Interaction of water vapour with twenty different poly-L-amino acids and their excess hydration in presence of sodium chloride

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Using the isopiestic vapour pressure technique, the magnitudes of excess binding of water and NaCl per mole of twenty different poly-L-amino acid residues, respectively in the presence of different bulk molefractions (X2) of NaCl have been evaluated from the mathematical expressions for the Gibbs surface excesses. At certain high ranges of NaCl concentration, the plot of −Γ1 vs. X1/X2 becomes linear, so that moles of water and NaCl, respectively bound per mole of amino acid residue can be evaluated. Γ1 is the excess moles of H2O per mole of amino acid residue and X1 and X2 stand for mole fractions of the water and NaCl, respectively in the sample system. Also, using the integrated form of the Gibbs absorption equation, the values of standard free energy change (ΔG°) for the excess adsorption of NaCl per kg of poly-L-amino acids have been evaluated. These values are all positive as a result of positive excess hydration of polyamino acids. The standard free energy of excess hydration ΔG°hyd (equal to −ΔG°) is negative due to spontaneous excess hydration of polyamino acid in the presence of a salt.

Extensive research work has been carried out on hydration of biopolymers in the presence of salts using various physico-chemical techniques. However, the role played by solvent and solute in controlling the process of hydration appears to be complex in nature. An useful thermodynamic concept of preferential hydration of biopolymer was developed by Schachman and Lauffer. This has further been elaborated by several other workers. The thermodynamic treatment elaborately discussed by Kuntz and Kauzmann has been used to calculate preferential interaction of water and solute with biopolymers of various types using sedimentation, light scattering, densitometry and other techniques.

The thermodynamic theory of excess adsorption or binding of solute and solvent at an interface was developed by Willard Gibbs as early as in 1876. Bull and Breese presented a theory of solute and water binding to proteins, using osmotically corrected isopiestic technique for the study of excess binding of solute and water to egg albumin. From a thermodynamic analysis, Chattoraj et al. have shown that the preferential binding of water developed by Schachman and Lauffer and the excess binding of solvent by a protein in the presence of salt are exactly similar with each other. Earlier, experimental method of Bull and Breese has been used by Chattoraj and co-workers to study the binding of water and solutes to proteins, nucleic acids, polysaccharides, lipids and fatty acids. This technique has been used now to measure the excess binding of water and NaCl to twenty different poly-L-amino acids (PAA’s). From these data, the excess binding of H2O and NaCl, respectively by side chain groups of amino acids have been evaluated. The values of standard free energy change (ΔG°) and related thermodynamic parameters, due to the excess hydration of PAA’s have been studied, using appropriate thermodynamic equations and their values have been critically compared.

Materials and Methods
Amino acid monomers forming different PAA’s are following: (I) PAA’s with non-polar side chain groups: alanine, valine, leucine, isoleucine, proline, phenyl alanine, tryptophan and methionine; (II) PAA’s with uncharged polar R groups: glycine,
serine, threonine, cbz-L-cystine, tyrosine, asparagine and glutamine; (III) PAA’s with ionic polar R groups: aspartate, glutamate, lysine bromide, arginine chloride and histidine chloride.

All PAA samples were from Sigma Chemical Co., USA. Na-Poly-L-glutamate, was a gift from Peptide Institute, Japan. NaCl (AR grade) was obtained from E. Merck (India). Double distilled water was used throughout the investigation.

