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# Volumetric properties of proteins and their analogs in diluted water solutions

## 1. Partial volumes of amino acids at 15–55 °C

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The apparent volumes of 14 amino acids in aqueous solutions at a concentration of 3 mg/ml were measured densitometrically within the temperature range 15–55 °C. The accuracy of measurements was ±0.3%. The decrease in volume of polar and charged atomic groups as well as the temperature dependences of the partial volumes was analysed. The differences in behaviour between charged, polar and nonpolar atomic groups were considered.

Amino acid; Partial volume; Temperature dependence; Solute-water interaction; Additive scheme

### 1. Introduction

Research on the volumetric properties of protein solutions was initiated about 50 years ago. The first systematic study on the thermodynamic properties of protein solutions, in particular of the partial volumes, was presented by Cohn and Edsall [1]. One of the main procedures for interpreting the physico-chemical properties of protein solutions is the comparative analysis of model compounds including substances of low molecular weight which contain the atomic groups specific for proteins. Thus, in ref. 1, the partial molar volumes of proteins were compared with the volumes of amino acids and peptides using a simple additive scheme presented by Traube [2]. Further investigations were directed at more accurate estimation of the contributions of amino acid residues to partial molar volumes in terms of the same scheme [3,4].

The derivatives of partial molar volumes, adiabatic compressibility and thermal expansibility of proteins in solutions were studied (see, for example, refs. 5–11). During the last decade, a new approach has been developed for investigation of protein volume, thermal expansibility and compressibility based on X-ray analysis [12–14].

Despite the wealth of information on the volumetric properties of protein solutions that has been published during the preceding half-century, a number of questions remain to be resolved.

One of the most important problems involves deriving the true values of density, compressibility and thermal expansibility of the interior of a protein molecule from the apparent values. This would be an insurmountable task without the correct estimation of the hydration effects.

Another problem has arisen in connection with the development of X-ray studies of volumetric properties. The partial volumes of amino acid residues in crystals and within protein molecules were found to be very large as compared with those in solutions [15,16]. In contrast, globule compressibility as observed by X-ray analysis [14] was determined to be 3–4-fold lower than that obtained from solution measurements [6,10,17]. Comparison between the crystallographic and

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solution data is complicated by the uncertainty in the contributions of surface atomic groups to the properties being studied. In this respect, correct estimation of the hydration effects is needed.

In order to resolve these problems, systematic studies on the volumetric properties of a wide spectrum of compounds, from those of low molecular weight to polymers, should be performed. We plan a series of reports on the partial volume and adiabatic compressibility of amino acids, peptides and proteins in water at various temperatures from investigations conducted in our institute [17–19]. The present paper is devoted to studying the partial volumes of amino acids in dilute aqueous solutions at 15–55 °C.

## 2. Materials and methods

All preparations utilized, except for lysine hydrochloride, were in the L-stereoisomeric form, being purchased from Sigma (U.S.A.) and used without further purification. Lysine hydrochloride (DL mixture, Reanal, Hungary) was recrystallized from alcohol prior to use.

Humidity of preparations was controlled according to a modification of the method of Fisher [20]. The moisture content was determined to an accuracy of ±0.3%. All solutions were made by weight with double-distilled water, degassed by boiling or being allowed to stand at low (< 0.1 atm) pressure. The degree of degassing was sufficiently high to prevent formation of bubbles in the measuring cell. The accuracy of the concentrations was limited by the moisture content of the preparations.

The densities were measured to a precision of $\pm 2 \times 10^{-6}$ g/cm$^3$ using a vibrating densimeter (DMA-60, Anton Paar, Austria) equipped with a measuring cell (DMA-602). The apparent volume, $\phi_V$, was calculated from the following equation:

$$\phi_V = \frac{M}{\rho} - \frac{\rho - \rho_1}{\rho \rho_1 m}$$

where $M$ denotes the molecular mass, $m$ the molar concentration, and $\rho$ and $\rho_1$ the densities of the solution and solvent, respectively.

For each estimation of $\phi_V$, two to six independent measurements were performed at a concentration of 3 mg/ml. Scatter in the data did not exceed the total instrumental error, estimated as 0.3% at this concentration.

Table 1

Apparent volume of amino acids, $\phi_V$ (cm$^3$/mol) [a]

