Partial molar volumes and expansibilities of some amino acids in water at 35°C

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Partial molar volumes of ten amino acids, dl-alanine, l-arginine, glycine, l-histidine, l-isoleucine, l-lysine-HCl, l-methionine, l-proline, l-serine, and dl-threonine, in water, have been calculated from density measurements made at 35°C. Amino acid-water interactions are interpreted from partial molar volume, $V^0$, data with particular reference to structural features of solute molecules, such as hydrogen bonding, side group interactions, etc. Comparison between present and previous data (at 25°C) shows that $V^0_{35}$ values are higher by 1-3 cm$^3$ mol$^{-1}$ than the respective $V^0_{25}$ data. Hydration volumes are estimated using different estimates of intrinsic volumes. Isobaric expansibilities, $E^0$, are also calculated which indicate correlation between $E^0$ and size, and between $E^0$ and hydrophobicity of solute molecules.

In aqueous medium, amino acids exist as dipolar ions manifesting a unique hydration behaviour which appears to be subtly linked to the vital biological phenomenon. Because of such subtle linkage study of amino acids, peptides and proteins are considered important in unfolding the role of dipolar ions in the living phenomenon. Although the subject of dipolar ion hydration has been extensively studied in the past1-15, studies concerning temperature and pressure effects on dipolar ion hydration are not as extensive and are needed for further understanding of the subject.

Several biological processes involve expansion and contraction of protein molecules resulting from temperature and pressure variations in the living systems. Such processes include fever, hypothermia, anaesthesia, etc16-17 and study of these processes requires fundamental information about volumetric properties of proteins. Amino acids are models well suited for the estimation of volumes and related properties of proteins.

Extensive volumetric data have been reported on amino acids at 25°C. We understand that much more relevance and significance can be achieved by studying compounds of biological importance at temperatures close to physiological temperatures 35°C being close to the optimum temperature of several living species offers a better choice for such experimentation.

In this work, we report partial molar volumes of ten amino acids, viz., dl-alanine, l-arginine, glycine, l-histidine, l-isoleucine, l-lysine-hydrochloride, l-methionine, l-proline, l-serine and dl-threonine, in water, at 35°C. The choice of temperature (35°C) was made in order to (a) examine the variation in hydration characteristics of amino acids, if any, at this temperature (b) obtain amino acid data for use in the computation of protein volumes at 35°C and (c) estimate amino acid expansibilities by comparing present and previous data at 25°C.

Materials and Methods

The compounds studied in this work were dl-alanine, l-isoleucine, l-lysine-HCl and l-methionine (Merck), l-arginine, l-histidine and l-serine (Sigma), glycine (Riedel), l-proline (BDH) and dl-threonine (Kento Chem.). These were further purified by recrystallising from ethanol-water mixtures. After recrystallisation compounds were dried under vacuum at room temperature. Water used in the experiments was doubly distilled, deionised and degassed.

Density measurements were carried out at 35°C using 20 ml pyknometer flasks (Pyrex), thermostated in a LAUDA RC3 thermostat bath providing a temperature control better than ± 0.01 °C. Calibration of pyknometers was done using water18 and NaCl19,20. Reproducible $\Delta d$ values were obtained through careful experimentation providing an accuracy of ± 0.5 $\times$ 10$^{-4}$ g cm$^{-3}$ in the density data. The molalities of solutions ranged from 0.01 to 0.35 m in most cases.

Results and Discussion

Apparent molal volumes ($\phi_v$, cm$^3$ mol$^{-1}$) were calculated using Eq. (1).
\[
\phi_v = \frac{100 (d_v - d) + M}{m d d_v} \quad \ldots \ (1)
\]

where \(d, d_v, m\) and \(M\) are the densities of solution, solvent, molality and the relative molar mass of solute, respectively. \(\phi_v\) data obtained from Eq. (1) were fitted to Eq. (2) using a weighted least squares fit program.

\[
\phi_v = \phi_v^0 + S_m \quad \ldots \ (2)
\]

Values of \(\phi_v^0\), i.e., the apparent molal volume at infinite dilution were taken as the partial molar volumes at 35°C, \(\phi_v^{35}\), and are reported in Table 1, along with values of partial molar volumes at 25°C, \(\phi_v^{25}\), selected from literature, for comparison.