All the PAA’s were dried completely in a desiccator containing conc. H\textsubscript{2}SO\textsubscript{4}, before use until their weights became constant. Isopiestic vapour pressure method\textsuperscript{12,13} was used for the measurement of hydration of polyamino acids in the presence of electrolyte. A definite amount (0.1 to 0.2 g) of a dry poly-L-amino acid along with a definite volume of NaCl solution of known molarity were taken in specially designed, previously weighed dry sample glass bottle. After removing lid, the weighing bottle containing the powdered sample was allowed to float on 100 ml of NaCl solution, taken in a specially designed desiccator with the help of a tripod stand, made of glass suitably dipped in the salt solution. The salt solution in the desiccator is termed as reference solution. The strengths of the reference solution at isopiestic equilibrium varied from 0.5 to 5.0 M. The desiccator was closed, evacuated appropriately and kept in an air thermostat for 7 days. At a constant temperature free exchange of water vapour occurred between the open sample bottle containing PAA and NaCl and the magnetically stirred reference salt solution until state of isopiestic equilibrium was attained by the system. The sample bottle was then taken out of the desiccator, quickly closed with the lid, washed from outside and dried with tissue paper. The bottle was then weighed and total moles (n\textsubscript{2}) of water associated per mole of residue (or per kg of dry sample) was obtained. Total mole (n\textsubscript{2}) of NaCl associated per mole of the residue (or per kg of PAA) in the sample at isopiestic equilibrium was also calculated from the known weight of the salt initially present along with known weight of polyamino acid in the sample bottle. The value of excess moles (\Gamma\textsubscript{2}) of NaCl adsorbed per mole of amino acid residue (or 1 kg PAA) was calculated using the following equation:

\[ \Gamma_2 = \frac{W_i}{1000} (m_2 - m_1) \quad \text{... (1)} \]

Here, m\textsubscript{1} is equal to \( \frac{1000n_1}{18n_1^M} \), so that its value can be calculated directly from the values of n\textsubscript{1} and n\textsubscript{2} per mole of residue (or per kg of PAA), which can be obtained from the experiments. W\textsubscript{i} is the total amount of water per mole of residue (per kg of PAA) in the sample bottle determined from experiment. The free molality of the solute dissolved in the free solvent in the sample bottle is m\textsubscript{2}. Assuming that polyamino acid in the sample bottle contributes negligibly to vapour pressure at isopiestic equilibrium, we can put m\textsubscript{2} = m\textsubscript{2}', so that value of \Gamma\textsubscript{2}' at a given value of m\textsubscript{2}' (or m\textsubscript{2}') can also be obtained using the experimental data. The validity of this assumption has been discussed later on. When m\textsubscript{1} > m\textsubscript{2}', then \Gamma\textsubscript{2} is positive, but \Gamma\textsubscript{2}' is negative when m\textsubscript{1} < m\textsubscript{2}'.

Replacing m\textsubscript{1}, m\textsubscript{2} and W\textsubscript{i} in Eq. (1) by \( \frac{1000n_2}{M_1n_1^M} \), \( \frac{1000n_2}{M_1n_1^M} \), and M\textsubscript{1}n\textsubscript{1}, respectively (M\textsubscript{1} being molecular wt. of H\textsubscript{2}O), equation (2) can be obtained:

\[ \Gamma_2' = n_2 - n_1 \left( \frac{n_2}{n_1} \right) \quad \text{... (2)} \]

or

\[ \Gamma_2' = n_2 - n_1 \left( \frac{X_2}{X_1} \right) \quad \text{... (3)} \]

where, X\textsubscript{1} and X\textsubscript{2} stand for mole fraction of the water and NaCl, respectively remaining free in the sample system.

Similarly, excess moles (\Gamma\textsuperscript{2}) of solvent per mole (or per kg) of PAA can be defined from Eq. (3) as:

\[ \Gamma_1^2 = n_1 - n_2 \left( \frac{X_1}{X_2} \right) \quad \text{... (4)} \]

or,

\[ \Gamma_1^2 = -\Gamma_2' \left( \frac{X_1}{X_2} \right) \quad \text{... (5)} \]

so that

\[ \Gamma_1^2 X_1 + \Gamma_2' X_2 = 0 \quad \text{... (6)} \]
and $\Gamma_1^2$ are thus not independent, but related to each other\textsuperscript{14-23}, through Eqs (5) or (6). The values of $\Gamma_1^2$ can be calculated, if values of $\Gamma_1^1$ are known.