| Compound [b] | $T$ (°C) | | | | |
|---|---|---|---|---|---|
| | 15 | 25 | 35 | 40 | 55 |
| 1. Gly | 42.4±0.1 | 43.3±0.1 | 43.8±0.1 | 43.9±0.1 | 44.3±0.1 |
| 2. Ala | 59.9±0.1 | 60.4±0.1 | 60.9±0.1 | 61.2±0.15 | 61.6±0.2 |
| 3. Nle | 107.0±0.2 | 107.6±0.3 | | 109.2±0.2 | 110.8±0.5 |
| 4. Pro | | 82.5±0.2 | | 83.6±0.2 | 84.5±0.3 |
| 5. Phe | 120.3±0.3 | 121.7±0.3 | | 123.5±0.4 | 124.9±0.5 |
| 6. Trp | 141.6±0.2 | 143.7±0.3 | | 145.6±0.4 | 147.8±0.5 |
| 7. His | 97.3±0.2 | 98.8±0.3 | 99.9±0.3 | 100.4±0.3 | 101.6±0.4 |
| 8. Met | 104.0±0.2 | 105.2±0.2 | | 107.0±0.2 | 108.1±0.5 |
| 9. Cys | 72.5±0.2 | 73.3±0.2 | | 74.7±0.2 | 75.0±0.3 |
| 10. Ser | 59.8±0.1 | 60.8±0.2 | | 61.7±0.2 | 62.2±0.3 |
| 11. Asn | 76.0±0.1 | 77.2±0.2 | | 78.8±0.2 | 79.5±0.3 |
| 12. Glu | 87.8±0.3 | 89.0±0.3 | | 90.8±0.3 | 91.8±0.4 |
| 13. Gln | 92.3±0.3 | 93.8±0.3 | | 94.9±0.3 | 96.3±0.4 |
| 14. Lys·HCl | 124.5±0.3 | 125.9±0.3 | | 127.1±0.3 | 126.6±0.4 |

[a] The mean square deviation of the average value is given as an error.

[b] Standard abbreviations are used, except for norleucine: Nle.

## 3. Results

The values for the apparent molar volume ($\phi_V$) are listed in table 1. The concentration dependences of $\phi_V$ for amino acids known from the literature [21–23] are weak, so that the difference between $\phi_V$ at 3 mg/ml and partial molar volumes at infinite dilution ($\bar{v}_2^0$) is less than experimental uncertainty. Therefore, below we do not discriminate between the terms 'partial volume' and 'apparent volume'. Our experimental uncertainty is almost 10-fold greater than that presented in many other works. It should be noted, however, that in the literature the value of reproducibility is usually taken as an error, that leads to underestimation of the absolute error. Indeed, the differences between the data presented by different authors, as a rule, exceed the declared errors (see table 2). Moreover, to a first approximation, the accuracy of our measurements seems to be sufficient for investigation of the temperature dependence of partial volumes.

Partial molar volumes of amino acids at 25°C reported in the literature are compared with our results in table 2. One observes that the data are in good agreement. The temperature dependences of $\phi_V$ are plotted in fig. 1. The solid lines represent the results of the approximation by the second polynomial. Also shown in fig. 1 are the data from dilatometric measurements (dashed lines) performed by Cabani et al. [24] on three amino acids. Their curves for serine are coincident with ours. However, the slope of the curves for glycine and alanine at 25°C is more than 2-fold greater. The discrepancy observed may be attributed to unidentified methodological difficulties in dilatometric measurements [25]. The temperature dependence of $\phi_V$ for glycine and some other nonpolar amino acids has been studied previously [26,27]. The results denoted by crosses in fig. 1 are in good agreement with our findings.

## 4. Discussion

### 4.1. The main component of the partial volume

One of the strictest representations for the partial volumes of organic molecules in water, $\bar{V}_2^0$, based on scaled particle theory [28], has been proposed by Pierotti [29]. $\bar{V}_2^0$ represents three distinct contributions:

(1) An ideal component, $\beta RT$, where $\beta$ is the isothermal compressibility of the solvent, and $RT$

Table 2

Comparison of apparent volume ($cm^3$/mol) at 25°C obtained in the present study with literature values

| Compound | This work | Literature |
|---|---|---|
| Gly | 43.3 | 43.5 [a]; 43.19 [b]; 43.19 [c]; 43.25 [d]; 43.3 [e]; 43.22 [f]; 43.22 [g]; 43.19 [h]; 43.39 [i] |
| Ala | 60.4 | 60.6 [a]; 60.47 [b]; 60.42 [c]; 60.45 [d]; 60.6 [e]; 60.40 [f]; 60.62 [i]; 60.47 [j] |
| Nle | 107.6 | 108.4 [a]; 107.93 [c]; 108.4 [4]; 107.72 [j] |
| Pro | 82.5 | 81.0 [a]; 82.83 [b]; 82.63 [c]; 82.65 [d]; 81.0 [e] |
| Phe | 121.7 | 121.3 [a]; 121.48 [b]; 122.2 [c]; 121.92 [d]; 121.2 [e]; 121.65 [f] |
| Trp | 143.7 | 144.1 [a]; 143.91 [b]; 143.8 [c]; 144.0 [d] |
| His | 98.8 | 99.3 [a]; 98.79 [b]; 98.3 [c]; 99.14 [d] |
| Met | 105.2 | 105.1 [a]; 105.35 [b]; 105.57 [c]; 105.3 [d] |
| Cys | 73.3 | 72.5 [a]; 73.44 [b]; 73.62 [d] |
| Ser | 60.8 | 60.8 [a]; 60.62 [c]; 60.62 [d]; 60.3 [e] |
| Asn | 77.2 | 78.0 [a]; 77.56 [c]; 77.18 [d] |
| Glu | 89.0 | 90.4 [a]; 85.88 [b]; 89.85 [c]; 89.36 [d] |
| Gln | 93.8 | 93.9 [a]; 93.61 [c]; 94.36 [d] |
| Lys·HCl | 125.9 | 124.76 [c]; 124.3 [k]; 125.6 [l] |