The accuracy in \(\phi_v\) and \(\phi_v^{35}\) data is a function of the accuracy in density and molality data. In comparison to the resolution obtained from the state-of-the-art techniques such as vibrating tube electronic densitometry\(^2\) where accuracy in density data is of the order of \(\pm \ 1.3 \times 10^{-6}\) g cm\(^{-3}\), present density values are accurate to within \(\pm 0.5 \times 10^{-4}\) g cm\(^{-3}\). Errors in the apparent molal volumes (\(\Delta \phi_v\)) were computed using differentiated form of Eq. 1 (with respect to density and molality), and were employed as weighting parameters in the least squares fit computations.

### Solute-solvent interactions

Volumetric studies of amino acids and other zwitter ions reported in literature at 25°C represent some important features of dipolar ion hydration\(^1\)\(^-\)\(^15\), summarised as follows: (i) \(\text{NH}_3^+\) and \(\text{CO}_2^-\) terminals, being charged ends produce strong electrostrictive compression around the solvent, while the intervening part of the molecule interacts with the solvent in a manner that depends largely on whether the residue is hydrophobic, hydrophilic or amphiphilic. (ii) The electrostrictive compression due to \(\text{NH}_3^+\) group is higher than that of the \(\text{CO}_2^-\) group by about 10 times\(^3\). (iii) Any substituent group falling near \(\text{N}\)-terminal produces greater value change than that produced by a group adjacent to the \(\text{C}\)-terminal as a result of the overlapping of neighbouring hydration cospheres.

Present data at 35°C appear to be consistent with these observations indicating the temperature independence of these qualitative features of dipolar-ion hydration. Comparison between \(\phi_v^{35}\) (present data) and \(\phi_v^{25}\) values (from literature), however, shows that present values are higher by 1-3 cm\(^3\) mol\(^{-1}\). In the case of \(l\)-arginine this difference is even higher (\(\approx 7\) cm\(^3\) mol\(^{-1}\)). This increase at higher temperatures is generally attributed to the increase in hydration volumes.

A view of \(\phi_v^{35}\) data indicates that a rough correlation exists between \(\phi_v^{35}\) and molar mass of the compounds. Deviations from this correlation, however, are also noted which reflect contributions of structural features of solute molecules towards solute-solvent interactions. Hydrogen bonding, for instance, is one notable contribution\(^28\). A view of solutes with and without hydrogen bonding sites illustrates this effect clearly. For example values of \(\phi_v^{35}\) of \(dl\)-alanine and \(l\)-serine are found to be about the same (61.9 cm\(^3\) mol\(^{-1}\)) while their molar masses differ by 16 mass units (\(dl\)-alanine = 89.09 g mol\(^{-1}\), \(l\)-serine = 105.1 g mol\(^{-1}\)). The side group in alanine is \(-\text{CH}_3\), and in serine it is \(-\text{CH}_2\text{OH}\). Keeping in view the comparative size of these groups a lower value of \(\phi_v^{35}\) for alanine was expected. On the contrary, nearly same values for both these compounds seem to represent the shrinkage due to hydrogen bonding between the \(-\text{OH}\) of serine and surrounding solvent.