Results and Discussion

Recently, Ghosh et al.\textsuperscript{23} have studied hydration of 20 different polyamino acids between the range of water activity ($a_1$) zero to unity using isopiestic vapour pressure method. No neutral salt was used in that study. At monolayer saturation state at an intermediate value of $a_1$, moles $n_1$ of water bound to one mole of amino acid residue obtained from the BET plot are observed to be relatively small. But, in the higher range of $a_1$, $n_1$ values sharply increase due to the formation of multilayer of water at the powdered PAA interface until at water activity, $a_1$ equal to 1, maximum value of $n_1$ becomes equal to $\Delta n_1^0$. Adsorbed layer of water molecules at $a_1$ equals to unity is inhomogeneous in character\textsuperscript{24}. The water molecules directly attached to the surface of solid powder, thus forming primary water layer at low values of $a_1$ are strongly attached to the PAA, by adsorption forces\textsuperscript{26}. Beyond monolayer state, the adsorbed molecules of water attached further to PAA may form secondary layer, which remains fixed within multilayers, due to the presence of relatively weak forces below $a_1$ equal to 0.92\textsuperscript{25-26}. For $a_1$ above 0.92, water molecules still associated with the surface are assumed to form a tertiary layer, when adsorption forces are very weak in character. These weakly attached water molecules may be responsible for swelling of powdered biopolymers. The inhomogeneous surface layer of water of various types may remain in equilibrium\textsuperscript{24} in contact with the bulk phase of water only at $a_1$ equal to unity. The nature of the inhomogeneous water layers to globular proteins at $a_1$ equal to unity has been discussed by Chattoraj and Mitra\textsuperscript{24}, earlier. The values of $\Delta n_1^0$ for different PAAs at unit water activity in the absence of the salt are presented in Table 1.

In the presence of equilibrium concentrations of NaCl, ranging from 1.0 to 5.0 $M$, the $a_1$ of the vapour in contact with PAA present the sample bottle will remain in the range of 0.90 to 0.99, depending upon values of $m_2$ during the experimental measurement. Let $\Delta n_1$ and $\Delta n_2$ moles of water and salt, respectively bound per mole of residue of PAA are in

<table>
<thead>
<tr>
<th>Poly-L-amino acids</th>
<th>$\Delta n_1$ mol (H$_2$O/per mol PAA)</th>
<th>$\Delta n_2$ mol (NaCl/PAA mol)</th>
<th>$\Delta n_1^0$ mole (to H$_2$O/mol PAA residue)</th>
<th>$m_2^{\text{zeo}}$ mol</th>
<th>$\Delta G^0$ (kJ/kg PAA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>5.52</td>
<td>0.407</td>
<td>1.57</td>
<td>3.99</td>
<td>61.0</td>
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<td>Arginine chloride</td>
<td>33.2</td>
<td>1.19</td>
<td>32.8</td>
<td>2.08</td>
<td>330</td>
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<tr>
<td>Asparagine</td>
<td>22.3</td>
<td>0.450</td>
<td>9.36</td>
<td>-</td>
<td>458</td>
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<tr>
<td>Aspartate</td>
<td>29.2</td>
<td>3.21</td>
<td>56.2</td>
<td>-</td>
<td>152</td>
</tr>
<tr>
<td>Cbz cysteine</td>
<td>0.950</td>
<td>0.053</td>
<td>0.57</td>
<td>3.23</td>
<td>60.0</td>
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<tr>
<td>Glutamate</td>
<td>54.9</td>
<td>2.20</td>
<td>26.4</td>
<td>2.25</td>
<td>99.3</td>
</tr>
<tr>
<td>Glutamine</td>
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<td>1.26</td>
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<td>2.49</td>
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<tr>
<td>Glycine</td>
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<td>0.50</td>
<td>1.05</td>
<td>1.42</td>
<td>70.2</td>
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<tr>
<td>Histidine chloride</td>
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<td>4.11</td>
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<tr>
<td>Isoleucine</td>
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<tr>
<td>Leucine</td>
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<td>0.020</td>
<td>1.47</td>
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<td>18.3</td>
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<tr>
<td>Lysine bromide</td>
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<td>1.40</td>
<td>17.3</td>
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<td>236</td>
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<td>Methionine</td>
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<td>0.090</td>
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<td>2.31</td>
<td>22.9</td>
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<td>Phenyl-alanine</td>
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<td>0.010</td>
<td>2.06</td>
<td>2.92</td>
<td>20.0</td>
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<tr>
<td>Proline</td>
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<td>0.110</td>
<td>2.27</td>
<td>2.92</td>
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<tr>
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<td>1.50</td>
<td>1.50</td>
<td>-</td>
<td>133</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.50</td>
<td>0.133</td>
<td>20.2</td>
<td>3.23</td>
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<tr>
<td>Tryptophan</td>
<td>19.3</td>
<td>1.42</td>
<td>2.79</td>
<td>3.04</td>
<td>43.0</td>
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<tr>
<td>Tyrosine</td>
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<td>1.47</td>
<td>3.92</td>
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</tr>
<tr>
<td>Valine</td>
<td>0.240</td>
<td>0.030</td>
<td>1.14</td>
<td>2.31</td>
<td>22.6</td>
</tr>
</tbody>
</table>