[a] Ref. 1; [b] ref. 21; [c] ref. 22, for Asn the value was obtained from Asn·$H_2O$ data by subtraction of 18.07 $cm^3$/mol (partial volume of $H_2O$); [d] refs. 23 and 51; [e] ref. 52; [f] ref. 27, for Phe average value of L- and D-isomers is presented; [g] ref. 53; [h] ref. 54; [i] ref. 55; [j] ref. 56; [k] ref. 57; [l] ref. 58, 20°C, calculated taking into account the partial volumes of HCl, 17.8 $cm^3$/mol, and of $H^+$ [60,61], ignoring the difference between 20 and 25°C.

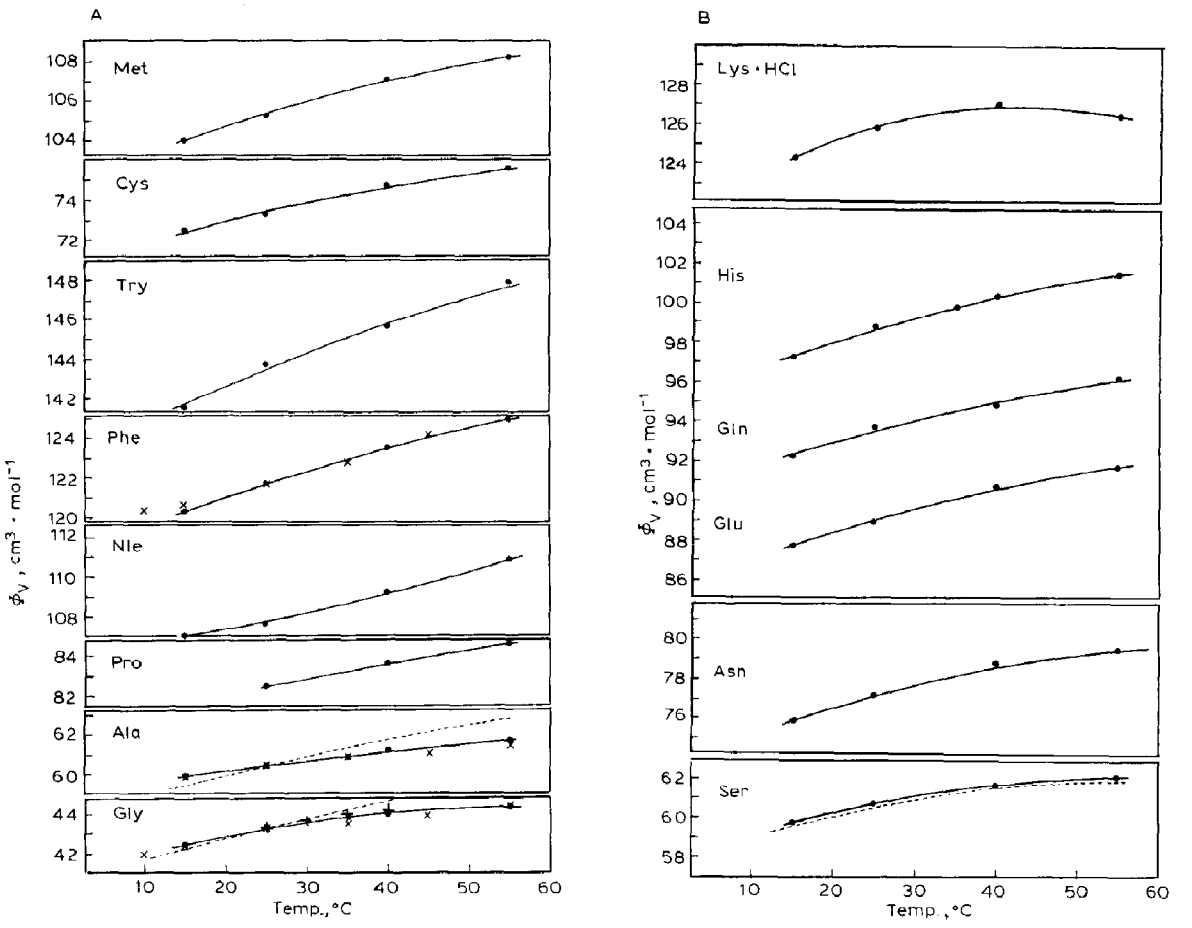


Fig. 1. Temperature dependences of apparent volumes of amino acids. Continuous lines represent the second polynomial approximation of the experimental data. Broken lines indicate data from ref. 24, and crosses denote data from refs. 26 (+) and 27 (×).

has its usual meaning. This value is small, 1.1 $cm^3/mol$ at 25° C, and is often overlooked.

