

Amino Acids under Hydrothermal Conditions: Apparent Molar Volumes of Aqueous α -Alanine, β -Alanine, and Proline at Temperatures from 298 to 523 K and Pressures up to 20.0 MPa

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The apparent molar volumes V_ϕ of aqueous α -alanine, β -alanine, and proline have been determined with platinum vibrating tube densitometers at temperatures from 298 to 523 K and at pressures in excess of steam saturation. Values of the standard partial molar volumes V° for the aqueous amino acids increase with temperature then deviate toward negative values at temperatures above 398 K, consistent with a lowering of the critical temperature in the solutions relative to water. This is opposite to the behavior predicted by the correlations developed by Shock and Helgeson (*Geochim. Cosmochim. Acta.* **1990** 54, 915–945) and Amend and Helgeson (*J. Chem. Soc., Faraday Trans.* **1997** 93, 1927–1941). The contribution to V° from the solvent polarization by the large dipole moment of the zwitterions deviates toward negative infinity as T_c is approached, in a manner similar to the V° values for each of the aqueous amino acids. While this agreement is qualitatively consistent, it is not quantitatively consistent, which suggests that either the nonelectrostatic hydration effects are of similar magnitude to the solvent polarization effects or that the equilibrium is significantly shifted toward the nonzwitterionic species at 523 K. Preliminary calculations suggest that the former is the case.

1. Introduction

The properties of amino acids in hydrothermal solutions are of intense interest to biochemists and geochemists interested in understanding the metabolic processes in thermophilic bacteria and the possible mechanisms for the origin of life at deep ocean vents.

Spies et al.¹ divide deep ocean vents into two broad classes: Galápagos-type vents and sulfide-mound hot-water vents. The Galápagos-type vents are the most common. These are characterized by 293 K vent water in 275 K surroundings. The fauna associated with the Galápagos-type vents include crabs, white clams, and giant tube worms and is their most visible feature. Sulfide-mound hot-water vents are characterized by 653 ± 30 K vent water in 275 K surroundings. The vent water is ejected through a limited number of chimneys or stacks with a relatively high velocity either as a clear fluid or as plume of white or black suspended particles. The discovery of these high-temperature vents marked the first time that liquid water at temperatures well in excess of 373 K had been found in open contact with the biosphere. Since the high pressures which permit these superheated conditions counteract some of the destructive effects of high temperature on biochemical systems, it is reasonable to wonder whether thermophilic organisms exist in high-temperature vents. The extreme environment resembles the conditions thought to have existed on primitive earth, and it has been postulated that hydrothermal vents were the source of life on earth. Bada et al.,² Baross and Deming,³ Crabtree,⁴ Miller and Bada,⁵ Ranganayaki et al.,⁶ Shock,^{7,8} Trent et al.,⁹ and Yanagawa and Kojima¹⁰ have considered various aspects of this hypothesis. However, all of these studies lack reliable, experimentally determined thermodynamic data for biomolecules, including amino acids, under hydrothermal conditions.

Amino acids exist in solution primarily as zwitterions. As such, their properties are intermediate between ions, which carry an overall nonzero charge, and neutral organic species, which have little or no localization of charge. The original versions of the Helgeson Kirkham Flowers (HKF) model^{11–13} for the standard partial molar properties of aqueous ionic species have been widely used to predict the thermodynamic properties of electrolytes at elevated temperatures and pressures. The HKF model was extended by Shock, Helgeson and their co-workers,^{7,8,14–16} in a purely empirical fashion, to predict the thermodynamic properties of aqueous organic species. Although this extended model includes predictions for amino acids, the predictive correlations are based on the very limited high-temperature data available in 1988. Moreover, the failure of these authors to consider the zwitterionic nature of the aqueous amino acids suggests that the extrapolated results are very uncertain at high temperatures. Except for the very recent measurements of V_ϕ for glycine by Hakin et al.,¹⁷ there are no experimental values for the thermodynamic properties of aqueous amino acids above 348 K in the literature. The lack of reliable, experimentally determined thermodynamic data for amino acids under hydrothermal conditions has allowed these predictions to remain untested.

Only a few studies to investigate the hydrothermal stability of amino acids have been reported. Abelson,¹⁸ Bada and Miller,¹⁹ Povoledo and Vallentyne,²⁰ Vallentyne,^{21,22} and White²³ have measured approximate Arrhenius rate constants for the first-order decomposition of several dilute aqueous amino acids at the temperatures and pressures used in this work (Table S1). Only five have sufficiently long half-lives to be suitable for measurements by flow densitometry at high temperatures. These are α -alanine, phenylalanine, glutamic acid, proline, and glycine.

The solubilities of the five candidate amino acids have been tabulated by Greenstein and Winitz.²⁴ Apparent molar volume

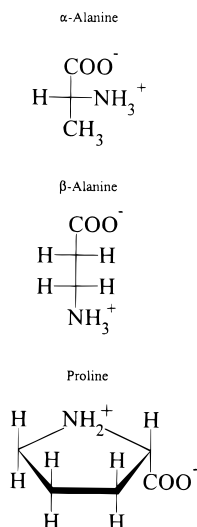


Figure 1. Structure of α -alanine, β -alanine, and proline.

measurements require molalities of at least $0.3 \text{ mol} \cdot \text{kg}^{-1}$ and, ideally, the solubility should not decrease with temperature to avoid the risk of precipitation in the densitometer. Phenylalanine and glutamic acid are insufficiently soluble in water to be of use, leaving α -alanine, proline, and glycine as the “best” model systems for studying the properties of amino acids under hydrothermal conditions. A series of batch experiments in small pressure vessels, described below in section 2.2, confirmed that α -alanine and proline are stable on the time scale required for our measurements at the temperatures, pressures, and molalities used in this work. Glycine was found to be significantly less stable. The structures of α -alanine and proline are illustrated in Figure 1, along with β -alanine which was included in this study for comparative purposes.

In this paper, we report the apparent molar volumes of α -alanine, β -alanine, and proline from 298 to 523 K at 0.1, 10.0, and 20.0 MPa obtained using a platinum/iridium vibrating tube densitometer recently reported by Xiao et al.²⁵ and Xiao and Tremaine.²⁶ The results are interpreted by a simple continuum model for dipole solvation.

2. Experimental Section

2.1 Materials. DL- α -Alanine was obtained from BDH (Assay 98.5% to 100.5%) and β -alanine was obtained from Aldrich (Assay 99+%). Before use, each alanine compound was recrystallized according to the method of Perrin and Armarego.²⁷ Briefly, α - and β -alanine were dissolved in a minimum amount of hot water ($T = 343 \text{ K}$). The resulting solutions were filtered while hot to remove any insoluble material. Hot ethanol ($T = 333 \text{ K}$) was then added slowly to the hot filtrates until 60/40 and 70/30 ethanol/water ratios were obtained for α -alanine and β -alanine, respectively. The resulting white precipitates were allowed to digest for 5 h, at which time they were collected and washed with cold water followed by cold ethanol. Each of the purified alanines was dried separately under vacuum, over Drierite, for 120 h at room temperature.

L-Proline was obtained from Aldrich (Assay 99+%). Before use, an attempt was made to recrystallize the proline according to the method of Perrin and Armarego.²⁷ This method failed to precipitate any purified proline and hence the following modified method was employed. The proline was dissolved in a minimum amount of hot anhydrous ethanol ($T = 333 \text{ K}$). The resulting solution was filtered while hot to remove any insoluble material. Warm, anhydrous diethyl ether was then added slowly to the

hot filtrate until the first permanent cloudiness was obtained. The resulting precipitate was allowed to digest for 5 h, at which time the white solid was collected and washed with cold ethanol followed by cold diethyl ether. The purified proline was dried under vacuum, over Drierite, for 120 h at room temperature.

NaCl (Fisher Scientific, Certified A. C. S. Crystal) was dried at 473 K for 20 h prior to use. DL- α -Alanine-DL- α -alanine (Aldrich, Assay 98%), butylamine (Aldrich, Assay 99+%), ethylamine hydrochloride (Aldrich, Assay 98%), L-prolyl-glycine (Sigma), and L-prolyl-hydroxy-L-proline (Sigma) were used without further purification. Degassed, Nanopure water (resistivity $> 8 \text{ M}\Omega \cdot \text{cm}$) was used to prepare all solutions. All solutions were prepared by mass.

2.2 Hydrothermal Stability Tests. Batch experiments to determine the hydrothermal stability of the aqueous amino acids were conducted in sealed Pyrex tubes ($\sim 17 \text{ cm}$ long, 6 mm o.d., 1 mm wall thickness) contained in an oven designed for this purpose.

The oven consisted of a cylindrical brass block into which thirteen holes had been drilled. The diameter of each hole was slightly larger than the diameter of the Pyrex tubes, and each hole was approximately 15 cm deep. The brass block was capped by a removable cylindrical aluminum block containing an identical set of holes with an approximate depth of 2.5 cm. The core was surrounded by a cylindrical ceramic heater which was controlled by an OMEGA CN76030 temperature controller. An insulated stainless steel container surrounded both the heater and the core to prevent heat loss and to contain explosion debris.

Aliquots of the amino acid solutions were measured into the Pyrex tubes such that the ratio of solution to gas space in the tube was approximately 1:2. This allowed more than sufficient space for thermal expansion and it reduced the frequency of explosion. At the start of each run, 10 tubes containing identical amino acid solutions were loaded into the oven at room temperature. Once the tubes were secured, the oven was heated to 523 K over a period of approximately 20 min. Several tubes were removed from the oven after 90 min, and the remaining tubes were removed after 72 h. Visual observations were made both when the solutions were hot (523 K) and at room temperature. The concentrations of amino acids and their decomposition products were measured at room temperature using a Beckman 121MB amino acid analyzer as described in Section 2.5.

2.3 High Temperature and Pressure Density Measurements. High temperature density measurements were made using a platinum vibrating tube densitometer. The design of the densitometer is essentially that given by Albert and Wood²⁸ as modified by Corti et al.²⁹ and Wood et al.³⁰ A detailed description of the densitometer has been given by Xiao et al.²⁵ Briefly, the densitometer consists of a U-shaped tube (0.90 Pt/0.10 Ir alloy), silver soldered into a cylindrical brass block. Two inconel rods, mounted on the tube with ceramic adhesive, rest between the poles of a permanent magnet. One of the rods carries the electrical current which drives the vibration of the tube. The other carries the induced current which is measured to sense the frequency of vibration. The temperature is controlled to $\pm 0.02 \text{ K}$ by a well-insulated brass oven, with a large thermal mass, that surrounds the densitometer cell.