\[\text{Table 1—Extents of water and NaCl binding to poly-L-amino acid values of } \Delta n_1^0 \text{ at 303 K} \]

[Data for polyglycine and Na-poly-L-glutamate were taken at 313 and that of poly-L-asparagine at 296 K]
equilibrium with \( n_1 \) and \( n_2 \) moles of free water and free NaCl in the bulk phase in the sample bottle at isopiestic equilibrium, so that one can write:

\[
\begin{align*}
\Delta n_1 &= n_1 + \Delta n_1 \\
\Delta n_2 &= n_2 + \Delta n_2
\end{align*}
\]

Inserting Eqs (7) and (8) in Eqs (3) and (4), relations (9) and (10) will be obtained:

\[
\Gamma_1^2 = \Delta n_2 - \Delta n_1 \frac{X_2}{X_1} \quad \ldots (9)
\]

and

\[
\Gamma_1^1 = \Delta n_1 - \Delta n_2 \frac{X_1}{X_2} \quad \ldots (10)
\]

In Figs 1 to 3, the values of \( \Gamma_1^2 \) calculated from Eqs (1) and (4) for different PAAs at several temperatures have been plotted as function \( \frac{X_1}{X_2} \). From these graphs, one can see the trend in hydration for each polymer in the presence of NaCl.
It is apparent that such plot for each PAA is linear in some wide range of solution composition $X_1/X_2$, so that positive values of $\Delta n_1$ and $\Delta n_2$ for each system have been estimated from the intercept and slope of these plots using Eq. (10). These values for different amino acids are presented in Table 1. The values of $\Delta n_1^0$ reported earlier are also presented for comparison.

On comparing $\Delta n_1^0$ and $\Delta n_1$ for each amino acid residue in the absence and presence of high concentrations of NaCl (1 to 5.0 M), in the free bulk phase present in sample bottle, it is found from Table 1 that for amino acid residues containing hydrophobic groups, the values of $\Delta n_1$ in the mole per mole unit vary from 0.25 to 19, depending upon the nature of hydrophobic groups attached to the side chain of the amino acids. The affinities of tryptophan and to some extent alanine for water are found to be considerably high possibly due to the large increase of hydrophobic hydration of these groups in the presence of excess NaCl in the bulk phase. The values of $\Delta n_1$ in mole per mole of amino acid residue stand in the following order:

tryptophan > alanine > proline > methionine > isoleucine > leucine > phenyl alanine > valine

It is interesting to note that the increasing trend in the values of $\Delta n_1^0$ and $\Delta n_1$ (Table 1) for different PAAs are not same. Alanine, valine, leucine and isoleucine have $\Delta n_1^0$ values 1.57, 1.14, 1.47 and 6.23, whereas $\Delta n_1$ for these PAAs are 5.52, 0.24, 1.51 and 1.89, respectively. Thus, for alanine and isoleucine residues $\Delta n_1 > \Delta n_1^0$, whereas for valine residue $\Delta n_1^0 > \Delta n_1$. For leucine, however, $\Delta n_1 \approx \Delta n_1^0$.