(2) The volume of the solvent cavity, $V_c$, which is formed under the action of repulsive forces in the solution. This contribution is readily calculable from scaled particle theory based on a hard-sphere potential.

(3) Finally, the so-called 'interaction volume', $V_I$, resulting from mutual attraction between solute and solvent molecules, is expected to make a contribution.

The major component of $V_C$ is the volume of the molecular core, $V_M$, which is impenetrable to the solvent. This value can be approximated by the van der Waals volume, $V_M \approx V_W$. Then

$$\overline{V}_2^0 = V_C + V_I + \beta RT = V_M + V_L + V_I + \beta RT \quad (1)$$

where $V_C = V_M + V_L \approx V_W + V_L$; $V_L$ is a function of temperature, and of the density and compressibility of the solvent, taking a positive value. The term $V_L$ was interpreted as representing the void or 'empty volume' around the solute molecules required for thermal motion to occur [30,31]. Such an interpretation is not strict but is very convenient due to its geometric clarity.

There are two empirical equations for a description of partial volumes. Of these equations, the one proposed by Terasawa et al. [30] is presented here in a modified form:

$$\bar{V}_2^0 = a_V V_W + b_V + V_I \quad (2)$$

where $a_V \approx 1.5$, $b_V \approx 10$ cm$^3$/mol being similar to Traube's co-volume [2]. When comparing the equations, one observes that the value of $(a_V - 1)V_W + b_V$ of eq. 2 corresponds to the value of $V_L + \beta RT$ from eq. 1. The other empirical equation for partial volume was proposed by Glueckauf [32] and used for organic solutes by Edward and Farrell [31,33]:

$$\bar{V}_2^0 = (4/3)\pi N_a (r + \Delta)^3 + V_I' \quad (3)$$

Here, $r$ denotes the radius of the molecular core, $N_a$ Avogadro's number and $\Delta$ (= 0.055 nm) the thickness of the void volume, $V_L$ around the molecule. The latter equation coincides quantitatively with the formulae of scaled particle theory [34]; the ideal term $\beta RT$ is actually contained in $V_I'$, which is defined practically as a deviation in the experimental value $\bar{V}_2^0$ from that predicted according to the first term in eq. 3, that in fact represents the term $V_C$ in eq. 1. As shown in ref. 33, eq. 2 describes properly cylindrical molecules and eq. 3 spherical ones. Taking this into account, one can easily demonstrate that for non-polar molecules (where $V_I = 0$) with an effective radius greater than 0.2 nm, the value $V_L$ is an approximately linear function of the surface area of the molecule, $S$ [17]:

$$V_L = a_S S + b_S. \quad (4)$$

Comparing eq. 4 with eqs. 1–3 for molecules whose van der Waals volumes range from 40 to 110 cm$^3$/mol (the range corresponding to amino acids), one can obtain $b_S = 4$ cm$^3$/mol, if $S$ is the molecular (van der Waals) surface area. $S$ may be either molecular or accessible surface (see below). The other empirical coefficient, $a_S$, can be estimated immediately from an approximation of the experimental data (see below).

Thus, it is useful to consider the experimental values of $\bar{V}_2^0$ as a function of the surface area of the solute molecule. From eqs. 1 and 4:

$$\bar{V}_2^0 = V_M + a_S S + V_I + (b_S + \beta RT) \quad (5)$$

where $a_S$ is the coefficient of proportionality between $S$ and $V_L$, $(b_S + \beta RT)$ is equal to 5 cm$^3$/mol if the molecular (van der Waals) surface is considered. When studying the empirical correlations without analysis of the finer details of the physical mechanisms, the type of surface may be chosen arbitrarily. We have used the van der Waals surface computed according to Bondi [35]. The use of the accessible surface * calculated for amino acids in ref. 36 entails no essential difference (see below).

Eq. 5 is adequate for a description of a molecule of any shape. This will be discussed in more detail elsewhere.

For a qualitative interpretation of the volumetric properties in terms of the hydration effect, the following simple equation is useful:

$$\bar{V}_2^0 = V_M + \Delta V_H = V_M + n_H(\bar{V}_H - \bar{V}_1) + \beta RT \quad (6)$$

where $\Delta V_H$ denotes the volume effect of hydration, which can be represented as the difference between the partial molar volumes of water in the hydration shell $\bar{V}_H$ and in bulk water $\bar{V}_1$, multiplied by the 'hydration number' $n_H$. This equation can be considered as a consequence of eq. 1 under the assumption that $V_L + V_I = \Delta V_H$. Eq. 6 differs from the commonly used simplest expression based on the continuous medium model [37] only in the ideal term $\beta RT$.

