The temperature of maximum density for amino acid aqueous solutions. An experimental and molecular dynamics study.

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Abstract

The temperature of maximum density (TMD) for aqueous solutions of seven amino acids has been experimentally determined by means of density measurements versus temperature. The selected amino acids have been arginine, cysteine, glutamic acid, glutamine, lysine, methionine, and threonine. The TMD dependence against composition has been obtained from the experimental data and characterized through the Despretz constant. All amino acids induce a depression in the TMD as compared with that of pure water. If the mole fraction is selected as composition variable, a clear dependence against amino acid molar mass is observed, which disappears when TMDs are represented versus mass fraction; almost all data shrink onto a single straight line. It must be pointed out that the TMD depressions for all studied amino acids are quite greater than those previously observed for proteins. This suggests that TMDs for proteins cannot be explained as a simple, additive result of its constituents, and, therefore complex cooperative phenomena seem to take place. The partial molar volume at infinite dilution has been obtained from density data, and a consistency test between its temperature dependence and that of temperature of maximum density versus composition has been performed, obtaining satisfactory results. A molecular dynamics study for all the studied systems has been also carried out. Amino acids have been modeled through the OPLS-AA force field, whereas the TIP4P/2005 model was used for water. The temperature of maximum density and partial molar volume have been calculated from the simulated density, and the results are compared with experimental data. Although the agreement is only fair, similar qualitative trends were obtained. The simulated Despretz constants are smaller than the experimental ones, a result that was already previously observed for methanol aqueous solutions. A structural analysis of water molecules in solution along the MD trajectories showed no enhancement of ice-like structures in complete agreement with the TMD decrease with concentration.

Keywords: Temperature of maximum density, water, amino acids, molecular dynamics.
1. Introduction.

Ice Ih is the solid form of water below the melting point at atmospheric pressure [1]. It is a hexagonal structure where each water molecule takes part in four hydrogen bonds [2]. The microscopic form of water after melting resembles this ice-type structure, although with a significant degree of distortion. As temperature increases from the melting point, a new form of water structure emerges, structurally more disordered and denser. The occupancy of both structures by water molecules gradually changes as temperature increases, being the high density one the predominant form at higher temperatures. This feature is responsible for one of the most famous anomalies in the behavior of water, namely, the presence of a density maximum for a given temperature at atmospheric pressure [3]. The shape of this T-\( \rho \) curve changes slightly with pressure: the low density ice-type structure is partially distorted by increasing pressure, favoring the formation of the high density structure. This shifts the temperature of maximum density, TMD, towards lower temperatures [4].

Addition of solutes to water also affects the location of the TMD. For more than six decades, a large body of work has focused on the study of the variation of the TMD due to the addition of solutes to water, both electrolytes [5-9] and non-electrolytes [10-25]. Wada and Umeda [11] have shown that the difference between the TMD of a mixture of solute mole fraction \( x \) from that of pure water, \( \Delta T = \text{TMD (mixture)} - \text{TMD (pure water)} \), can be split into two contributions

\[
\Delta T = \frac{\alpha_1 v_1^{\%} 273.15}{2(1-x)} - \frac{dv^E/dT}{2(1-x)}
\]

by considering the molar volume of the pure solute (component 1) and pure water (component 2) in the neighborhood of the TMD is given by

\[
v_1^{\%} = v_1^{\%} 273.15 (1 + \alpha_1 (T - 273.15)),
\]

\[
v_2^{\%} = v_2^{\%} 277.13 (1 + \alpha_2 (T - 277.13))^2,
\]

respectively, where \( v^{\%} \) denotes molar volume of pure component, \( \alpha \) is the thermal coefficient of the molar volume, and \( v^E \) is the excess molar volume. The first term of the second member of equation (1) is the ideal contribution \( \Delta T^{id} \), which defines the dependence on the solute mole fraction, \( x \), (a monotonous decrease of \( \Delta T \) as the solute mole fraction increases) when \( v^E \) is zero. The second term
represents the structural contribution $\Delta T_{st}$ (non-ideal contribution) and it allows the classification of
the solutes as “structure makers” (those that promote the low density ice-like water structure) if $\Delta T_{st}$
is positive and “structure breakers” (those that promote the high density disordered structure) if $\Delta T_{st}$
is negative. This classification is, however, not unique [26] and in some cases $\Delta T$ is used instead
$\Delta T_{st}$. This last option is easier to apply since it does not require the previous knowledge of the
parameters of Equation (2), which are not easily available for solid solutes such as electrolytes or
biological macromolecules.

The characterization of the protein-water interaction is one of the great challenges of the
biological sciences. The influence of small amounts of protein on the structure of solvation water
will conversely affect the way in which hydrophobic and hydrophilic effects compete, and
subsequently lead to protein folding in solution. As previously commented, the TMD variation is a
good indicator of the changes induced by the solute in the structure of water. It is then of utmost
importance to understand how single amino acids influence the structure of water and subsequently
alter its TMD in order to deepen our understanding of water-protein interactions [27]. This work
was initiated by Kuppers [28] and Kaulguld et al. [29], and recently continued by Romero et al. [30]
and by Troncoso et al. [31], but to date the full 20 amino acid set present in natural proteins has yet
to be completely studied experimentally. It is the aim of this work to contribute further to fill this
gap, both experimentally and with the aid of molecular simulation.