The values of $\Delta n_1$ for PAAs containing polar, but non-ionic side chains (Table 1) are higher in order than those of non-polar side chain, except tryptophan. The $\Delta n_1$ in mole per mole unit for PAAs with polar and uncharged side chains stand in the order:

glutamine > serine > tyrosine > asparagine > glycine > threonine > cbz cysteine.

This order is not same for $\Delta n_1^0$, but magnitude in two cases are significantly different from each other (vide Table 1). In fact, $\Delta n_1$ values are significantly larger than $\Delta n_1^0$, except for glutamine and threonine, where $\Delta n_1^0 > \Delta n_1$.

The values of $\Delta n_1$ for PAAs containing ionic side chains (Table 1) vary between 30 to 55 moles per mole residue, and are quite high compared to $\Delta n_1$, values observed for non-polar and polar non-ionic residues. The order of $\Delta n_1$ for ionic residues is as follows:

glutamate > histidine > arginine > lysine > aspartate

For aspartate residue, $\Delta n_1^0 \gg \Delta n_1$, but for glutamate, lysine and histidine residues, $\Delta n_1 > \Delta n_1^0$.

From Table 1, it is observed that $\Delta n_2$ for all PAAs are lower than $\Delta n_1$ by 10 to 20 times or more. We also note that 1 to 4 moles of NaCl may remain associated by adsorption process with one mole of PAAs, containing ionic side chain groups. In all probability, as a result of electrostatic interaction, major amounts of $Na^+$ and $Cl^-$ (as counter ions and co-ions) will be present close to the primary layer of water associated with ionic side chain. Small fraction of these cations and anions may also remain dissolved in the secondary and tertiary layers of water bound to PAA in the presence of neutral salt. The values of $\Delta n_2$ are found to vary between 0.01 to 1.7 moles of NaCl per mole of residue of PAAs with polar and non-ionic residues (vide Table 1). It is expected that major fraction of NaCl remains dissolved in secondary and tertiary layers of adsorbed water in these cases. Except PAAs containing tryptophan and alanine residues, all other PAAs containing hydrophobic side chains possess very small values of $\Delta n_2$. The complex gradient of adsorbed layer of water associated with different PAAs in the presence of NaCl depend upon the nature of the side chain groups of the polymer, as well as on the dissolved neutral salt distributed unequally in primary, secondary and tertiary regions of the bound water forming the inhomogeneous surface phase. Besides, the concentration of NaCl in the free bulk aqueous phase may be as high as 5.0 molar. Thus, bulk water exerts salting out effect on surface bound water, modifying the gradient existing in the inhomogeneous phase. Compared to this, the structure of the inhomogeneous phase in the absence of NaCl appears...
to be probably simple in nature. Here, as water activity \( a_1 \) is altered from zero to unity, water molecules in the primary, secondary and tertiary layers may be filled up systematically. The density and other properties of water may change continuously in the inhomogeneous surface phase until at \( a_1 \) equal to unity, the bulk water surrounds the bound water in the surface phase\(^{26}\). Similar aspects of inhomogeneous surface phase for different proteins in the absence of salt have been discussed by Chattoraj et al.\(^{25}\) earlier from thermodynamic standpoint.

Because of these fundamental difference in the nature of water gradients in the absence and presence of excess salt, the order of \( \Delta n_2 \) and \( \Delta n_1 \) for different amino acid residues do not agree with each other in many situations.

A major feature of the plot of \( \Gamma_1^2 \) against \( \frac{X_2}{X_1} \) in Figs. 1 to 3 (or 55.5/m\(_2\)) for each system is that the values of water excesses \( \Gamma_1^2 \) at \( m_2 \) less than a critical value \( m_{azeo} \) are positive, whereas above this critical value, \( \Gamma_1^2 \) values are all negative. The critical value of \( m_{azeo} \), at which \( \Gamma_1^2 \) becomes zero (vide Figs 1 to 3) for different systems are given in Table 1. According to Eq. (10), at the azeotropic state:

\[
\frac{\Delta n_2}{\Delta n_1} = \frac{X_2}{X_1} = \frac{m_2}{55.5}
\]

or,

\[
m_2 = 55.5 \frac{\Delta n_2}{\Delta n_1} = m_{azeo}^2 \quad \ldots \quad (12)
\]

At the surface azeotropic state, the apparent molality, \( m_{azeo}^2 \) of solute at the interfacial phase becomes equal to the molality \( m_{azeo}^2 \) of the bulk phase.