### 4.2. Volume effects of charged and polar groups

In fig. 2, $(\bar{V}_2^0 - V_W)$ of the amino acids is plotted vs. the area of the van der Waals surface, $S_W$. $V_W$ and $S_W$ are calculated according to Bondi [35]. An increase in the surface area of the non-polar portion of the amino acids results in an increase in $(\bar{V}_2^0 - V_W)$, that indicates the contri-

* The accessible surface is defined as the locus of the center of the probe ball of radius 0.14 nm (the effective radius of a water molecule) in contact with the surface of the solute molecule.

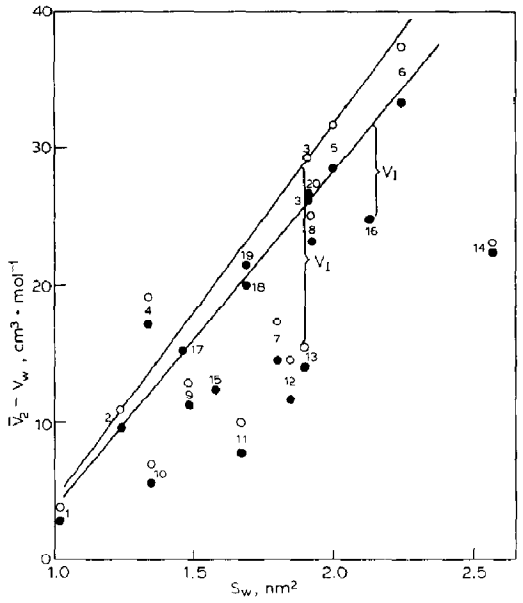


Fig. 2. Dependence of the hydration part of the partial volume on the van der Waals surface area at 25 °C (●) and 55 °C (○). Numbers indicated beside the circles correspond to those listed for the amino acids in table 1 except for threonine, N 15 [24]; tyrosine, N 16 [22–24]; α-aminobutyric acid, N 17 (averaged from refs. 1, 22, 24 and 52); valine, N 18 (averaged from refs. 1, 21–24, 27, 52 and 53); norvaline, N 19 (averaged from refs. 1 and 22); and leucine, N 20 (averaged from refs. 21–24, 27, 52 and 56). See also footnote f in table 3.

bution of $V_L$ to the partial volume, provided that the contribution of the nonpolar groups to $V_I$ is neglected [30,31,33]. The slope of the dependence is equal to 24.9 cm³/mol per nm² at 25 °C. In order to estimate the volume decrease, $V_I$, due to specific interaction of the hydrophilic groups with water, a hypothetical nonpolar group of equal surface area can be chosen as reference.

Thus, the $V_I$ values of the amino acid side chain radicals can be estimated from the deviation of the points indicating the amino acids from the straight line plotted for the nonpolar amino acids in fig. 2. As shown in fig. 2, the points corresponding to amino acids with a polar group in the side chain radical lie below the straight line, that indicates the volume decrease due to the polar group-water interaction. The point for proline lies above this line. This suggests that the zwitterion electrostriction decreases due to the reduced accessibility of charged imino groups as compared with that of the α-amino groups of the other amino acids. For glycine, a slight deviation from the line toward a volume decrease implies that electrostriction of the glycine zwitterion is stronger than that of the other amino acids having a side chain group. A similar effect of side chain substituents on amino acid zwitterions has been discussed in studies of the apparent compressibility [10,17,38,39].

In order to estimate $V_I$ for glycine, a hypothetical nonpolar molecule with an identical surface area was considered and the value $(b_S + \beta RT)$ was taken into account (see eq. 5).

The values of $V_I$ obtained in a similar manner are listed in table 3. As is evident, $V_I$ for side chain radicals possessing a carboxylate or amide group is 2-fold greater, in absolute value, than that in the case of a side chain hydroxyl group. Therefore, for every polar atom of these groups, $V_I = -6$ cm³/mol. All of the nitrogen atoms of the histidine side chain group have a somewhat smaller volume effect, $V_I = -4$ cm³/mol. (It should be noted once more that the quantitative data being considered were obtained with the van der Waals surface as the selected molecular surface; see table 3 for differences between $V_I$ obtained from the van der Waals and accessible surfaces). A similar volume decrease is observed for the side chain groups of methionine and cysteine and, hence, sulfur atoms, >S and -SH behaving as polar groups.