An Isco 260D high-pressure pump injected water continuously into the system at a constant volumetric flow rate ($0.0117 \text{ cm}^3 \cdot \text{s}^{-1}$ for aqueous proline and aqueous β -alanine, $0.00833 \text{ cm}^3 \cdot \text{s}^{-1}$ for aqueous α -alanine). A two-position six-port valve was used to direct the flow of water either directly to the densitometer to act as a reference fluid or into a 15 cm^3 injection

TABLE 1: Visual Observations of the α -Alanine, Proline, and Glycine Solutions after 0, 1.5, and 72.0 h at 523 K

	initial solution	t = 1.5 h	t = 72.0 h
α -Alanine			
visual appearance			
hot	clear, colorless	clear, colorless	clear, slight yellow coloration
cold	clear, colorless	clear, very slight yellow coloration	colorless liquid, white precipitate
amino acid analysis	—	51% thermally reacted	100% thermally reacted
Proline			
visual appearance			
hot	clear, colorless	clear, slight yellow coloration	yellow liquid, white precipitate
cold	clear, colorless	clear, slight yellow coloration	yellow liquid, white and yellow precipitate
amino acid analysis	—	18% thermally reacted	100% thermally reacted
Glycine			
visual appearance			
hot	clear, colorless	clear, yellow-brown coloration	yellow-brown liquid, white precipitate
cold	clear, colorless	yellow-brown liquid, white precipitate	yellow liquid, white precipitate

loop constructed from 3.2 mm o.d. PEEK tubing (Upchurch Scientific) to force the sample solution into the densitometer. The sample solution was loaded into the injection loop using a second two-position six-port valve and a filling syringe, then the loop was pre-pressurized to the system pressure by another HPLC pump. The pressure of the flow system was maintained by a nitrogen cylinder and a back-pressure regulator (Tescom model 26–1700) connected to the pressurized reservoir at the exit of the densitometer cell. The system pressure was measured by means of an Omega PX623 pressure transducer traceable to NIST standards and an Omega DP41-E process indicator. The accuracy of the pressure measurement was confirmed to be within the manufacturer's specified error limit by determining the bubble point of water at 300 °C.²⁵

The densitometer was calibrated daily with a standard 1 mol·kg^{−1} NaCl solution and pure water, using the standard reference values compiled by Archer³¹ and Hill,³² respectively. The combined uncertainty in the measured relative densities, ($\rho - \rho_w$), arising from the sensitivity limits of the instrument itself ($\pm 0.00004 \text{ g}\cdot\text{cm}^{-3}$) and the accuracy of the reference data, is estimated to be $\pm 0.0002 \text{ g}\cdot\text{cm}^{-3}$.

Seven to thirteen 15 mL sample solutions of α -alanine and proline were injected into the densitometer along with approximately 300 mL of water from the Isco pump for each temperature and pressure at which measurements were made. The effluent from the densitometer was collected in a stainless steel reservoir under an atmosphere of nitrogen at the operating pressure of the densitometer. At the conclusion of a set of measurements, the reservoir was emptied into a sample vial under an atmosphere of nitrogen at ambient pressure so that the solutions could be analyzed for thermal decomposition products.

The procedure for β -alanine was similar, except that the effluent samples were collected in a 3 cm³ sampling loop, constructed from 3.2 mm o.d. PEEK tubing (Upchurch Scientific), which had been added to the output side of the densitometer. A two-position six-port valve was used to direct the flow of the effluent either directly into the stainless steel reservoir or into the reservoir through the sampling loop. Samples were withdrawn from the loop using a nitrogen filled syringe, then analyzed for the presence of thermal reaction products.

2.4 Density Measurements at Room Temperature. The density measurements made at 298.15 K and 0.1 MPa were obtained using a Sodev 03D vibrating tube densitometer equipped with platinum cells. The design of the densitometer is essentially that given by Picker et al.³³ A Sodev CT-L circulating bath was used to maintain the temperature of the densitometer to within ± 0.01 K. The temperature of the densitometer was measured by an Omega 44107 thermistor

which had been calibrated with a Hewlett-Packard 2804A quartz-crystal thermometer traceable to NIST standards. Nano-pure water and standard solutions of aqueous NaCl were used to calibrate the densitometer.

2.5 Analytical Methods. A Beckman 121MB amino acid analyzer was used to measure approximate amino acid concentrations in the batch experiments and to analyze for hydrothermal decomposition products in the effluent from the densitometer. The amino acid analyzer detects ninhydrin-active species over a wide range of concentrations, with the optimal concentration being 3 mmol·kg^{−1}. Separation is achieved by ion chromatography of the $R_3C(NH_3)^+$ species in a dilute solution of acid containing trace amounts of ninhydrin as a colorimetric indicator.

For α -alanine, β -alanine, and proline the primary thermal decomposition reactions are known to be amino acid condensation^{34–36} and decarboxylation.²¹ The primary condensation products in our systems are α -alanyl- α -alanine, from α -alanine; β -alanyl- β -alanine, from β -alanine; and prolyl-proline, from proline. Decarboxylation of α -alanine and β -alanine would yield ethylamine, while decarboxylation of proline would yield butylamine. These thermal reaction products are all ninhydrin-active and can be detected by the amino acid analyzer. The instrument was calibrated by making standard additions of the following expected decomposition products or closely related compounds, all prepared as 3 mmol·kg^{−1} standard solutions: L-prolyl-hydroxy-L-proline, L-prolyl-glycine (these two compounds have molar masses just above and below the molar mass of prolyl-proline and therefore should indicate the approximate position of a prolyl-proline peak), butylamine, proline, α -alanyl- α -alanine (which has the same molar mass as β -alanyl- β -alanine and should indicate the approximate position of the β -alanyl- β -alanine peak), ethylamine, α -alanine, and β -alanine. Aliquots of the expected decomposition products were added to 3 mmol·kg^{−1} solutions of each amino acid (L-prolyl-hydroxy-L-proline, L-prolyl-glycine, and butylamine to the proline solution; α -alanyl- α -alanine and ethylamine to both the α - and β -alanine solutions), such that their concentrations were approximately 10% and 1% of the amino acid concentration. All except ethylamine in α -alanine were detected at the 1% level. Hence, the detection limit for all of the hydrothermal reaction products was taken to be $< 0.03 \text{ mmol}\cdot\text{kg}^{-1}$, except ethylamine in α -alanine solutions whose detection limit was $< 0.3 \text{ mmol}\cdot\text{kg}^{-1}$.

3. Results

3.1 Amino Acid Decomposition. The results of the batch experiments described in section 2.2 are summarized in Table 1. It was found that 51% of the α -alanine and 18% of the proline

had undergone thermal reaction in the first 1.5 h of heating at 523 K, and neither amino acid solution contained any precipitate. After 72.0 h of heating, a precipitate was observed in both solutions. These results confirmed that α -alanine and proline are stable on the time scale required for our measurements at the temperatures, pressures, and molalities required for this work. After 1.5 h of heating at 523 K, a precipitate was observed in the glycine solution, suggesting that glycine is considerably less stable than the other two candidate compounds.

No decomposition products were detected in the α -alanine or proline solutions that had passed through the densitometer at any of the temperatures or pressures used in this study. Although the effluent from both the α -alanine and proline measurements at $T = 523$ K was clear and colorless while under a nitrogen atmosphere, the solutions developed a yellow coloration over a period of 30 min on exposure to air. The concentrations of the colored impurities were below the detection limit of the amino acid analyzer. No decomposition products were detected in the β -alanine effluent at temperatures below 423 K. Although the effluent from the β -alanine measurements at 473 K remained clear and colorless upon exposure to air, the amino acid analysis indicated that approximately 26% of the β -alanine had been converted to ammonia.

An attempt to measure the densities of α -alanine solutions at $T = 573$ K yielded erratic results for V_ϕ that deviated to high positive values at low molalities. Analysis of the effluent solutions from the densitometer at 20 and 10 MPa revealed that there had been decomposition to ammonia (10.9 and 13.4 mol percent, respectively), ethylamine (23.3 and 32.0 mol percent, respectively), and traces of dialanine. These results suggest that the rate of thermal decomposition decreases with increasing pressure.

3.2 Apparent Molar Volumes. The density of the fluids in the vibrating tube densitometer was determined from the usual expression:

$$\rho = \rho_w + K(\tau^2 - \tau_w^2) \quad (1)$$

where ρ and ρ_w are the densities of the fluid and water, respectively; τ and τ_w are the resonance periods for the fluid and water, respectively; and K is a characteristic constant which is determined by calibration with two reference fluids, water and aqueous NaCl solutions. The densities of water and the standard solutions of NaCl were obtained from the equations of state reported by Hill³² and Archer,³¹ respectively.

Apparent molar volumes, V_ϕ , were calculated from these densities according to the definition:

$$V_\phi = \left(\frac{1000(\rho_w - \rho)}{m\rho\rho_w} \right) + \left(\frac{M}{\rho} \right) \quad (2)$$

where m is the molality and M is the molar mass (89.093 g·mol⁻¹ for α - and β -alanine, 115.131 g·mol⁻¹ for proline). The experimentally determined relative densities ($\rho - \rho_w$) and apparent molar volumes for aqueous α -alanine, β -alanine, and proline are listed in Tables 2, 3, and 4, respectively.

Simple polynomial expressions were used to fit the molality dependence of the apparent molar volume data for each amino acid studied. A quadratic function was required for α -alanine and β -alanine:

$$V_\phi = V^\circ + bm + cm^2 \quad (3)$$

where V_ϕ is the apparent molar volume, V° is the standard partial molar volume, m is the molality of the amino acid solution, b

and c are functions which depend on temperature and/or pressure. A linear function was adequate for proline:

$$V_\phi = V^\circ + bm \quad (4)$$

The V_ϕ results are plotted in Figures 2–7. Values of V° , b , and c determined by fits to the isothermal data are listed in Table 5.

Except for the measurements made by Hakin et al.¹⁷ on glycine, there are no experimental values of V_ϕ for aqueous amino acids above 348 K in the literature with which to compare the results obtained in this work. However, our data are completely consistent with literature reports of measurements made at lower temperatures. For example, the temperature and pressure dependence of the standard partial molar volumes V° for α -alanine, β -alanine, and proline, shown in Figures 8–10, follow the trend established by the data reported by Hakin et al.,³⁷ Chalikian et al.,³⁸ and Hakin et al.³⁹ respectively, within the combined experimental error. Figure 8 includes our experimental value for V° of α -alanine at 298.15 K and 0.1 MPa. This value agrees with the literature values of Kikuchi et al.,⁴⁰ Chalikian et al.,⁴¹ Hakin et al.,³⁷ and Ogawa et al.⁴² within the combined experimental uncertainties.

3.3 “Equations Of State” for Aqueous α -Alanine, β -Alanine, and Proline. Two different models were used to represent the temperature and pressure dependencies of the apparent molar volume data. The first model is the revised Helgeson Kirkham Flowers model, as adapted to organic species by Shock and Helgeson:¹⁴

$$V^\circ = A_1 + \left[A_2 + \left(\frac{A_3}{(p + \Psi)} \right) \right] \left(\frac{1}{(T - \Theta)} \right) - \omega_e Q \quad (5)$$

$$\kappa^\circ_T = \left(\frac{A_3}{(p + \Psi)^2} \right) \left(\frac{1}{(T - \Theta)} \right) + \omega_e \left(\frac{\partial Q}{\partial p} \right)_T \quad (6)$$

$$b = B_1 + \left(\frac{B_2}{(T - \Theta)} \right) - B_3 Q \quad (7)$$

$$c = C_1 + \left(\frac{C_2}{(T - \Theta)} \right) \quad (8)$$

Here Ψ is a solvent parameter equal to 260.0 MPa, Θ is a solvent parameter equal to 228 K, Q is a Born function equal to $(1/\epsilon^2)(\partial\epsilon/\partial p)_T$ where ϵ is the dielectric constant of water, and $A_1, A_2, A_3, B_1, B_2, B_3, C_1$, and C_2 are fitting parameters. It was necessary to use the effective Born coefficient ω_e as a fitting parameter, although Shock and Helgeson¹⁴ gave an expression for its estimation. An expression for the standard partial molar isothermal compressibility was included in this model to ensure a good fit to the small pressure dependence observed in the measured apparent molar volumes.