The apparent standard free energy change (\( \Delta G_{ap}^\circ \)) due to the simultaneous interactions of NaCl and water to 1 kg of a polyamino acid, thus forming one or two phase systems may be calculated\(^{14,30}\), using the equation:

\[
\Delta G_{ap}^\circ = -2RT \left[ \int_{0}^{X_{X_2}^{\pm}} \frac{\Gamma_2^{\pm}}{f_2^{\pm} X_2^{\pm}} df_2^{\pm} \ln f_2^{\pm} + \Gamma_1^{\pm} \ln \left( \frac{f_2^{\pm} X_2^{\pm}}{X_2} \right) \right]
\]

\( \ldots \quad (13) \)

\( \Gamma_1^{\pm} \), here is expressed in moles of NaCl present per kg of PAA. The value of the mean mole fraction, \( X_\pm \) of NaCl at a given value of \( m_2 \) can be calculated using the relation:

\[
X_\pm = \frac{m_2}{2m_2 + 55.5}
\]

Also, the mean activity coefficient \( f_\pm \) of NaCl at a given value of \( m_2 \) in the practical scale can be obtained from the standard table\(^{31}\), so that its value in the rational scale can be computed by multiplication with appropriate conversion factors. The values of \( \Gamma_1^2 \) can be obtained directly from experimental data using Eq. 1 so that values of \( \Gamma_1^2 \) at a given value of \( m_2 \) (or \( X_2/X_1 \)) may be calculated using Eq. (4).

At a given value of \( m_2 \) (or \( X_\pm \)), the value of \( \Delta G_{ap}^\circ \) can thus be computed using relations (13, and (14). The values of \( \Delta G_{ap}^\circ \) are observed to change with increase of \( X_\pm \). In Fig. 4, the values of \( \Delta G_{ap}^\circ \) for

![Fig. 4—Plot of \( \Delta G_{ap}^\circ \) vs \( 1/\sqrt{X_2} \) for (○) Na poly-L-aspartate (22°C), (△) Na-poly-L-aspartate (30°C), (□) poly-L-lysine bromide (22°C), and (●) poly-L-bromide (30°C) all in the presence of NaCl](image-url)
different PAAs have been plotted against \( \frac{1}{\sqrt{X_+}} \). The
extrapolated value of the linear region of the plot at
unit mean mole fraction of NaCl (\( X_+ = 1 \)) stands for
the standard free energy change (\( \Delta G^o \)) for the excess
negative adsorption of NaCl per kg of PAA \(^{14,30} \).
These values presented in Table 1 are all positive,
which indicate that excess negative adsorption of
NaCl (Figs 1-3) results from the positive excess
hydration of PAAs. It has been shown from
thermodynamic treatment\(^ {30} \) that the standard free
energy of excess hydration (\( \Delta G^o_{hy} \)) is equal to \( -\Delta G^o \).

The values of \( \Delta G^o_{hy} \) in Table 1 are negative due to the
spontaneous excess hydration of PAAs in the
presence of salt. \( \Delta G^o_{hy} \) results from the change of
bulk activity of \( H_2O \) from zero to unity in the mole
fraction scale.