A weak dependence of $V_I$ on the nature of the polar groups and on temperature can be understood when it is taken into account that the volume decrease is due to the ability of solute molecules to form hydrogen bonds with water. Quantitatively, the volume effect of hydrogen bond formation can be estimated with the use of the empirical formula of Glueckauf (eq. 3). If the centers of two spherical molecules of radius 0.168 nm are situated at a distance equal to the average length of a hydrogen bond, i.e., 0.280 nm, then the spheres of radius $(r + \Delta)$ overlap, which results in a decrease in volume by about 5 cm³/mol. The value 0.168 nm corresponds to the effective radius of a water molecule, as estimated from the van der Waals volume using Bondi's radii [35]; water has been

Table 3

Volume decrease, $V_I$ ($cm^3$/mol), of glycine and side chain groups of other amino acids

| Compound [a] | WS [b] | | AS [c] | Sph [d] |
|---|---|---|---|---|
| | 25°C | 55°C | 25°C | 25°C |
| Ser | −7 | −7 | −4.5 | −5.9 (−OH) |
| Thr [e] | −5.5 | | −4 | −5.9 (−OH) |
| Tyr [e] | −6.5 | | −5 | −5.9 (−OH) |
| Asn | −13 | −13 | −9 | −11.3 ($-CONH_2$) |
| Glu | −13 | −13 | −9.5 | −10.7 (−COOH) |
| Gln | −12 | −13.5 | −7.5 | −11.3 ($-CONH_2$) |
| His | −9 | −9 | −9.5 | −8.7 (>NH×2) |
| Try | −1 | −1 | −2.5 | −4.3 (>NH) |
| Cys | −4.5 | −4.5 | −4 | |
| Met | −3 | −4.5 | −3.5 | 0 (>S) |
| $Lys \cdot H^+ Cl^-$ [f] | −20 | −24.5 | −28 | |
| $^+Gly^-$ [g] | −27 | −29 | | |

[a] Side chain groups, except for glycine.

[b] Obtained using the van der Waals surface (WS) computed according to Bondi [35].

[c] Determined using the accessible surface (AS) according to Shrake and Rupley [36].

[d] Values taken from ref. 43 for a spherical approximation (Sph) of amines and amides; the corresponding atomic groups are given in parentheses.

[e] Calculated from literature values for $\bar{V}_2^0$: 76.8 for Thr (averaged from refs. 22 and 23); and 123.7 for Tyr (averaged from refs. 22–24).

[f] For calculating $V_M$ and $V_I$ of lysine hydrochloride, $Cl^-$ was approximated by a sphere of radius 0.181 nm [59].

[g] Value calculated for the whole molecule with regard to the contributions of $\beta RT = 1.1$ $cm^3$/mol and of constant $b_S$ being equal to 4 $cm^3$/mol (see text). Temperature dependences of these terms are negligible.

chosen as a representative example of the molecule or 'atomic group' capable of hydrogen bonding. Using a simple quasi-chemical model for the equilibrium between the hydrogen bonds being formed and those being broken in pure water, one can easily see that the formation of hydrogen bonds with water is always accompanied by a shift in equilibrium for the solvent, so that the appearance of every new hydrogen bond is partially compensated by the rupture of about one-half of a hydrogen bond in water. The value of $V_I = -6$ $cm^3$/mol obtained experimentally for polar atoms, therefore, indicates that every polar atomic group of the solute molecule can form a hydrogen bond with approximately two water molecules, one hydrogen bond in water being broken. If a slightly polar atomic group forms a hydrogen bond with only one solvent molecule, the value of $V_I$ should be 50% lower.

For a tryptophan molecule, having one polar nitrogen atom on a side chain group, the volume decrease was found to be abnormally low. This is likely to be due not only to the characteristic property of this nitrogen, but also to inaccuracy in estimating the molecular surface area and impenetrable volume $V_M$ of this large complex molecule.

As one would expect, the greatest volume decrease is observed for charged molecules as a result of electrostriction. For glycine, $V_I$ was found to be $-27$ $cm^3$/mol. The same value was obtained for the side chain group of lysine hydrochloride.

The conventional method of estimating the electrostrictive effect of amino acids involves comparison of the partial volumes of the amino acids with the volumes of their uncharged isomers [40–42]. The values thus obtained ($-10$ and $-15$ $cm^3$/mol) are considerably lower than our estimates mainly due to the fact that the method mentioned does not consider the volume effect of the polar groups of the isomers. From table 3 one can see that the electrostriction effect is more pronounced at 55°C than at 25°C. This is explained by the fact that the partial volume of charged groups increases with temperature more

slowly than that of the nonpolar groups used as a reference for the determination of the electrostriction effect (see section 4.3).

By using the accessible surface (according to Shrake and Rupley [36]) instead of the van der Waals form, other values of $V_I$ are obtained, however, the difference is not significant for our consideration (table 3). The dependence of the estimates on the type of surface chosen arises from the fact that the value of $V_I$ is not exactly a linear function of the surface area, but is also determined by the curvature of the surface. If one postulates the void volume around the molecule to have a constant thickness, $\Delta$, independent of the curvature, the estimates of $V_I$ will be found to fall between the values for the two side chains presented in table 3. This question will be considered in more detail elsewhere.