The second model is an extension of the “density” treatment used by Mesmer et al.⁴³ to describe the temperature and pressure dependence of $\log K$ under hydrothermal conditions:

$$V^\circ = A_1 + \left[\left(\frac{A_2}{T} \right) + A_3 + A_4 T \right] (R\beta_w) \quad (9)$$

$$\kappa^\circ_T = -R \left[\left(\frac{A_2}{T^2} \right) + A_3 + A_4 T \right] \left(\frac{d\beta_w}{dp} \right)_T \quad (10)$$

$$b = B_1 + (B_2 RT\beta_w) \quad (11)$$

$$c = C_1 + (C_2 RT\beta_w) \quad (12)$$

TABLE 2: Densities Relative to Water ($\rho - \rho_w$) and Apparent Molar Volumes V_ϕ for Aqueous Solutions of α -Alanine vs Molality m

T/K	p/MPa	$m/\text{mol}\cdot\text{kg}^{-1}$	$10^2 \times (\rho - \rho_w)/\text{g}\cdot\text{cm}^{-3}$	$V_\phi/\text{cm}^3\cdot\text{mol}^{-1}$	T/K	p/MPa	$m/\text{mol}\cdot\text{kg}^{-1}$	$10^2 \times (\rho - \rho_w)/\text{g}\cdot\text{cm}^{-3}$	$V_\phi/\text{cm}^3\cdot\text{mol}^{-1}$
$T_{\text{average}} = 298.10 \text{ K}; p_{\text{average}} = 0.10 \text{ MPa}$									
298.10	0.100	0.95258	2.529	61.10	298.10	0.100	0.40562	1.122	60.83
298.10	0.100	0.83574	2.237	61.05	298.10	0.100	0.31134	0.8677	60.76
298.10	0.100	0.63154	1.717	60.94	298.10	0.100	0.16204	0.4572	60.62
298.10	0.100	0.52269	1.433	60.89	298.10	0.100	0.10203	0.2889	60.58
$T_{\text{average}} = 333.21 \text{ K}; p_{\text{average}} = 10.047 \text{ MPa}$									
333.20	10.033	1.00958	2.555	62.64	333.21	10.045	0.40562	1.082	62.17
333.19	10.041	0.80030	2.059	62.54	333.21	10.051	0.31134	0.8384	62.08
333.20	10.041	0.63154	1.648	62.42	333.22	10.055	0.16204	0.4431	61.90
333.20	10.045	0.52269	1.378	62.31	333.22	10.055	0.10203	0.2809	61.81
$T_{\text{average}} = 381.70 \text{ K}; p_{\text{average}} = 10.057 \text{ MPa}$									
381.69	10.060	1.00958	2.596	63.31	381.71	10.062	0.40562	1.108	62.57
381.70	10.062	0.80030	2.106	62.98	381.70	10.060	0.31134	0.8592	62.41
381.70	10.058	0.63154	1.684	62.88	381.70	10.054	0.16204	0.4553	62.13
381.71	10.059	0.52269	1.410	62.73	381.70	10.052	0.10203	0.2892	61.96
$T_{\text{average}} = 422.42 \text{ K}; p_{\text{average}} = 10.036 \text{ MPa}$									
422.15	10.064	1.00958	2.749	62.69	422.27	10.018	0.40562	1.170	61.87
422.19	10.013	0.80030	2.224	62.39	422.28	10.014	0.31134	0.9081	61.68
422.28	10.079	0.80030	2.225	62.39	422.27	10.093	0.16204	0.4814	61.33
422.59	10.007	0.63154	1.782	62.21	422.27	10.097	0.10203	0.3054	61.18
422.27	10.016	0.52269	1.490	62.07					
$T_{\text{average}} = 477.24 \text{ K}; p_{\text{average}} = 10.058 \text{ MPa}$									
477.15	10.045	1.00958	3.078	60.08	477.30	10.061	0.40562	1.303	59.15
477.10	10.045	0.80030	2.479	59.85	477.36	10.068	0.31134	1.009	58.96
477.16	10.053	0.63154	1.982	59.65	477.36	10.070	0.16204	0.5358	58.42
477.28	10.064	0.52269	1.658	59.43					
$T_{\text{average}} = 523.36 \text{ K}; p_{\text{average}} = 10.063 \text{ MPa}$									
523.33	10.071	1.00958	3.469	55.26	523.39	10.060	0.40562	1.479	53.43
523.39	10.075	0.80030	2.800	54.79	523.36	10.054	0.31134	1.147	53.08
523.40	10.073	0.63154	2.248	54.23	523.33	10.049	0.10203	0.3849	52.21
523.38	10.072	0.52269	1.882	53.86					
$T_{\text{average}} = 334.65 \text{ K}; p_{\text{average}} = 19.977 \text{ MPa}$									
334.61	19.976	1.00958	2.519	62.90	334.71	19.978	0.40562	1.069	62.39
334.60	19.977	0.80030	2.033	62.75	334.67	19.979	0.31134	0.8322	62.17
334.61	19.979	0.63154	1.628	62.62	333.65	19.977	0.16204	0.4380	62.11
334.65	19.976	0.52269	1.362	62.51					
$T_{\text{average}} = 383.20 \text{ K}; p_{\text{average}} = 19.939 \text{ MPa}$									
383.23	19.940	1.00958	2.612	63.01	383.20	19.944	0.40562	1.103	62.58
383.23	19.942	0.80030	2.103	62.91	383.19	19.941	0.31134	0.8553	62.43
383.21	19.944	0.63154	1.678	62.86	383.15	19.927	0.10203	0.2873	62.06
383.21	19.944	0.52269	1.405	62.71					
$T_{\text{average}} = 423.47 \text{ K}; p_{\text{average}} = 19.976 \text{ MPa}$									
423.45	19.973	1.00958	2.739	62.67	423.50	19.980	0.40562	1.159	62.08
423.45	19.973	0.80030	2.202	62.59	423.49	19.979	0.31134	0.8990	61.89
423.41	19.977	0.63154	1.763	62.42	423.50	19.977	0.10203	0.3024	61.40
423.45	19.981	0.52269	1.475	62.27					
$T_{\text{average}} = 478.67 \text{ K}; p_{\text{average}} = 19.967 \text{ MPa}$									
478.68	19.958	1.00958	3.037	60.51	478.71	19.968	0.40562	1.288	59.54
478.68	19.962	0.80030	2.441	60.36	478.69	19.967	0.31134	0.9974	59.36
478.67	19.969	0.63154	1.956	60.10	478.64	19.974	0.16204	0.5284	58.93
478.71	19.966	0.52269	1.636	59.89	478.63	19.971	0.10203	0.3355	58.70
$T_{\text{average}} = 523.39 \text{ K}; p_{\text{average}} = 19.934 \text{ MPa}$									
523.43	19.933	1.00958	3.349	57.02	523.39	19.936	0.40562	1.436	55.06
523.42	19.934	0.80030	2.713	56.39	523.38	19.936	0.31134	1.119	54.46
523.41	19.932	0.63154	2.178	55.89	523.36	19.936	0.16204	0.5939	53.75
523.40	19.935	0.52269	1.827	55.46	523.36	19.935	0.10203	0.3778	53.32

Here β_w is the compressibility of water, $\beta_w = -(1/V_w)(\partial V_w/\partial p)_T$, and $A_1, A_2, A_3, A_4, B_1, B_2, C_1$, and C_2 are fitting parameters. The addition of the term containing A_1 , absent in the original model, increases the flexibility of the fitting functions thereby improving its ability to reproduce the temperature and pressure dependence of the experimental data at $T < 373 \text{ K}$.

To increase the temperature range and accuracy of the fitting parameters obtained from each model, a number of complementary data sets were included, along with the apparent molar volumes obtained in this work. These include the values of V°

for α -alanine and β -alanine, determined by Hakin et al.³⁷ and Chalikian et al.,³⁸ respectively; values of V_ϕ for proline determined by Hakin et al.;³⁹ and values of κ°_T for α -alanine and proline calculated from isentropic compressibilities κ°_S reported by Chalikian et al.⁴¹ and Kharakoz.⁴⁴

Following Xiao and Tremaine,⁴⁵ the experimental values of V_ϕ were assigned weighting factors proportional to the molality in the least-squares fit. A similar analysis yielded the following weighting factors W_{298} for the V° and κ°_T values at 298.15 K: $W_{298} = 3.0$ for $V^\circ(\alpha\text{-alanine})$ from Hakin et al.³⁷ and this work;

TABLE 3: Densities Relative to Water ($\rho - \rho_w$) and Apparent Molar Volumes V_ϕ for Aqueous Solutions of β -Alanine vs Molality m

T/K	p/MPa	$m/\text{mol}\cdot\text{kg}^{-1}$	$10^2(\rho - \rho_w)/\text{g}\cdot\text{cm}^{-3}$	$V_\phi/\text{cm}^3\cdot\text{mol}^{-1}$
$T_{\text{average}} = 334.59 \text{ K}; p_{\text{average}} = 10.314 \text{ MPa}$				
334.64	10.318	0.099806	0.2935	59.91
334.62	10.318	0.20334	0.5842	60.42
334.60	10.320	0.30406	0.8726	60.28
334.63	10.318	0.40204	1.132	60.66
334.59	10.320	0.49491	1.387	60.64
334.57	10.315	0.59810	1.658	60.79
334.57	10.308	0.79238	2.158	60.98
334.58	10.309	0.92083	2.475	61.14
334.56	10.304	1.1858	3.114	61.38
$T_{\text{average}} = 383.34 \text{ K}; p_{\text{average}} = 10.355 \text{ MPa}$				
383.41	10.343	0.099806	0.3040	59.69
383.40	10.350	0.20334	0.6123	59.87
383.36	10.354	0.30406	0.9053	60.06
383.30	10.351	0.40204	1.177	60.43
383.28	10.354	0.49491	1.446	60.33
383.30	10.355	0.59810	1.728	60.49
383.31	10.362	0.79238	2.246	60.77
383.32	10.367	0.92083	2.580	60.90
383.32	10.365	1.1858	3.247	61.17
$T_{\text{average}} = 423.60 \text{ K}; p_{\text{average}} = 10.290 \text{ MPa}$				
423.57	10.289	0.20334	0.6446	58.92
423.57	10.292	0.30406	0.9547	59.08
423.57	10.290	0.40204	1.250	59.26
423.58	10.290	0.49491	1.524	59.43
423.58	10.291	0.59810	1.824	59.57
423.58	10.292	0.79238	2.379	59.77
423.60	10.293	0.92083	2.728	60.01
423.59	10.292	1.1858	3.434	60.32
$T_{\text{average}} = 478.78 \text{ K}; p_{\text{average}} = 10.326 \text{ MPa}$				
478.76	10.318	0.099806	0.3461	56.43
478.78	10.323	0.30406	1.035	56.85
478.77	10.316	0.40204	1.356	57.02
478.77	10.324	0.49491	1.656	57.20
478.78	10.323	0.59810	1.974	57.57
478.78	10.331	0.79238	2.574	57.86
478.80	10.341	0.92083	2.969	57.92
478.81	10.343	1.1858	3.739	58.34
$T_{\text{average}} = 423.63 \text{ K}; p_{\text{average}} = 20.533 \text{ MPa}$				
423.56	20.515	0.099806	0.3134	59.36
423.58	20.523	0.20334	0.6311	59.57
423.58	20.530	0.30406	0.9339	59.75
423.64	20.537	0.40204	1.223	59.91
423.64	20.540	0.49491	1.492	60.05
423.64	20.540	0.59810	1.784	60.21
423.65	20.542	0.79238	2.321	60.49
423.65	20.549	0.92083	2.675	60.54
423.67	20.551	1.1858	3.355	60.96
$T_{\text{average}} = 478.61 \text{ K}; p_{\text{average}} = 20.577 \text{ MPa}$				
478.54	20.538	0.099806	0.3362	57.64
478.61	20.551	0.20334	0.6783	57.85
478.59	20.577	0.30406	1.005	58.05
478.60	20.579	0.40204	1.315	58.27
478.60	20.594	0.49491	1.606	58.42
478.60	20.591	0.59810	1.923	58.59
478.62	20.590	0.79238	2.503	58.92
478.62	20.594	0.92083	2.877	59.13
478.63	20.598	1.1858	3.623	59.51