### Table 1—Partial molar volumes and expansibilities of amino acids studied

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\phi_v^{35}) ((\text{cm}^3\text{ mol}^{-1}))</th>
<th>(\phi_v^{25}) ((\text{cm}^3\text{ mol}^{-1}))</th>
<th>(E^0) ((\text{cm}^3\text{ mol}^{-1}\text{K}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(dl)-Alanine</td>
<td>61.99 (\pm 1.19)</td>
<td>60.42 (\pm 0.02)</td>
<td>0.16</td>
</tr>
<tr>
<td>(l)-Arginine</td>
<td>131.10 (\pm 0.05)</td>
<td>123.86 (\pm 0.09)</td>
<td>0.72</td>
</tr>
<tr>
<td>Glycine</td>
<td>44.52 (\pm 0.03)</td>
<td>43.19 (\pm 0.02)</td>
<td>0.13</td>
</tr>
<tr>
<td>(l)-Histidine</td>
<td>100.35 (\pm 0.99)</td>
<td>98.30 (\pm 0.10)</td>
<td>0.21</td>
</tr>
<tr>
<td>(l)-Isoleucine</td>
<td>107.13 (\pm 0.96)</td>
<td>105.80 (\pm 0.07)</td>
<td>0.13</td>
</tr>
<tr>
<td>(l)-Lysne HCl</td>
<td>126.43 (\pm 1.44)</td>
<td>124.76 (\pm 0.08)</td>
<td>0.17</td>
</tr>
<tr>
<td>(l)-Metionine</td>
<td>108.11 (\pm 1.03)</td>
<td>105.57 (\pm 0.02)</td>
<td>0.26</td>
</tr>
<tr>
<td>(l)-Proline</td>
<td>85.13 (\pm 0.96)</td>
<td>82.63 (\pm 0.05)</td>
<td>0.25</td>
</tr>
<tr>
<td>(l)-Serine</td>
<td>61.96 (\pm 1.22)</td>
<td>60.62 (\pm 0.03)</td>
<td>0.13</td>
</tr>
<tr>
<td>(dl)-Threonine</td>
<td>78.04 (\pm 0.40)</td>
<td>76.83 (\pm 0.04)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

a: From ref. 10; b: figures in brackets represent number of points used in least squares fit of data.
molecules, an interaction missing in the case of alanine.

Similar discrepancies are seen in some other cases, as well, e.g., isoleucine ($V_{35}^0 = 107.13 \text{ cm}^3 \text{ mol}^{-1}$, molar mass = 131.1 g mol$^{-1}$) and methionine ($V_{35}^0 = 108.13 \text{ cm}^3 \text{ mol}^{-1}$, molar mass = 194.1 g mol$^{-1}$). A difference of 63 mass units seems to disappear in their $V_{35}^0$ values. $V_{35}^0$ (methionine) is much smaller than expected on the basis of $V^0$-molar mass correlation, which is presumably due to hydrogen bonding between $\text{S}^-$ of methionine and the surrounding solvent molecules. The hydrophobic portion of these molecules do not seem to be very dissimilar from each other and hence volume change due to these may not be too dissimilar as well. Data concerning lysine-arginine pair and threonine-proline pair also tend to highlight similar specific interactions by the side groups.

**Hydration volumes**

On molecular level, partial molar volumes are conventionally described in terms of intrinsic and hydration volumes of solute molecules, $V_i$ and $V_h$, respectively.

$$V^0 = V_i^0 + V_h^0 \quad \ldots (3)$$

The hydration volume, is the volume between solute boundary and the peripheral sheath of solvent molecules and hence a function of the distance of closest approach taken as minimum. Equation 3 helps in estimating $V_h$ values if appropriate values of intrinsic volumes are available.

Estimation of intrinsic (skeletal) volume of a solute, however, is not always easy. Ideally, such an estimation implies measurement of the entire unsmooth and bumpy surface of large and twisted solute molecule, a task that appears to be more hypothetical than empirical. In literature, practical measures of skeletal volumes are, however, described in terms of either van der Waals' volumes (often calculated by the addition of group contributions) or crystal volumes obtained by averaging volumes of crystal lattices.