Our estimates of $V_I$ are in agreement with those of Shahidi et al. [42,43] (see table 3, column 5). However, the considerable volume decrease for the thioester group, which we have determined, was not observed by those authors [42,43]. The discrepancy is probably due to the oversimplified spherical approximation employed by Shahidi et al., which does not consider the actual surface area of the molecules.

### 4.3. Temperature dependences

Figs. 3 and 4 demonstrate plots of $\partial\bar{V}_2^0/\partial T$ and $\partial^2\bar{V}_2^0/\partial T^2$ as a function of the van der Waals surface. $\partial\bar{V}_2^0/\partial T$ is an almost linearly proportional function of area for all amino acids except norleucine having a large aliphatic side chain and lysine hydrochloride with a charged side chain radical. The slope of the regression line depicted in fig. 3 is equal to 0.09 $cm^3\ mol^{-1}\ K^{-1}\ nm^{-2}$. With the exception of aliphatic amino acids and lysine hydrochloride, all the amino acids have similar negative second derivatives (fig. 4).

Some general remarks should be made before interpretation of the results obtained. The temperature dependence of the partial volumes of amino acids is not infrequently attributed to changes in the number of water molecules in the hydration shell. The changes in hydration properties of water are, however, local and embrace no more than two

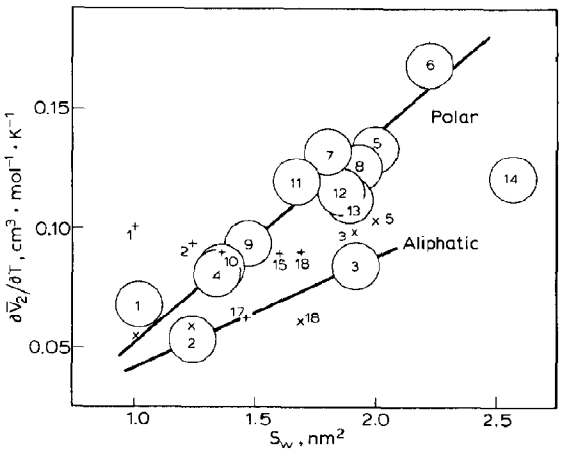


Fig. 3. First temperature derivative as a function of surface area at 25 °C. Crosses designate literature data from refs. 24 (+) and 27 (×). See legend to fig. 2 for numbering.

coordination spheres [44]. It is therefore natural to assume that the boundary of the hydration shell is coincident with that of the coordination sphere (the minimum of a radial correlation function). Clearly, then, the temperature dependence of the hydration number must be smaller than the coefficient of expansion for liquids, $10^{-3}\ K^{-1}$ on average, and hence, the temperature dependence of the

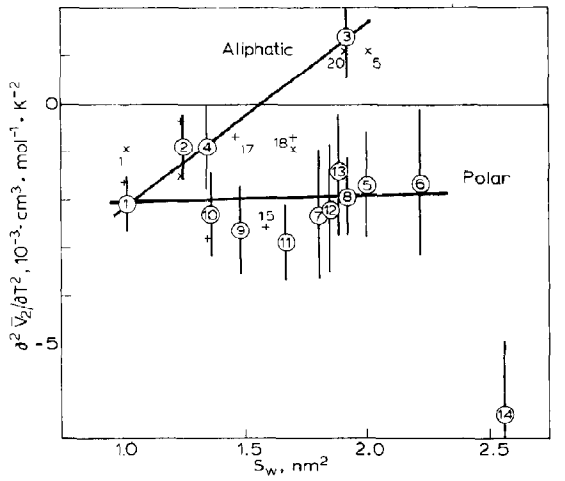


Fig. 4. Second temperature derivative as a function of surface area within the temperature range 15–55 °C. Crosses designate literature data from refs. 24 (+) and 27 (×). See legend to fig. 2 for numbering.

hydration number can be ignored. Consequently, differences in the temperature dependence of the partial volumes observed in experiments can be ascribed to variations in the properties of water molecules in the hydration shell and in pure solvent rather than to change in the hydration number. From eq. 6 it follows that, at $n_H = \text{const}$,

$$\frac{\partial \bar{V}_2^0}{\partial T} = \frac{\partial V_M}{\partial T} + n_H\left(\frac{\partial \bar{V}_H}{\partial T} - \frac{\partial \bar{V}_1}{T}\right) + \frac{\partial(\beta RT)}{\partial T} \quad (7)$$

and

$$\frac{\partial^2 \bar{V}_2^0}{\partial T^2} = \frac{\partial^2 V_M}{\partial T^2} + n_H\left(\frac{\partial^2 \bar{V}_H}{\partial T^2} - \frac{\partial^2 \bar{V}_1}{\partial T^2}\right) + \frac{\partial^2(\beta RT)}{\partial T^2} \quad (8)$$

As is well known, pure water has a strongly nonlinear temperature dependence with an abnormally small first derivative and abnormally large second, positive, derivative, as compared with normal liquids. The explanation for this behavior is that the temperature rise involves not only an increase in the amplitude of thermal motion, but also structural changes of the liquid: the proportion of the strong hydrogen bonds providing an open packing of water molecules decreases with rising temperature. If the solute suppressed the lability of the water structure, the temperature dependence would become more linear, as occurs for normal liquids. In this case, as indicated by eqs. 7 and 8, the second derivative of the partial volume would be negative and the first derivative positive. On the contrary, if the lability of the water structure is increased, the curvature of the temperature dependence can also increase, thus resulting in a positive value for the second derivative.