$W_{298} = 3.0$ for κ°_T (α -alanine) from Chalikian et al.;⁴¹ $W_{298} = 5.0$ for V° (β -alanine) from Chalikian et al.;³⁸ $W_{298} = 5.5$ for κ°_T (proline) from Kharakoz.⁴⁴ Weighting factors for V° at other temperatures W_T were assigned from the relationship:

$$W_T = W_{298} \left(\frac{\sigma_{V_{298}}}{\sigma_{V_T}} \right)^2 \quad (13)$$

where $\sigma_{V_{298}}$ is the uncertainty associated with V° at 298.15 K

and σ_{V_T} is the uncertainty associated with V° at temperatures other than 298.15 K. Weighting factors for κ°_T were taken to be independent of temperature ($W_T = W_{298}$).

The fitting parameters obtained using Model 1 are tabulated in Table 6, along with their standard deviations. The corresponding values for Model 2 are tabulated in Table 7. The fitted isotherms, V_ϕ vs m , from both models are plotted in Figures 2–7, for comparison with the experimental data. The standard partial molar volumes V° obtained from Models 1 and 2 are plotted in Figures 8, 9, and 10, where they are compared to those obtained from fitting eq 3 or 4 to each set of isothermal data. The fitted values of κ°_T for α -alanine and proline are plotted in Figures 11 and 12, along with the experimental values from Chalikian et al.⁴¹ and Kharakoz,⁴⁴ respectively.

3.4 Relative Success of Model 1 Versus Model 2. The overall weighted standard deviations of V_ϕ , σ , for each model are included in Tables 6 and 7. The values of σ from Model 2 are consistently lower than those from Model 1, despite the fact that Model 2 has fewer adjustable parameters. This is particularly true for the proline data, where Model 1 gives a weighted standard deviation of $0.18 \text{ cm}^3\cdot\text{mol}^{-1}$, significantly higher than the value $\sigma = 0.13 \text{ cm}^3\cdot\text{mol}^{-1}$ obtained from Model 2. Attempts to improve the fits by using alternative expressions for the temperature dependence of b and c were unsuccessful.

The values of V_ϕ for α -alanine obtained from Models 1 and 2 are compared with the experimental data in Figures 2 and 3. The fitting functions obtained from both models lie within the statistical scatter. Likewise, the standard partial molar volumes from both models agree with the values of V° from the isothermal fits in Figure 8 to within the experimental uncertainty. As illustrated in Figure 11, Model 2 yielded a superior fit to the experimental values for κ°_T determined from the data reported by Chalikian et al.⁴¹

The experimental and fitted values of V_ϕ for β -alanine are compared in Figure 4. While values from both models fit the experimental values at high temperatures within the experimental scatter, the curvature in the molality dependence given by Model 1 is opposite to that exhibited by the values measured at the lowest temperatures. This problem is reflected in the poor agreement between the values of V° from Model 1 with those from the isothermal fits in Figure 9. Model 2 was found to fit the data to within the statistical scatter in V_ϕ over the entire temperature range and to reproduce the values of V° , b , and c from the isothermal fits.

Neither Model 1 nor 2 fitted the experimental V_ϕ data for proline in Figures 5, 6, and 7 as well as was the case for α - or β -alanine. However, Model 2 reproduced the experimental data better than Model 1, which produced a fitting function for V° with an inverted pressure dependence at $T \leq \sim 425 \text{ K}$. The failure of Model 1 to fit the pressure-dependent data is illustrated in Figures 10 and 12, in which values of V° and κ°_T from the two models are compared with those obtained from isothermal fits and the experimental values reported by Kharakoz,⁴⁴ respectively.

3.5 Comparison with HKF Model Predictions. Amend and Helgeson¹⁵ have used the revised HKF model, with the correlations derived for neutral organic species, to predict the standard partial molar volumes V° of aqueous α -alanine and proline as a function of temperature. The predicted values of V° for α -alanine at 19.96 MPa are compared to the experimentally determined V° data in Figure 13. Proline and β -alanine show similar behavior. Unlike the experimental values of V° which deviate toward negative values at temperatures above 398

TABLE 4: Densities Relative to Water ($\rho - \rho_w$) and Apparent Molar Volumes V_ϕ for Aqueous Solutions of Proline vs Molality m

T/K	p/MPa	$m/\text{mol}\cdot\text{kg}^{-1}$	$10^2\cdot(\rho - \rho_w)/\text{g}\cdot\text{cm}^{-3}$	$V_\phi/\text{cm}^3\cdot\text{mol}^{-1}$	T/K	p/MPa	$m/\text{mol}\cdot\text{kg}^{-1}$	$10^2\cdot(\rho - \rho_w)/\text{g}\cdot\text{cm}^{-3}$	$V_\phi/\text{cm}^3\cdot\text{mol}^{-1}$
$T_{\text{average}} = 334.56 \text{ K}; p_{\text{average}} = 10.157 \text{ MPa}$									
334.58	10.142	0.14512	0.4435	84.91	334.51	10.167	0.44101	1.311	85.02
334.61	10.160	0.20055	0.6099	84.92	334.53	10.165	0.49425	1.462	85.03
334.60	10.164	0.24921	0.7543	84.94	334.55	10.164	0.59753	1.751	85.08
334.59	10.163	0.29640	0.8931	84.96	334.60	10.098	0.71024	2.058	85.13
334.59	10.078	0.30165	0.9086	84.96	334.57	10.160	0.78254	2.254	85.15
334.53	10.165	0.34868	1.045	84.99	334.60	10.108	0.79617	2.290	85.16
334.51	10.166	0.39582	1.182	85.00					
$T_{\text{average}} = 383.39 \text{ K}; p_{\text{average}} = 10.168 \text{ MPa}$									
383.36	10.125	0.20055	0.6093	86.66	383.42	10.187	0.44101	1.309	86.79
383.39	10.148	0.24921	0.7536	86.69	383.16	10.101	0.71024	2.054	86.94
383.14	10.108	0.30165	0.9076	86.70	383.36	10.197	0.78254	2.246	87.00
383.41	10.185	0.39582	1.180	86.78	383.12	10.095	0.79617	2.283	86.99
$T_{\text{average}} = 423.71 \text{ K}; p_{\text{average}} = 10.173 \text{ MPa}$									
423.68	10.168	0.20055	0.6331	87.14	423.73	10.179	0.39582	1.226	87.28
423.70	10.169	0.29640	0.9270	87.21	423.73	10.182	0.44101	1.360	87.31
423.56	10.130	0.30165	0.9426	87.22	423.55	10.142	0.71024	2.134	87.49
423.72	10.174	0.34868	1.085	87.25	423.56	10.140	0.79617	2.371	87.58
$T_{\text{average}} = 479.04 \text{ K}; p_{\text{average}} = 10.115 \text{ MPa}$									
479.01	10.134	0.20055	0.6878	86.60	479.06	10.098	0.44101	1.476	86.93
479.03	10.126	0.24921	0.8499	86.70	479.07	10.088	0.49425	1.645	87.00
479.03	10.118	0.34868	1.177	86.82	479.05	10.094	0.59753	1.966	87.18
479.05	10.110	0.39582	1.331	86.87	479.06	10.092	0.78254	2.533	87.33
$T_{\text{average}} = 524.07 \text{ K}; p_{\text{average}} = 10.094 \text{ MPa}$									
524.05	10.056	0.14512	0.5565	83.28	523.98	10.118	0.39582	1.487	83.50
524.06	10.059	0.20055	0.7659	83.31	524.06	10.123	0.44101	1.651	83.55
524.06	10.070	0.24921	0.9476	83.38	524.16	10.122	0.49425	1.842	83.62
524.08	10.085	0.29640	1.123	83.40	524.08	10.116	0.59753	2.208	83.71
524.05	10.103	0.34868	1.315	83.46	524.09	10.112	0.78254	2.848	83.90
$T_{\text{average}} = 334.93 \text{ K}; p_{\text{average}} = 20.199 \text{ MPa}$									
334.92	20.200	0.20055	0.6041	85.00	334.97	20.20	0.59753	1.734	85.15
334.89	20.202	0.29640	0.8845	85.04	334.90	20.22	0.71024	2.038	85.20
334.97	20.192	0.44101	1.298	85.10	334.92	20.20	0.78254	2.231	85.23
335.00	20.188	0.49425	1.448	85.11					
$T_{\text{average}} = 383.66 \text{ K}; p_{\text{average}} = 20.157 \text{ MPa}$									
383.67	20.184	0.14512	0.4400	86.63	383.65	20.165	0.49425	1.451	86.76
383.63	20.163	0.24921	0.7485	86.66	383.63	20.152	0.59753	1.739	86.79
383.64	20.153	0.29640	0.8860	86.69	383.21	20.212	0.71024	2.044	86.84
383.68	20.141	0.34868	1.037	86.71	383.68	20.144	0.78254	2.239	86.86
383.71	20.135	0.39582	1.173	86.71	383.22	20.207	0.79617	2.274	86.86
383.63	20.159	0.44101	1.302	86.73					
$T_{\text{average}} = 423.90 \text{ K}; p_{\text{average}} = 20.205 \text{ MPa}$									
424.00	20.197	0.14512	0.4570	87.12	423.80	20.209	0.59753	1.801	87.41
423.89	20.194	0.24921	0.7768	87.18	423.53	20.204	0.71024	2.117	87.48
423.86	20.200	0.29640	0.9196	87.22	423.94	20.215	0.78254	2.318	87.53
423.82	20.204	0.30165	0.9350	87.22	423.40	20.207	0.79617	2.354	87.54
423.90	20.207	0.39582	1.217	87.27					
$T_{\text{average}} = 479.15 \text{ K}; p_{\text{average}} = 20.194 \text{ MPa}$									
479.15	20.195	0.14512	0.4939	86.80	479.14	20.190	0.44101	1.458	87.13
479.18	20.200	0.20055	0.6793	86.83	479.15	20.195	0.49425	1.625	87.19
479.12	20.200	0.24921	0.8397	86.91	479.19	20.197	0.59753	1.945	87.30
479.13	20.190	0.29640	0.9938	86.97	478.60	20.136	0.71024	2.285	87.44
478.59	20.136	0.30165	1.010	86.99	479.13	20.198	0.78254	2.501	87.52
479.15	20.179	0.34868	1.163	87.02	478.61	20.135	0.79617	2.539	87.53
479.14	20.184	0.39582	1.314	87.08					
$T_{\text{average}} = 523.98 \text{ K}; p_{\text{average}} = 20.240 \text{ MPa}$									
523.96	20.223	0.14512	0.5422	84.44	523.99	20.249	0.44101	1.603	84.85
523.94	20.239	0.20055	0.7457	84.50	524.02	20.245	0.49425	1.788	84.93
523.98	20.248	0.24921	0.9222	84.58	524.02	20.235	0.59753	2.141	85.07
523.90	20.250	0.29640	1.092	84.64	523.41	20.183	0.71024	2.518	85.22
523.37	20.177	0.30165	1.110	84.66	524.06	20.245	0.78254	2.757	85.32
523.94	20.248	0.34868	1.279	84.69	523.41	20.184	0.79617	2.799	85.36
523.93	20.247	0.39582	1.445	84.79					