$V_i$ values obtained through these practical measures are prone to errors due to approximations used in correction terms, e.g., fluctuation volumes$^{22}$, reduction factors in van der Waals' volumes$^{20}$ (overlapping of groups), packing voids in crystals$^{24}$ etc. Different estimates of skeletal volumes, therefore, give rise to different values of hydration volumes when used in Eq. 3. Studies using these practical measures of $V_i$ show that such intrinsic volumes are found higher than respective $V^0$ values, giving rise to negative values of hydration volumes.

The discrepancies inherent in estimating various practical measures of $V_i$ emphasize the need for more realistic intrinsic volumes.

Guided by the intuition that intrinsic volumes ought to be smaller than any other measure of solute volume, one can try obtaining a situation where solute shrinks to the lowest limit of volume close to the required estimate of a minimum intrinsic volume. Ideally, molar volume at 0 °K might serve as the best estimate of the lowest volume limit, yet practically this amounts to the method of crystal volumes and again prone to the errors mentioned earlier. Another approximation may be a situation where solute experiences the highest compression from surroundings shrinking down to a minimum volume limit. This model may either be realised (i) theoretically through computational procedures, e.g., involving simulations of a probe (solvent) molecule travelling around the solute surface, with the distance of closest approach taken as minimum, or (ii) through an empirical situation where the solute is compressed by the solvent at maximum density. The holes and crevices that are inaccessible by the probe (or the solvent at its maximum density), of course, remain as part of the built-in voids. Among these two possibilities the empirical situation described in (ii) above can be taken as a convenient approximation to the minimum solute volume. Partial molar volume of solute at the temperature of maximum density, $V_{\text{tmd}}$, may, therefore be taken as a realistic substitute of $V_i$.

Using this approach, we calculated $V_h$ values of selected amino acids using $V_{\text{tmd}}$ as the intrinsic volumes. In aqueous systems, the temperature of maximum density is 3.98°C at amino acid concentrations tending to zero$^{26,27}$.

$V_{\text{tmd}}$ values in selected cases were obtained using regression parameters reported in literature$^9$ and were used as $V_i$ in Eq. 3. $V_h$ values calculated in this manner are shown in Table 2 and are found as positive, as compared to the negative hydration volumes obtained through previous methods$^4$. A comparison between $V_h$ values obtained from these two approaches is not possible because of obvious differences in the choice of $V_i$ data. Notwithstanding possible objections in treating $V_{\text{tmd}}$ as $V_i$, we place this proposition open for further comments and investigations.

**Isobaric expansibilities**

Considering the temperature derivative of Eq. 3, it can be seen that the change in intrinsic volume due to temperature may not be perceptible within the small temperature range under study (25 to 35 °C) and
Table 2—Various estimates of \( V_i \) (intrinsic) and \( V_h \) (hydration) volumes

<table>
<thead>
<tr>
<th>Compound</th>
<th>( V_{35}^i )</th>
<th>( V_i^a )</th>
<th>( V_i^{mol} )</th>
<th>( V_h^c )</th>
<th>( V_h^d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>dl-Alanine</td>
<td>61.99 ± 1.19</td>
<td>64.98</td>
<td>58.58</td>
<td>-9.74</td>
<td>3.41</td>
</tr>
<tr>
<td>l-Arginine</td>
<td>131.10 ± 0.05</td>
<td>131.47</td>
<td>-14.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycine</td>
<td>44.52 ± 0.03</td>
<td>46.93</td>
<td>40.75</td>
<td>-6.89</td>
<td>3.77</td>
</tr>
<tr>
<td>l-Histidine</td>
<td>100.35 ± 0.99</td>
<td>109.91</td>
<td>-21.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>l-Isoleucine</td>
<td>107.13 ± 0.96</td>
<td>109.23</td>
<td>104.10g</td>
<td>-13.47</td>
<td>3.03</td>
</tr>
<tr>
<td>l-Lysine HCl</td>
<td>126.43 ± 1.44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>l-Methionine</td>
<td>108.11 ± 1.03</td>
<td>113.81</td>
<td>-17.55</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>l-Proline</td>
<td>85.13 ± 0.96</td>
<td>83.67</td>
<td>-7.25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>l-Serine</td>
<td>61.96 ± 1.22</td>
<td>66.43</td>
<td>58.1</td>
<td>-4.97</td>
<td>3.86</td>
</tr>
<tr>
<td>dl-Threonine</td>
<td>78.04 ± 0.40</td>
<td>79.47</td>
<td>75.54</td>
<td>-9.70</td>
<td>2.50</td>
</tr>
</tbody>
</table>