The behavior of amino acid solutions can be analysed in terms of this simple qualitative model.

In the neighborhood of charged atomic groups of the zwitterion skeleton of an amino acid, $^+NH_3CHCOO^-$, water molecules are exposed to a strong electric field. As a result, water ceases to display abnormal properties, exhibiting a more linear temperature dependence of the volume. As a consequence, $\partial^2 \bar{V}_2^0/\partial T^2 < 0$ for the majority of amino acids. Such behavior of the partial volume of amino acids is similar to that of electrolytes [45,46]. Assuming that the volume of the hydration complex is a linear function of temperature, $\partial^2(V_M + n_H \bar{V}_H)/\partial T^2 = 0$, one can easily calculate the hydration number $n_H$ from eq. 4. Similar calculations have been carried out for electrolytes [47]. For uni/univalent electrolytes, $n_H$ was found to be 20–30. This value slightly exceeds the number of water molecules that can occupy the first coordination spheres of a pair of ions. Using the same assumptions, a value of $n_H = 14 \pm 4$ was calculated for glycine from eq. 4 by using $\partial^2 V_1/\partial T^2 = 1.6 \times 10^{-4}$ and $\partial^2(\beta RT)/\partial T^2 = 1.5 \times 10^{-4}$ cm$^3$/mol per K$^2$ [48]. The value obtained was shown to be slightly lower than the number of water molecules, 18, closely packed in the immediate vicinity of the glycine molecule. (The latter number was calculated from the accessible surface area of the amino acid atomic group [36] on the basis of one water molecule occupying about 0.10 nm$^2$ of surface.)

For lysine hydrochloride, an additional pair of charges results in a further decrease in the second derivative (fig. 4). Aliphatic radicals, as seen from fig. 4, contribute positively to $\partial^2 \bar{V}_2^0/\partial T^2$. This property of aliphatic radicals has long been known, being interpreted as the result of the 'structure-forming' effect of these radicals [45,49]. The validity of using the sign of $\partial^2 \bar{V}_2^0/\partial T^2$ as a criterion to assess whether a structure-forming ($> 0$) or structure-breaking ($< 0$) effect on water takes place needs critical consideration [17,44]. This problem will be dealt with in a later report. In any case, the positive contribution of the second derivative indicates that, for the hydration shell of aliphatic groups, the curvature for the temperature dependence of the volume increases compared with that of pure water, i.e., water becomes more 'abnormal'.

Amino acids having polar side chain radicals do not differ from glycine in $\partial^2 \bar{V}_2^0/\partial T^2$ within the experimental uncertainties. Therefore, the side chain radicals practically do not affect the curvature for the temperature dependence of the water volume. This is likely to be due to mutual compensation of the opposing effects of polar and aliphatic atoms of the radical.

The behavior of the aromatic amino acids, phenylalanine and tryptophan, was found to be

somewhat surprising. As judged from the temperature dependence, $\bar{V}_2^0$, they behave like typical polar amino acids (figs. 3 and 4), whereas on the basis of the absolute values of $\bar{V}_2^0$, they are more characteristic of nonpolar aliphatic amino acids (fig. 2). Whether such behavior is related to the particular properties of the interactions of $\pi$-electrons with water, or is due to differing behavior of small and large molecules and atomic groups [50] upon hydration remains to be elucidated. However, according to ref. 27, phenylalanine shows noncontradictory behavior: both the absolute value of $\bar{V}_2^0$ and its temperature derivatives are within the range for nonpolar amino acids in figs. 2–4. Additional independent measurements are required.

## 5. Conclusion

In summary, volume data have been obtained for aqueous solutions of amino acids within a relatively wide temperature range. The differences in behavior between charged, polar and nonpolar atomic groups have been considered. The approach presented for the evaluation of volume effects of polar and charged groups is, in a physical sense, similar to those proposed by Edward and Farrell [31,33] and Terasawa et al. [30], who also used a typically nonpolar molecule as a reference for the evaluation of volume effects. However, it is a distinctive feature of our approach that the surface area of the molecule, in addition to the van der Waals volume, is considered as an essential parameter in comparing polar and nonpolar molecules. In my opinion, this will allow one to construct a more adequate additive scheme for a description of the partial volumes of molecules with a complex configuration, in particular proteins. Then, the qualitative and quantitative data on volume effects obtained here can be used in analysing the hydration contribution to protein volume as well as the relationship between the temperature dependences of partial volumes and the structure of amino acids.

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