K, the predicted values of V° continue to become more positive as the critical temperature of water is approached. Quantitative errors are known to arise from the very limited high-temperature database on which the extension of the revised HKF model to

organic species was based.^{46,47} In the discussion that follows, we suggest that the discrepancy between the predicted and experimental behavior of V° in Figure 13 reflects the neglect of the zwitterionic nature of the aqueous amino acids.

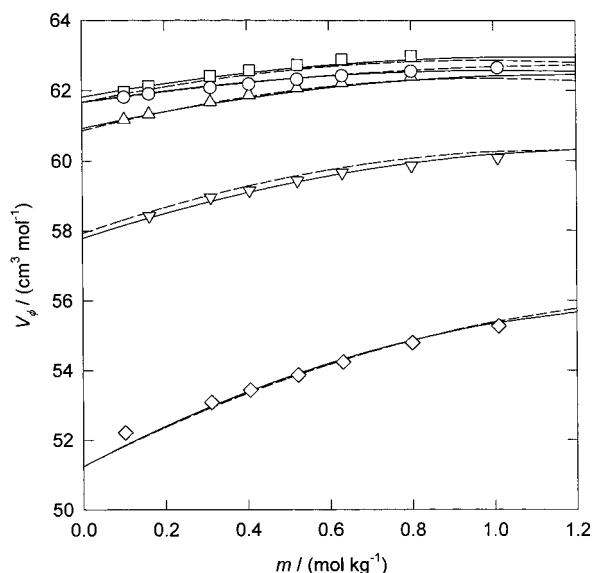


Figure 2. The apparent molar volumes V_ϕ of α -alanine from 333.2 to 523.4 K at 10.05 MPa plotted against molality. Symbols are experimental results: \square , 381.7 K; \circ , 333.2 K; \triangle , 422.4 K; ∇ , 477.2 K; \diamond , 523.4 K. Lines are fitted values: ---, Model 1; —, Model 2.

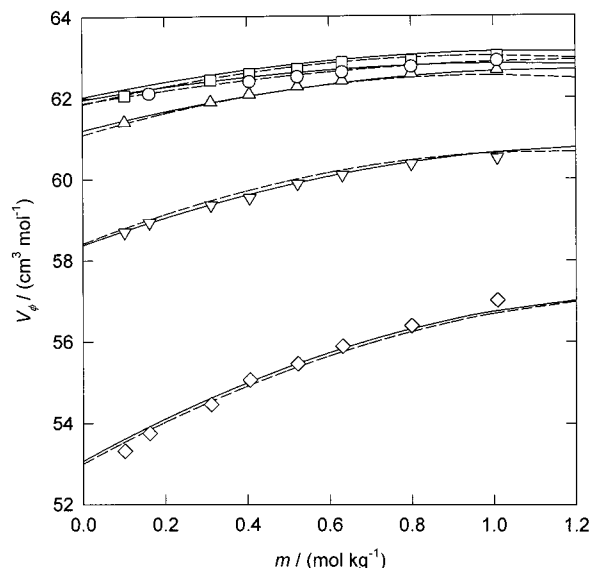
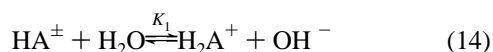


Figure 3. The apparent molar volumes V_ϕ of α -alanine from 334.6 to 523.4 K at 19.96 MPa plotted against molality. Symbols are experimental results: \square , 383.2 K; \circ , 334.6 K; \triangle , 423.5 K; ∇ , 478.7 K; \diamond , 523.4 K. Lines are fitted values: ---, Model 1; —, Model 2.

4. Discussion

4.1 Speciation at Elevated Temperature and Pressure. In aqueous solution, amino acids can exist as either the zwitterion HA^\pm , the neutral species HA^0 , the deprotonated ion A^- , or the protonated ion H_2A^+ . The equilibria between the zwitterionic, neutral, and ionic forms can be summarized as follows:



where K_1 , K_2 , and K_3 are the ionization constants for reactions 22, 23, and 24, respectively. Values for the ionization constants

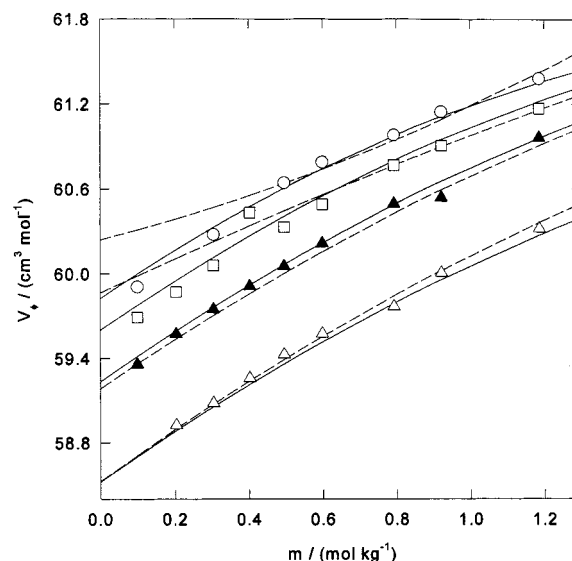


Figure 4. The apparent molar volumes V_ϕ of β -alanine from 334.6 to 423.6 K at 10.32 MPa and at 423.6 K and 20.53 MPa plotted against molality. Empty symbols are experimental results at 10.32 MPa: \circ , 334.6 K; \square , 383.3 K; \triangle , 423.6 K. Solid symbol is the experimental result at 20.53 MPa: \blacktriangle , 423.6 K. Lines are fitted values: ---, Model 1; —, Model 2.

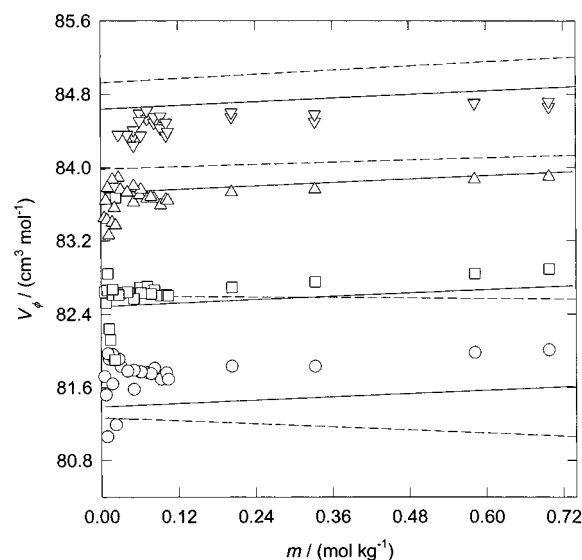


Figure 5. The apparent molar volumes V_ϕ of proline from 288.15 to 328.15 K at 0.1 MPa plotted against molality. Symbols are the experimental values of Hakin et al.:³⁹ ∇ , 328.15 K; \triangle , 313.15 K; \square , 298.15 K; \circ , 288.15 K. Lines are the fitted values: ---, Model 1; —, Model 2.

of the zwitterions at elevated temperatures were estimated from room-temperature values of $\ln K$, ΔH° , and ΔC_p° for eqs 14 and 15 using the isocoulombic forms of these equilibria⁴⁶ according to the expression:

$$\ln K_T = \ln K_{298} + \Delta H_{298}^\circ [(1/298.15) - (1/T)]/R + \Delta C_{p,298}^\circ [\ln(T/298.15) - 1 + (298.15/T)]/R \quad (17)$$

Eq 17 is based on the observation that the values of $\Delta C_{p,298}^\circ$ for most isocoulombic reactions are independent of temperature and that the effect of pressure on $\ln K_T$ is quite small below 300 °C. In the absence of literature data, K_3 was assumed to be approximately equal to $(K_1 \cdot K_2)$. Details are given in Table S1.

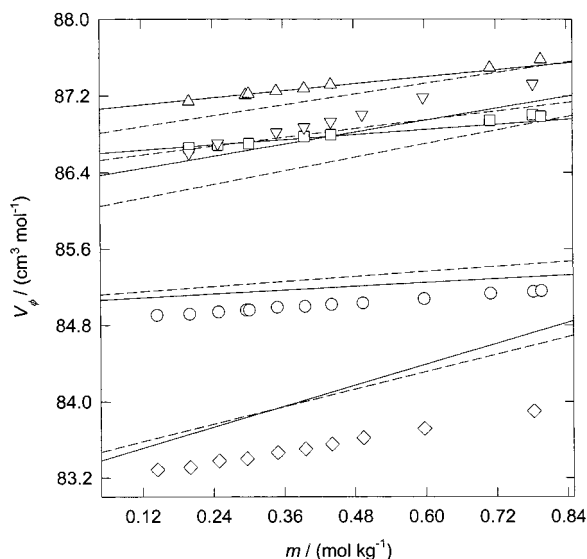


Figure 6. The apparent molar volumes V_ϕ of proline from 334.6 to 524.1 K at 10.14 MPa plotted against molality. Symbols are experimental results: Δ , 423.7 K; ∇ , 479.0 K; \square , 383.4 K; \circ , 334.6 K; \diamond , 524.1 K. Lines are fitted values: ---, Model 1; —, Model 2.