Due to the strong interaction of water with ionic
side chain groups of amino acid residues, the values
of \( \Delta G^o_{hy} \) are relatively high. These values stand in the
order:
arginine > histidine > lysine > aspartate > glutamate
\( \Delta G^o_{hy} \) for non-polar hydrophobic PAAs with non-
polar residues, on the other hand, are considerably
lower (vide Table 1), than those of residues
containing ionic side chains. The values of \( \Delta G^o_{hy} \) for
polar non-ionic residues are expected to occupy
intermediate position between hydrophobic and ionic
amino acid residues. But, there are few exceptions.
Among the PPAs, \( -\Delta G^o_{hy} \) value is lowest for poly-L-
cbz-cysteine. This may be possibly due to the
benzoylation of side chain sulphur group. On the
other hand, asparagine shows the highest \( -\Delta G^o_{hy} \)
value. The \( -\Delta G^o_{hy} \) value for glutamine is nearly as
high as that of ionic lysine and arginine side chain.

We shall now focus our attention to more extensive
data obtained for eleven poly-L-amino acids (vide
Table 2) studied at 295 and 303 K. Using Eq. (13)
apparent standard free energy changes, \( \Delta G^o_{ap} \), have
been calculated for different values of \( X_+ \). From linear
extrapolation of \( \Delta G^o_{ap} \) vs \( \frac{1}{\sqrt{X_+}} \), the values of
standard free energy change (\( \Delta G^o \)) at \( X_+ =1 \) have been
evaluated for these PAAs at two different
temperatures. The values of \( \Delta G^o \) are found to differ
significantly with alteration of temperature by 8° C.

The standard enthalpy change per kg of PAA can be
evaluated from the equation\(^ {29} \):

\[
\frac{\Delta G^o_2}{T_2} - \frac{\Delta G^o_1}{T_1} = \Delta H^o_{av} \left( \frac{1}{T_2} - \frac{1}{T_1} \right)
\]  \hspace{1cm} \ldots (15)

Here \( \Delta G^o_1 \) and \( \Delta G^o_2 \) are the evaluated values of
standard free energy change at temperatures \( T_1 \) and
\( T_2 \), respectively. \( \Delta H^o_{av} \) stands for average value of
standard enthalpy change at average temperature

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**Table 2**—Thermodynamic parameters for binding of water and NaCl to different poly-L-amino acids at average 299K

<table>
<thead>
<tr>
<th>Poly-L-amino acids</th>
<th>( \Delta G^o_{av} ) (kJ/kg)</th>
<th>( \Delta H^o_{av} ) (kJ/kg)</th>
<th>( T_\circ \Delta S^o_{av} ) (kJ/kg/K)</th>
<th>( \Delta S^o_{av} ) (kJ/kg/K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>83.0</td>
<td>1760</td>
<td>1680</td>
<td>5.63</td>
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<tr>
<td>Arginine chloride</td>
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<td>-3070</td>
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<td>-11.2</td>
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<td>2970</td>
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<td>120</td>
<td>113</td>
<td>0.376</td>
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<tr>
<td>Glutamine</td>
<td>348</td>
<td>6140</td>
<td>5790</td>
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<tr>
<td>Histidine chloride</td>
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<td>-11.2</td>
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<tr>
<td>Isoleucine</td>
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<td>-1360</td>
<td>-1390</td>
<td>-4.67</td>
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<tr>
<td>Lysine bromide</td>
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<td>-1690</td>
<td>-1900</td>
<td>-6.39</td>
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<tr>
<td>Threonine</td>
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<tr>
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<td>-418</td>
<td>-1.40</td>
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<tr>
<td>Tyrosine</td>
<td>119</td>
<td>-1290</td>
<td>-1410</td>
<td>-4.73</td>
</tr>
</tbody>
</table>
The values of $\Delta H^o_{av}$ for different PAAs are presented in Table 2. Corresponding average values of $\Delta G^o_{av}$ equal to

$$\frac{1}{2} (\Delta G_1^o + \Delta G_2^o)$$

at 299 K have also been calculated (vide Table 2), so that average values of $T_{av} \Delta S^o_{av}$ equal to $\Delta H^o_{av} - \Delta G^o_{av}$ for different PAAs, as well as average changes of entropy $\Delta S^o_{av}$ have been calculated (Table 2). One finds from the Table that magnitude of $\Delta H^o_{av}$ and $T_{av} \Delta S^o_{av}$ are very close to each other, indicating the existence of entropy-enthalpy compensation effect for the hydration of PAAs in the presence of salt. Such compensation effect is shown more precisely by the linear plot of $\Delta H^o_{av}$ against $\Delta S^o_{av}$ (Fig. 5). Ghosh et al.\textsuperscript{23} have earlier shown that the compensation effect of hydration of PAAs in the absence of salt remains valid also from detailed thermodynamic analysis.