\( a: \) From ref. 4 & 25, \( b: \) calculated from regression coefficients reported in ref. 9, \( c: \) calculated using \( V_i = (0.7)(0.634)V_c \), as reported in ref. 4, \( d: \) calculated from eq. 3 using \( V_i = V_{mol} \); \( e: \) from ref. 13.

Therefore, observed increase in \( V^0 \) would be essentially due to the increase in hydration volume. The increase in thermal agitation of solvent molecules at higher temperatures results in the increase in the distance of closest approach between solute and solvent molecules, widening the intervening space between the two, hence producing greater hydration volumes.

Assuming that a linear variation of \( V^0 \) occurs within this temperature range, partial molar expansibilities, \( E^0 \) (= \( \frac{\partial V^0}{\partial T} \)), of these compounds were calculated by taking \( \partial V^0 \) as \( (V^0_{35} - V^0_{25}) \) and \( \partial T = 10^\circ C \). \( E^0 \) values obtained in this manner are shown in Table 2. In terms of accuracy these values fall within the experimental uncertainties and therefore do not appear to be very significant. Nevertheless, they seem to represent two important correlations (i) between \( E^0 \) and solute size, and (ii) between \( E^0 \) and solute hydrophobicity. Glycine, for instance, being the smallest molecule in this series (molar mass = 75.07 g mol\(^{-1}\)), has the expansibility value of 0.13 cm\(^3\) mol\(^{-1}\) K\(^{-1}\) while large sized molecule such as histidine (molar mass = 155.2 g mol\(^{-1}\)) has a value of \( E^0 = 0.21 \) cm\(^3\) mol\(^{-1}\) K\(^{-1}\). Another comparison between histidine and methionine highlights the \( E^0 \)-hydrophobicity correlation. Methionine (molar mass = 149.21 g mol\(^{-1}\)) has the expansibility value of 0.27 cm\(^3\) mol\(^{-1}\) K\(^{-1}\), higher than the \( E^0 \) of histidine. Despite being smaller molecule, methionine carries a more hydrophobic residue, \( \text{CH}_3\text{S}--\text{CH}_2--\text{CH}_2-- \) than that of histidine. Lysine HCl shows a relatively smaller \( E^0 \) value (0.17 cm\(^3\) mol\(^{-1}\) K\(^{-1}\)) despite being the largest in size among the compounds studied (molar mass = 182.65 g mol\(^{-1}\)). The ionic character of this compound seems to offset the size effect.
The correlation of expansibility with size and hydrophobicity of solutes has been noted in some previous studies, as well. Reading and Hedwig's recent data on peptides at different temperatures indicate similar correlations. E° values calculated from their data show that E° of a hydrophobic peptide (glycyl-dl-leucine, E° = 0.13 cm⁻³ mol⁻¹ K⁻¹) was higher than an amphiphilic (glycylglycine, E° = 0.08 cm⁻³ mol⁻¹ K⁻¹) and a hydrophilic peptide (glycyl-dl-serine, E° = 0.044 cm⁻³ mol⁻¹ K⁻¹).

Coefficient of thermal expansion, α = (V°/V)(∂V°/∂T), is a quantity that eliminates volume dimension from expansibility property and reflects on the qualitative characteristics of solute hydration with respect to temperature. A problem about α values, however, is the loss of precision in calculating this quantity from V° data. In this work we attempted calculation of α values, but the results being insignificant were not reported. A survey of α values of similar compounds reported in literature also shows wide disagreements indicating the extent of uncertainty associated with α data.

References
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