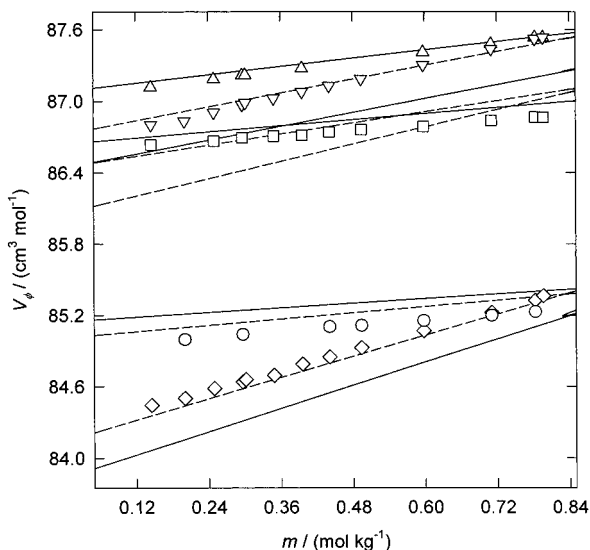


Figure 7. The apparent molar volumes V_ϕ of proline from 334.9 to 524.0 K at 20.20 MPa plotted against molality. Symbols are experimental results: Δ , 423.9 K; ∇ , 479.1 K; \square , 383.7 K; \circ , 334.9 K; \diamond , 524.0 K. Lines are fitted values: ---, Model 1; —, Model 2.

The degree of dissociation of α -alanine to form A^- , H_2A^+ , and HA^0 is plotted in Figure 14. Both β -alanine and proline give similar plots for the degree of dissociation as a function of temperature. Approximately 7% of the zwitterionic α -alanine is dissociated at 523 K according to this estimate, while the degree of dissociation at 523 K for both β -alanine and proline is approximately 2%. Similar estimates for glycine, made by Haken et al.¹⁷ with a group additivity model,¹⁵ yield much smaller values for the degree of dissociation.

4.2 Contributions to V° . The standard partial molar volume of an aqueous species V_{aq}° is usually considered to contain contributions from the intrinsic volume of the species itself V_{intr}° , plus terms associated with transferring the species from an ideal gas into the solution at infinite dilution:

$$V_{aq}^\circ = V_{intr}^\circ + \Delta_{solv} V^\circ + \Delta_{ss} V^\circ \quad (18)$$

TABLE 5: Values of V° , b , and c for α -Alanine, β -Alanine and Proline Obtained from Fitting Eq 3 or 4 to Each Set of Isothermal Data

T (K)	p (MPa)	V° ($\text{cm}^3 \cdot \text{mol}^{-1}$)	b ($\text{cm}^3 \cdot \text{kg} \cdot \text{mol}^{-2}$)	c ($\text{cm}^3 \cdot \text{kg}^2 \cdot \text{mol}^{-3}$)
α-Alanine				
334.65	19.977	61.89 ± 0.02	1.41 ± 0.06	-0.41 ± 0.04
383.20	19.939	61.86 ± 0.07	2.18 ± 0.23	-1.03 ± 0.18
423.47	19.976	61.11 ± 0.01	2.95 ± 0.02	-1.39 ± 0.01
478.67	19.967	58.30 ± 0.06	3.89 ± 0.22	-1.68 ± 0.17
523.39	19.934	52.81 ± 0.11	6.05 ± 0.39	-1.88 ± 0.30
333.21	10.047	61.65 ± 0.01	1.55 ± 0.05	-0.56 ± 0.04
381.70	10.057	61.72 ± 0.03	2.65 ± 0.12	-1.32 ± 0.12
422.42	10.036	60.89 ± 0.01	2.95 ± 0.01	-1.35 ± 0.01
477.24	10.058	57.91 ± 0.07	3.72 ± 0.25	-1.57 ± 0.18
523.36	10.063	51.68 ± 0.07	4.89 ± 0.22	-1.31 ± 0.17
β-Alanine				
423.63	20.533	59.26 ± 0.09	1.68 ± 0.28	-0.22 ± 0.19
334.59	10.315	59.72 ± 0.06	2.09 ± 0.17	-0.59 ± 0.11
383.34	10.355	59.57 ± 0.12	1.86 ± 0.37	-0.43 ± 0.25
423.60	10.290	58.60 ± 0.07	1.72 ± 0.21	-0.23 ± 0.14
Proline				
334.93	20.199	84.92 ± 0.01	0.39 ± 0.01	
383.66	20.157	86.58 ± 0.01	0.36 ± 0.01	
423.90	20.205	87.02 ± 0.01	0.65 ± 0.01	
479.15	20.194	86.63 ± 0.01	1.14 ± 0.01	
523.98	20.240	84.22 ± 0.01	1.42 ± 0.01	
334.56	10.157	84.84 ± 0.01	0.40 ± 0.01	
383.39	10.168	86.54 ± 0.01	0.57 ± 0.02	
423.71	10.173	87.00 ± 0.01	0.72 ± 0.02	
479.04	10.115	86.40 ± 0.03	1.22 ± 0.07	
524.07	10.094	83.12 ± 0.01	1.00 ± 0.01	
288.15	0.10	81.71 ± 0.03	0.44 ± 0.06	
298.15	0.10	82.60 ± 0.04	0.41 ± 0.09	
313.15	0.10	83.64 ± 0.02	0.38 ± 0.05	
328.15	0.10	84.44 ± 0.03	0.40 ± 0.05	

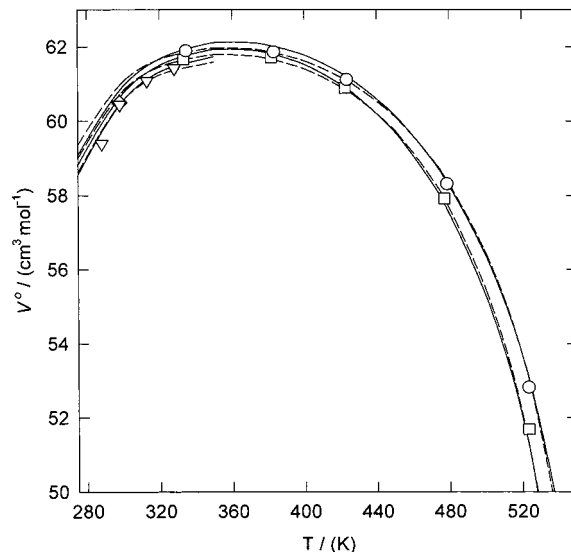


Figure 8. The standard partial molar volumes V° of α -alanine from 0.1 to 19.96 MPa plotted against temperature. Symbols are the values obtained from the isothermal fit of eq 3 to the experimental data at each temperature: \circ , 19.96 MPa; \square , 10.05 MPa; \diamond , 0.1 MPa. ∇ , 0.1 MPa from Hakin et al.³⁷ Lines are the fitted values: ---, Model 1; —, Model 2.

The volume of solvation $\Delta_{solv} V^\circ$ arises from time-averaged configurational changes in the water caused by introducing the solute from a fixed point in the gas phase into a fixed point in the solution. These are commonly identified with two major effects, the long-range polarization of water caused by the localized charge distribution within the solute, $\Delta_{pol} V^\circ$, and short-

TABLE 6: Fitting Parameters for Model 1, Eqs 5–8

	α -alanine	β -alanine	proline
A_1 (cm ³ ·mol ⁻¹)	65.483 (0.179) ^a	64.615 (0.345)	91.213 (0.258)
A_2 (K·cm ³ ·mol ⁻¹ ·10 ²)	1.91 (1.39)	18.41 (2.95)	-10.21 (1.67)
A_3 (MPa·K·cm ³ ·mol ⁻¹ ·10 ⁵)	-1.224 (0.350)	-5.681 (0.766)	1.164 (0.415)
ω_e (MPa·cm ³ ·mol ⁻¹ ·10 ⁴)	17.388 (0.246)	24.94 (2.13)	7.506 (0.387)
B_1 (cm ³ ·kg·mol ⁻²)	3.082 (0.500)	0.455 (0.909)	1.296 (0.432)
B_2 (K·cm ³ ·kg·mol ⁻² ·10 ¹)	-18.83 (6.20)	-5.1 (11.2)	-9.72 (3.74)
B_3 (kg·mol ⁻¹ ·10 ³)	-46.06 (3.49)	-88.9 (31.1)	-7.22 (6.88)
C_1 (cm ³ ·kg ² ·mol ⁻³)	-2.514 (0.451)	-0.967 (0.754)	
C_2 (K·cm ³ ·kg ² ·mol ⁻³ ·10 ²)	1.987 (0.631)	1.33 (1.01)	
σ (cm ³ ·mol ⁻¹)	0.08	0.09	0.18

^a The standard deviation for each parameter is given in parentheses. The standard deviations associated with eqs 5–8 can be estimated from equations in Table S4.

TABLE 7: Fitting Parameters for Model 2, Eqs 9–12

	α -alanine	β -alanine	proline
A_1 (cm ³ ·mol ⁻¹)	68.419 (0.342)	74.66 (1.14)	88.146 (0.449)
A_2 (K ² ·10 ⁶)	-1.3735 (0.0571)	0.917 (0.323)	-3.5752 (0.0965)
A_3 (K·10 ³)	4.327 (0.423)	-12.19 (2.24)	15.886 (0.708)
A_4	-6.163 (0.562)	15.96 (3.02)	-18.112 (0.093)
B_1 (cm ³ ·kg·mol ⁻² ·10 ⁻²)	59.0 (29.3)	169.9 (87.7)	-5.2 (13.7)
B_2 (kg·mol ⁻¹ ·10 ⁻¹)	9.49 (1.16)	0.70 (5.97)	3.164 (0.509)
C_1 (cm ³ ·kg ² ·mol ⁻³ ·10 ⁻¹)	-5.15 (2.47)	-5.63 (6.62)	
C_2 (kg ² ·mol ⁻² ·10 ⁻¹)	-2.666 (0.909)	1.20 (4.26)	
σ (cm ³ ·mol ⁻¹)	0.07	0.08	0.13

^a The standard deviation for each parameter is given in parentheses. The standard deviations associated with eqs 9–12 can be estimated from equations in Table S4.

