dilution, $K_{T,2}^o$ {$K_{T,2}^o = -(\partial V_2^o/\partial p)_T$}, for a solute in aqueous solution to be determined from speed of sound measurements as a function of pressure.

Previous papers of this series [19–22] report volumetric properties for tripeptides of sequence glycyl-X-glycine (gly-X-gly), where X represents one of the 20 amino acids, in aqueous solution at ambient pressure. These small peptides are of interest primarily because they are ideal compounds with which to model the amino acid side-chains of proteins. The single side-chain of the amino acid X in each tripeptide is flanked by two peptide groups, which is structurally identical to that found in proteins. The partial molar volumes for the various amino acid side-chains, which can be obtained from V_2^o data for these tripeptides [22–24], are useful in group additivity schemes to estimate the partial molar volume of

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the completely unfolded random-coil form of a protein's unfolded state [25].

The partial molar compression of a globular protein in aqueous solution is essentially comprised of two terms [26–28]. The first is the so-called intrinsic compression of the protein, which is due to intra-chain interactions and packing effects. The second term arises from the hydration of the various amino acid functional groups and the peptide backbone that are exposed to the solvent. Since globular proteins are large complex molecules, a better understanding of the hydration component of protein compression can be gleaned from the study of model compounds, such as the gly-X-gly tripeptides.

Herein, we report the results of speed of sound measurements at $T = 298.15$ K and at the pressures $p = (10, 20, 40, 60, 80, 100,$ and $120)$ MPa for aqueous solutions of the tripeptides glycylglycylglycine (glyglygly), glycylalanylglycine (glyalagly), glycylleucylglycine (glyleugly), glycylasparagylglycine (glyasngly), glycylthreonylglycine (glythrgly) and glycyltyrosylglycine (glytyrgly). The volumetric properties V_2^o, and $K_{T,2}^o$ for these tripeptides, that were derived from the sound speeds, were used to calculate the contributions made to the thermodynamic properties by the various amino acid side-chains.

2. Experimental

General information about the tripeptides used in this work is given in Table 1. The samples of glyglygly, glyalagly, glytyrgly, and the peptide monohydrate glythrgly·H_2O used were purified solids remaining from previous studies [23,29,30]. The sample of glyasngly was material that had been recovered from solutions used in a previous study [31], recrystallized from (water + ethanol), and dried under vacuum at room temperature. Since this peptide had been well characterized in earlier work [22], solution density was used as a criterion of purity. The density at $T = 298.15$ K for an aqueous solution of glyasngly was in agreement, within the combined experimental uncertainties, with that calculated using previous density data [22]. A new sample of glyleugly, purchased from Bachem Feinchemikalien, was recrystallized from (water + ethanol) to give a hemihydrate, as confirmed by alkalimetric titration [32,33]. A hemihydrate was also found for our synthesized glyleugly sample used in earlier studies [24]. All the anhydrous peptides were dried exhaustively under vacuum at room temperature before use. The water used to prepare solutions and as the reference solvent was purified by reverse osmosis and deionization using an Ondeo Purite Select water purification system, and was thoroughly degassed immediately prior to use. All solutions were prepared by mass using a Mettler Toledo AX205 analytical balance (readability 0.01 mg), and corrections were made for the effect of air buoyancy. The standard uncertainties for the solution molalities were typically $\pm 1 \times 10^{-5}$ mol·kg^{-1}.

The instrument used for the sound speed measurements at high pressures was designed and constructed at the University of Bergen. It uses pulsed sound methodology with the sound generated and received by a 10 MHz piezoceramic transducer. A 10 MHz rubium oscillator serves as a time scale to which major time operations are synchronized. The apparatus and operational procedures used have been described in detail in previous work [18]. The operating temperature $\{T = (298.15 \pm 0.01)$ K$\}$ was stable to ±0.001 K, and the pressure, which has a standard uncertainty of ±0.2 MPa, was adjustable to within ±0.15 MPa of any nominal value [18]. The estimated uncertainty for a measured sound speed was ±0.03 m·s^{-1}.

3. Results

3.1. Thermodynamic formalism

Although the background thermodynamic derivation of the relationship for the calculation of solution densities at high pressures has been given in our previous papers [18,34,35], it is, nevertheless, useful to summarize the derivation herein. The difference between the isothermal compressibility, κ_T $\{\kappa_T = -(\partial V/\partial p)_T/V\}$, and the isentropic compressibility, κ_S $\{\kappa_S = -(\partial V/\partial p)_S/V\}$, which is usually represented by the symbol δ [36,37], can be written in the form [36–38]

$$\delta = \kappa_T - \kappa_S = (T \cdot \alpha^2 \cdot V)/C_p = (T \cdot \alpha^2)/\sigma, \quad (1)$$

where C_p is the isobaric heat capacity, σ is the heat capacity per unit volume, and α is the isobaric expansibility [38], which is defined by the equation

$$\alpha = (\partial V/\partial T)_p/V. \quad (2)$$

Since the difference between C_p and the isochoric heat capacity, C_v, is given by [38]

$$C_p - C_v = (T \cdot \alpha^2 \cdot V)/\kappa_T, \quad (3)$$

it follows from Eqs. (1) and (3) that the ratio of C_p to C_v, which is often expressed using the symbol γ, is given by

$$\gamma = C_p/C_v = \kappa_T/\kappa_S. \quad (4)$$

The isentropic compressibilities of fluids are conveniently evaluated from speed of sound measurements. In the absence of sound dispersion, the isentropic compressibility is related to the speed of sound, u, by the Newton–Laplace equation [39]

$$\kappa_S = 1/(u^2 \cdot \rho), \quad (5)$$

where ρ is the density of the fluid. Combining the isothermal compressibility recast in terms of density, $\{\kappa_T = (\partial\rho/\partial p)_T/\rho\}$, with Eqs. (4) and (5) leads to the equation

Table 1
Source, purification method, and mass fraction purity of each tripeptide.

Chemical name	Source	Purification method	Mass fraction purity	Method of analysis
Glycylglycylglycine	Sigma	Recrystallization	⩾0.99	CHN[a], titrimetry[b]
Glycyl-L-alanylglycine	Synthesized[c]	Recrystallization	⩾0.99	CHN, titrimetry
Glycyl-L-leucylglycine	Bachem	Recrystallization	⩾0.99	Titrimetry
Glycyl-L-asparagylglycine	Synthesized[d]	Recrystallization	⩾0.99	CHN, titrimetry
Glycyl-DL-threonylglycine	Synthesized[d]	Recrystallization	⩾0.99	CHN, titrimetry
Glycyl-L-tyrosylglycine	Sigma[e]	Recrystallization	⩾0.99	CHN, titrimetry

[a] Elemental analyses for C, H, and N.
[b] Details of peptide analysis by alkalimetric titration are given in Refs. [32,33].
[c] The procedure used for the synthesis is described in Ref. [43].
[d] The procedure used for the synthesis is described in Ref. [22].
[e] This peptide was originally obtained in 1997 as a customer accommodation through Sigma. See: Ref. [23].