In the absence of the neutral salt, the water vapour adsorbed by PAA sample leads to the formation of hydrated biogel having swelling pressure. $\Pi_{sw}$. $\Pi_{sw}$ is equal to $n_1 RT \ln \frac{p_o}{p}$, where $n_1$ stands for moles of water adsorbed per mole of PAA at water activity $a_1$. As $p/p_o$ or $a_1$ decreases, $\Pi_{sw}$ significantly increases. But, when $p/p_o$ becomes close to 0.90 to unity, the values of $\Pi_{sw}$ are negligibly small. In the presence of NaCl in the sample system, total pressure, $\Pi_{total}$ in the system is equal to $\Pi_{sw} + \Pi_{os}$. Here, $\Pi_{os}$ stands for osmotic pressure exerted by high concentration of NaCl (1.0 to 5.0 M) present in free solution in contact with sample. Also, $p/p_o$ for such concentration of NaCl ranges from 0.90 to 0.98, so that $\Pi_{sw}$ must be very low compared to high values of $\Pi_{os}$. Thus, the contribution of hydrated PAAs (signified by $\Pi_{sw}$) to the equilibrium vapour pressure, $\frac{p}{p_o}$ will be negligibly small and experimental value of $p/p_o$ is solely contributed by the value of $\Pi_{os}$, so that $m_2 = m_1$.

Isopiestic hydration of $\beta$-lactoglobulin and lysozyme was studied by Chattoraj et al.\textsuperscript{32,35} at 30°C in the presence of excess NaCl. Experimental values of $\Delta n_1$ for these two proteins reported by them are 712 and 2928 moles of H$_2$O per mole of protein, respectively of known molecular weights and amino-acids composition. The theoretical values of $\Delta n_1$ equal to $(\Delta n_1)_{th}$ of above two proteins in mole per mole unit can be calculated using the following equation:

$$(\Delta n_1)_{th} = \sum N_{i}^{AA} (\Delta n_i),$$ \hspace{1cm} (16)

Here $N_{i}^{AA}$ stands for the number of $i$th amino acid residue present in one molecule of protein. $(\Delta n_i)$ represents the moles of water bound per mole of different amino acid residues which can be obtained at 30°C from Table 1 with neglect of the effect of temperature difference in the cases of glutamate, serine, asparagine and glycine residues. Estimated values of $(\Delta n_1)_{th}$ for $\beta$-lactoglobulin and lysozyme using Eq. (16) are 6460 and 2260 moles H$_2$O per mole of protein, respectively. For $\beta$-lactoglobulin, it can be concluded that significant dehydration of its amino acid residues occurs in folding of protein from random structure to globular form in the presence of NaCl. The values of $(\Delta n_1)_{th}$ for lysozyme calculated in this manner is close to the measured value of $\Delta n_1$ for this protein. The closeness of these two values indicates that both positive and negative effects during protein folding from unfolded states almost
cancel each other, so that in the final state of hydration of folded and unfolded proteins become close to each other.

Recently\textsuperscript{23}, we have compared measured values of $\Delta n_i^0$ for several proteins in presence of pure H$_2$O with $\langle \Delta n_i^0 \rangle_{\text{theo}}$, theoretically computed from the linear summation of $\Delta n_i^0$ for various residues forming these proteins of definite amino acid composition (vide also Eq. (16). The values of $\langle \Delta n_i^0 \rangle_{\text{theo}}$ are always found to be significantly higher than the experimentally observed value of $\Delta n_i^0$. This is in agreement with our results with $\beta$-lactoglobulin in the presence of NaCl.

Acknowledgement

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References

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