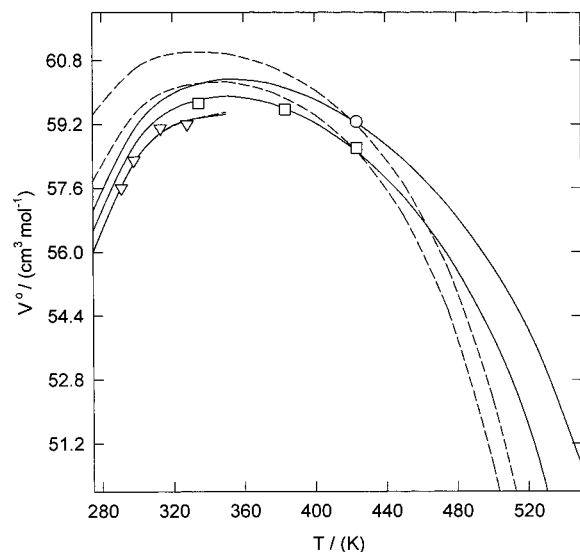


Figure 9. The standard partial molar volumes V° of β -alanine from 0.1 to 20.53 MPa plotted against temperature. Symbols are the values obtained from the isothermal fit of eq 3 to the experimental data at each temperature: \circ , 20.53 MPa; \square , 10.32 MPa; ∇ , 0.1 MPa. Lines are the fitted values: ---, Model 1; —, Model 2.

range hydration effects arising from the hydrogen-bonded “structure” of water in the immediate vicinity of the solute, $\Delta_{\text{hydr}}V^\circ$, so that $\Delta_{\text{soln}}V^\circ$ can be expressed as:

$$\Delta_{\text{soln}}V^\circ = \Delta_{\text{pol}}V^\circ + \Delta_{\text{hydr}}V^\circ \quad (19)$$

The term $\Delta_{\text{ss}}V^\circ$, which accounts for solute translational effects, arises from the difference in standard states between the gas phase (1 bar, ideal gas) and solution (hypothetical 1 molal solution) and is given by the expression:

$$\Delta_{\text{ss}}V^\circ = RT\beta_w$$

where β_w is the compressibility of the solvent water.^{49,50}

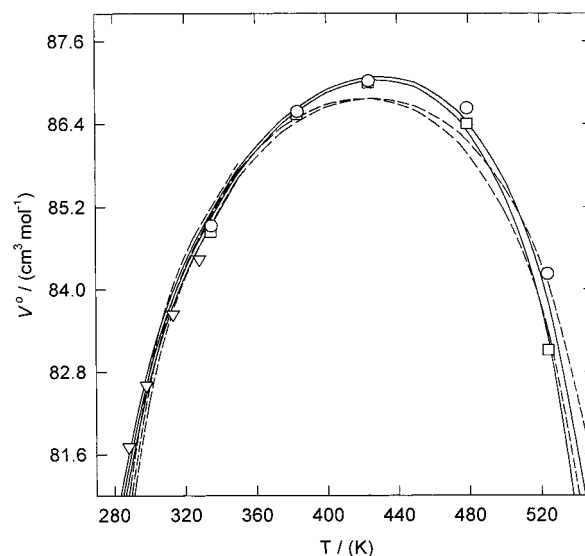


Figure 10. The standard partial molar volumes V° of proline from 0.1 to 20.20 MPa plotted against temperature. Symbols are the values obtained from the isothermal fit of eq 4 to the experimental data at each temperature: \circ , 20.20 MPa; \square , 10.14 MPa; ∇ , 0.1 MPa. Lines are the fitted values: ---, Model 1; —, Model 2.

4.3 Partial Molar Volume of Polarization by Neutral Dipolar Species. Many authors have represented $\Delta_{\text{pol}}V^\circ$ by extensions of the Born model^{51–53} in which the dissolved species is represented by a collection of discrete charges contained in a cavity which is imbedded in a structureless polarizable dielectric continuum.

$$\Delta_{\text{pol}}G^\circ = \Delta_{\text{Born}}G^\circ + \Delta_{\text{dipole}}G^\circ + \Delta_{\text{quadrupole}}G^\circ + \Delta_{\text{octopole}}G^\circ + \dots \quad (21)$$

Here $\Delta_{\text{Born}}G^\circ$ is the contribution of the ionic charge to the Gibbs free energy of polarization, while $\Delta_{\text{dipole}}G^\circ$, $\Delta_{\text{quadrupole}}G^\circ$ and

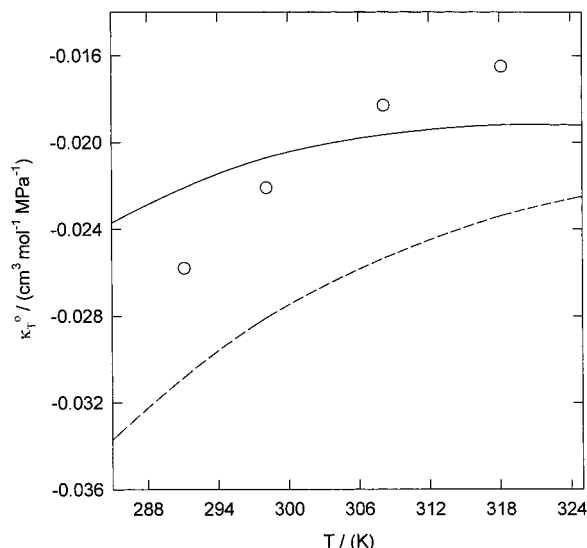


Figure 11. The standard partial molar isothermal compressibilities κ_T° of α -alanine at 0.1 MPa plotted against temperature. Symbols are the experimental values of Chalikian et al.⁴¹ Lines are fitted values: ---, Model 1; —, Model 2.

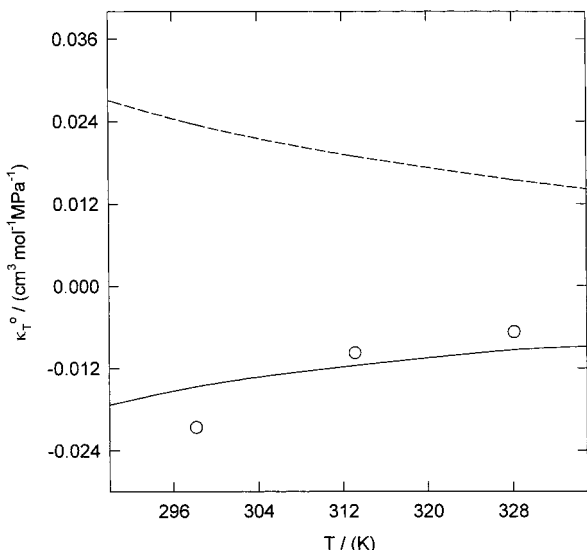


Figure 12. The standard partial molar isothermal compressibilities κ_T° of proline at 0.1 MPa plotted against temperature. Symbols are the experimental values of Kharakoz.⁴⁴ Lines are fitted values: ---, Model 1; —, Model 2.

$\Delta_{\text{octopole}} G^\circ$ are the contributions from the dipole, quadrupole, and octopole moments. The analogous terms for volume can be derived through the identity $(\partial \Delta G^\circ / \partial p)_T = \Delta V^\circ$, to yield:

$$\Delta_{\text{pol}} V^\circ = \Delta_{\text{Born}} V^\circ + \Delta_{\text{dipole}} V^\circ + \Delta_{\text{quadrupole}} V^\circ + \Delta_{\text{octopole}} V^\circ + \dots \quad (22)$$

The hydration term $\Delta_{\text{hydr}} V^\circ$ is difficult to model and is represented in this work by an empirical function.

For many ionic species, only the first term in eq 21 is significant. This leads to the well-known Born equation⁵¹ for the Gibbs free energy of polarization:

$$\Delta_{\text{Born}} G^\circ = \frac{-(Ze)^2 N_A}{8\pi\epsilon_0} \left(\frac{(\epsilon - 1)}{\epsilon} \right) \quad (23)$$

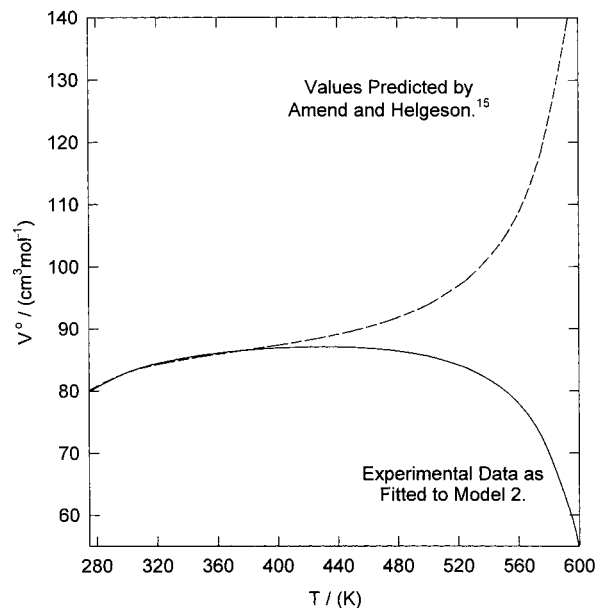


Figure 13. The predicted and experimental standard partial molar volumes V° of α -alanine at 19.96 MPa.

from which is derived:

$$\Delta_{\text{Born}} V^\circ = \frac{(Ze)^2 N_A}{8\pi\epsilon_0 a} \left(\frac{-1}{\epsilon^2} \right) \left(\frac{\partial \epsilon}{\partial p} \right)_T \quad (24)$$

Here Z is the ionic charge, e is the charge on an electron, N_A is Avogadro's number, ϵ is the solvent static dielectric constant, ϵ_0 is the permittivity of free space, and a is the "effective" radius of the dissolved species, all in SI units. The second term in the expansion of $\Delta_{\text{pol}} G^\circ$ is the analogous expression for a dissolved neutral dipolar species:⁵³

$$\Delta_{\text{dipole}} G^\circ = \frac{\mu^2 N_A}{4\pi\epsilon_0 a^3} \left(\frac{1 - \epsilon}{2\epsilon + 1} \right) \quad (25)$$

$$\Delta_{\text{dipole}} V^\circ = \frac{\mu^2 N_A}{4\pi\epsilon_0 a^3} \left(\frac{-3}{(2\epsilon + 1)^2} \right) \left(\frac{\partial \epsilon}{\partial p} \right)_T \quad (26)$$

where μ is the dipole moment arising from the charge distribution within the cavity.

A number of authors have reported temperature-dependent values of the dipole moment μ for α -alanine,^{54–58} β -alanine,^{54,55,57,59,60} and proline.^{61,62} Most of these are estimates of the effective dipole moment in dilute solutions from molar dielectric increments δ , as defined by the Kirkwood equation:^{63,64}

$$\mu = 0.1910 T^{1/2} \delta^{1/2} \quad (27)$$

where μ is expressed in Debye and δ is expressed in $\text{m}^3 \cdot \text{mol}^{-1}$. In the Kirkwood model, μ includes contributions from the mean dipole moment of the molecule itself in solution and the vector sum of the dipole moment of the central amino acid with the immediate neighboring water molecules. For our calculations, values of μ for α -alanine, β -alanine, and proline were extrapolated to elevated temperatures by fitting an empirical equation to temperature-dependent data reported by Aaron and Grant⁵⁵ and Shepherd and Grant:⁶¹

$$\mu = \mu_0 [A(T/T_c)^j + 1]^{0.5} \quad (28)$$

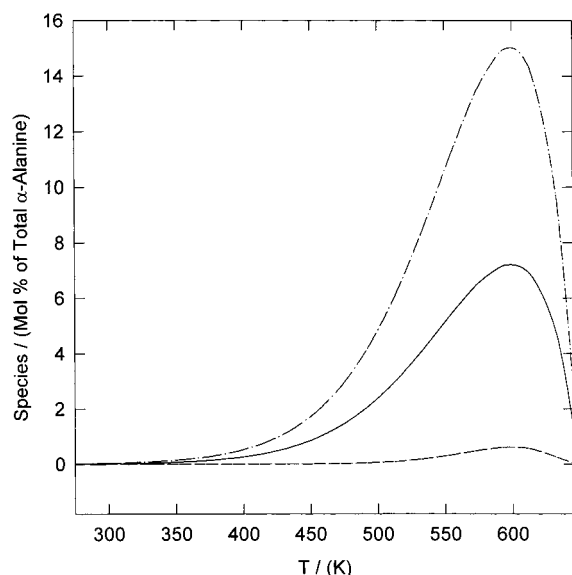


Figure 14. The values of $[H_2A^+]$, $[H_2A^+]$, $[A^-]$, and $[HA^0]$ for α -alanine as a function of temperature, where the sum of $[H_2A^+]$, $[H_2A^+]$, $[A^-]$, and $[HA^0]$ is $1 \text{ mol} \cdot \text{kg}^{-1}$. Lines represent the following: —, $[H_2A^+] = [A^-]$; ---, $[HA^0]$; - · - · -, $[H_2A^+] + [A^-] + [HA^0]$. The solid and dotted lines are coincident.

TABLE 8: Values of V_{intr}° , d , μ_0 , A , and j for α -Alanine, β -Alanine, and Proline

amino acid	V_{intr}° $\text{cm}^3 \cdot \text{mol}^{-1}$	d m	μ_0 Debye	A	j
α -alanine	33.5	2.67×10^{-10}	12.87	4.153×10^{-2}	3.475
β -alanine	59.8	1.98×10^{-10}	9.50	1.884	0.6194
proline	78.2	3.26×10^{-10}	15.68	3.106×10^{-5}	12.52

where μ_0 is the gas-phase dipole moment, $T_c = 647.14 \text{ K}$ is the critical temperature of water, and A and j are fitting parameters. Equation 28 is similar in form to an expression recently used to fit dielectric constant data for liquid water at steam saturation.⁶⁵ The gas-phase dipole moment μ_0 was estimated from X-ray crystallographic data.^{66,67} The positive and negative charges were assumed to reside on the nitrogen atom and on the oxygen atom with the longer carbon–oxygen bond, respectively. The conformations with the closest approach between the ammonium and carboxylate groups were chosen for α -alanine and β -alanine. The charge separation in proline is fixed by its ring structure. The values of d , μ_0 , A , and j determined for α -alanine, β -alanine, and proline are summarized in Table 8. The variation of μ with temperature for α -alanine is shown in Figure 15.

The intrinsic standard partial molar volumes of the amino acids in solution were calculated from the expression for a hard sphere:

$$V_{\text{intr}}^\circ = \left(\frac{4}{3}\right) N_A \pi r^3 \quad (29)$$

using the radii of α -alanine ($r = 2.37 \text{ \AA}$), β -alanine ($r = 2.87 \text{ \AA}$), and proline ($r = 3.14 \text{ \AA}$) estimated from the crystal structures.^{67–69} The corresponding values of V_{intr}° are listed in Table 8.

Values of $\Delta_{\text{dipole}} V^\circ$ for α -alanine, β -alanine, and proline were calculated from eq 26 with the assumption that the cavity radius a was equal to the molecular radius r .

4.4 Amino Acid Hydration at Elevated Temperatures.

Figure 16 illustrates the relative contributions of $\Delta_{\text{dipole}} V^\circ$, $\Delta_{\text{ss}} V^\circ$,

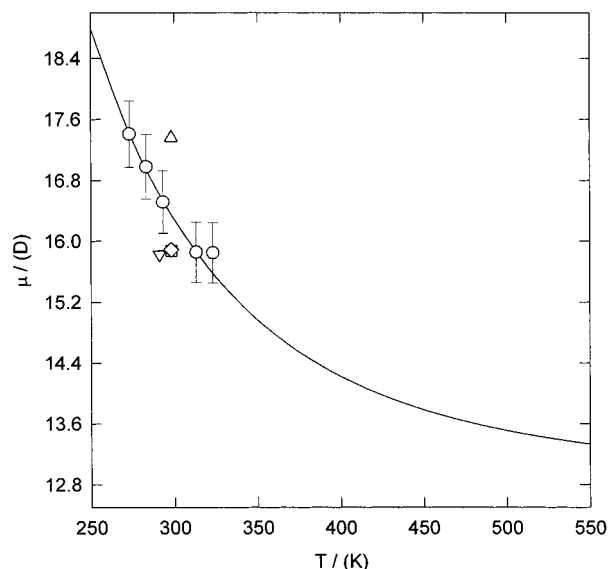


Figure 15. The aqueous dipole moment μ of α -alanine plotted against temperature. Symbols are the experimental values of: \square , Wyman and McMeekin;⁵⁴ \circ , Aaron and Grant;⁵⁵ \triangle , Devoto;⁵⁶ ∇ , Hederstrand;⁵⁷ \diamond , Osborn.⁵⁸ The solid line represents the least-squares fit obtained using eq 28 and the data of Aaron and Grant.⁵⁵

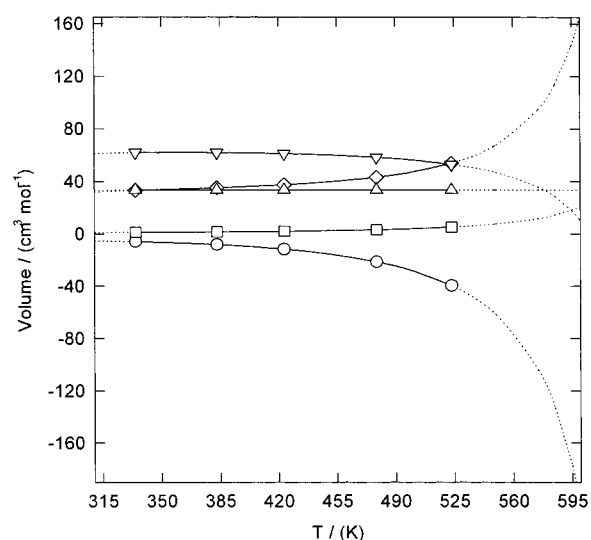


Figure 16. The values of V_g° , $\Delta_{\text{dipole}} V^\circ$, $\Delta_{\text{ss}} V^\circ$, and $\Delta_{\text{hydr}} V^\circ$ calculated for α -alanine at 19.96 MPa . Symbols are the calculated values at the temperatures and pressures associated with the experimental values of V° : \triangle , V_g° ; \circ , $\Delta_{\text{dipole}} V^\circ$; \square , $\Delta_{\text{ss}} V^\circ$; \diamond , $\Delta_{\text{hydr}} V^\circ$; ∇ , V° .

and V_{intr}° for α -alanine at $p = 19.96 \text{ MPa}$ as a function of temperature, along with the experimental values of V° from the isothermal fits. Similar results were obtained for α -alanine at $p = 10 \text{ MPa}$, and for β -alanine and proline at $p = 10 \text{ MPa}$ and $p = 20 \text{ MPa}$. The difference between V° and the contributions of $\Delta_{\text{dipole}} V^\circ$, $\Delta_{\text{ss}} V^\circ$, and V_{intr}° may be attributed to the short-range hydration term, $\Delta_{\text{hydr}} V^\circ$, with the assumption that $\Delta_{\text{pol}} V^\circ \approx \Delta_{\text{dipole}} V^\circ$ so that:

$$\Delta_{\text{hydr}} V^\circ = V_{\text{aq}}^\circ - [V_{\text{intr}}^\circ + \Delta_{\text{dipole}} V^\circ + \Delta_{\text{ss}} V^\circ] \quad (30)$$

Values of $\Delta_{\text{hydr}} V^\circ$ are also plotted in the Figure.

The contribution from $\Delta_{\text{dipole}} V^\circ$ deviates toward $-\infty$ as the temperature approaches the critical point at steam saturation, and it is apparent from Figure 16 that the effect of the dipole polarization term is to overpredict the observed decrease in the experimental values of V° with increasing temperature. As a

result, the residual term $\Delta_{\text{hydr}}V^\circ$, which is assumed to arise from hydrophobic hydration, displays an increasingly large positive deviation which cancels out some of the contribution from dipole polarization in the range of our measurements. While the behavior $\Delta_{\text{hydr}}V^\circ$ is consistent with the behavior of many nonelectrolytes,^{14,46,47} its magnitude is larger than other molecules of similar size.

The temperature dependence of $\Delta_{\text{hydr}}V^\circ$ may be significantly reduced if a larger cavity radius ($a > r$) is used in eq 26 to estimate $\Delta_{\text{dipole}}V^\circ$. It has long been known that the use of crystallographic radii in the Born equation overpredicts the contribution of electrostriction to the partial molar volumes of aqueous ions, because the dipolar reorientation of water molecules in the primary solvation sphere is severely restricted. Helgeson and Kirkham¹¹ have noted that an effective Born cavity radius $a = r + 0.94Z$ yields approximate agreement with the behavior of C_p° and V° for cations of the type M^{z+} at elevated temperatures and pressures. Although it is generally assumed that the magnitude of $\Delta_{\text{dipole}}V^\circ$ is small relative to $\Delta_{\text{Born}}V^\circ$, a comparison of eqs 24 and 26 shows that this is not the case when the charge separation distance in the zwitterion is of similar magnitude to the cavity radius. The use of an effective radius $a = r + 0.9$ in eq 26 yields very good agreement with the high temperature behavior of α -alanine, along with a hydration term that is almost independent of temperature in the range of our data. Similar agreement was obtained with the data obtained for glycine by Hakin et al.¹⁷ The use of this effective radius in calculations with our proline data yielded a hydration term $\Delta_{\text{hydr}}V^\circ$ that retained a significant temperature dependence, possibly because proline is a larger molecule so that its zwitterion forms an off-centered dipole in the solvation cavity.

An estimate of the effect of the ionic species A^- and H_2A^+ , on V° was carried out using eqs 24 and 26, along with the speciation estimates plotted in Figure 14. The contributions of solvent polarization by A^- , H_2A^+ and HA^\pm are all of similar magnitude when $r = a$, so that the effect of the amino acid ions on the calculated values of $\Delta_{\text{dipole}}V^\circ$ is minor unless the speciation estimates are grossly in error. Experiments to measure the equilibrium speciation at elevated temperatures by Raman spectroscopy are now underway.

5. Conclusions

This work reports the first experimentally determined apparent molar volumes V_ϕ for aqueous α -alanine, β -alanine, and proline between the temperatures $T = 343$ and 523 K at pressures in excess of steam saturation. This paper and the recent work by Hakin et al.¹⁷ on glycine are the first reported measurements of V° for any amino acid at temperatures above 353 K. Standard partial molar volumes V° derived from the experimental V_ϕ data from both studies compare extremely well with the published values obtained near ambient temperature. At progressively higher temperatures V° increases to a shallow maximum near 398 K, then decreases toward increasingly negative values in a manner that is similar to the behavior of large univalent aqueous electrolytes. This behavior is almost undoubtedly caused by the zwitterionic nature of the amino acids, and it may be described semiquantitatively by the primitive model for dipole-solvent polarization, eq 26, with a suitable choice of cavity radius. The major uncertainties are the treatment for dipole moment, which is approximate, and the distribution of species at elevated temperature and pressure, which must be determined by direct measurement.

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Supporting Information Available: Table S1, amino acid decomposition rate constants, Table S2, listing of data used in speciation calculations with supporting references,^{70–77} Table S3, listing of calculated values for contributions to V° , and Table S4, equations for standard deviations, are available as supplementary information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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