Molecular simulations are nowadays a very powerful tool to facilitate a microscopic
interpretation of macroscopic experimental results. The great increase of the computational power
and improvements in modeling algorithms during the last decade has made feasible the simulation
of complex systems, such as biological macromolecules, using fairly realistic molecular
descriptions. Application of this methodology requires the use of accurate force fields (there is a
wide variety of choices, such as OPLS-AA [32], AMBER [33], CHARMM [34], and MARTINI
[35], among others) that adequately describe the inter and intramolecular interactions. The common
practice is assessing first the ability of the force field to reproduce the required experimental
macroscopic behavior for a given set of properties and thermodynamic states. This is crucial in
mixtures, since most force field are usually developed for pure components and their application to
mixtures requires the use of somewhat arbitrary combining rules. In our case, we want to analyze
the “structure maker/breaker” character of various amino acids from a microscopic stand point. This
implies that the variation of the TMD with the solute concentration is the key quantity to be
reproduced.
In this work we have thus focused on aqueous solutions of arginine, cysteine, glutamic acid, glutamine, lysine, methionine, and threonine in the dilute region. On the one hand, the variation of the TMD of water due to the addition of these seven amino acids was experimentally determined. This was done by carrying out density measurements in the temperature interval (273.65-283.15) K in 0.5 K steps using a vibrating tube densimeter DMA5000 from Anton Paar. Density data were also used to evaluate the partial molar volumes of the amino acids at infinite dilution. With this, we have performed a thermodynamic consistency check with the TMD values following the scheme devised by Armitage et al. [14]. On the other hand, extensive NpT Molecular Dynamics (MD) simulations of the studied systems, in the temperature range 238.15-308.15 K and at several solute concentrations \((x = 0.0, 0.0025, 0.0050, 0.0075, 0.01)\), were performed using the OPLS-AA force field for amino acids together with the TIP4P/2005 model for water [36]. The purpose of the simulations was to evaluate the ability of the chosen model to account for the variation of the TMD with the solute concentration and also to analyze the promotion/destruction of ice-like water structure during mixing. This last point was carried out in a quantitative way by performing a specific structural analysis of water molecules in solution along the MD trajectories. We will see that although the simulation reproduces the correct qualitative trends, it provides a stronger depression of TMD with the addition of a solute. Structural analysis showed no enhancement of the ice-like water structure confirming the ‘structure-breaker’ character of the solutes.

2. Experimental

Table 1 shows the the CAS number, mass fraction purity and supplier of the studied amino acids. They were used as supplied, without further purification. Milli Q water, degassed under vacuum, was used for preparing the mixtures. Amino acid aqueous solutions were done using an AE-240 Mettler balance with an uncertainty of \(\pm 0.0001\) g in the mass determination. The mixture compositions were quantified using the mass concentration \(c\) in milligram of amino acid per gram of water \((\text{mg} \cdot \text{g}^{-1})\) or the mole fraction \(x\); both used throughout the paper. Uncertainty in \(c\) is estimated in 0.05 mg·g\(^{-1}\).

Densities of the samples were measured using the atmospheric pressure vibrating tube densimeter Anton Paar DMA5000. Its working principle is based on the determination of the resonant vibration period of a borosilicated glass U-tube filled with the sample and clamped at its ends. The sample density is related with the square of this period through a simple linear relation whose parameters are estimated by calibration. The most common calibration procedure for these instruments is the
so-called “classical” calibration [37, 38], which is based on the determination of the vibration period for two liquids with well-known densities in the studied temperature range. In this work, we choose this option using Milli-Q water and octane, taking their density values from literature [39, 40]. Uncertainty in the density measurement was previously estimated [31] in 0.00005 g·cm⁻³.

3. Models and computational details

A molecular representation of the amino acids of this work is given in Figure 1. They have been modeled using the OPLS-AA force field, from W. L. Jorgensen [32]. Each atom of the molecule is considered as a different interaction site for the potential energy computation of the system. The potential energy is split into bonded and non-bonded contributions. The bonded term comprises only the intramolecular energy, with two harmonic components due to the linear bond stretching and angle bending potentials and a dihedral angle contribution expressed in the Ryckaert-Bellemans form. The explicit expression for the bonded term reads:

\[ U_{\text{bonded}} = U_{\text{linear}} + U_{\text{angle}} + U_{\text{dihedral}} \]  (4)

\[ U_{\text{linear}} = \frac{1}{2} \sum_{a,b} k_{ab} (r_{ab} - r_{ab}^0)^2 \]  (5)

\[ U_{\text{angle}} = \frac{1}{2} \sum_{a,b,c} k_{abc} (\theta_{ab} - \theta_{abc}^0)^2 \]  (6)

\[ U_{\text{dihedral}} = \sum_{a,b,c,d} (\sum_{n=0}^{5} C_n (\phi_{abcd} - 180^\circ)^n) \]  (7)

where the \( U \) refers to a potential energy contribution, \( a, b, c, d \) denote atoms in the molecule, the \( k \)'s are the spring constants, with \( \theta \) being the bond angle, \( \phi \) the dihedral angle, \( C_n \) are the Ryckaert-Bellemans constants, and superindex 0 refers to the value in equilibrium. The \( r_{ab} \) are the interatomic intramolecular distances. The non-bonded interaction comprises the energy between atoms of different molecules and also between atoms in the same molecule, but separated by at least three linear bonds. In the present instance, the non-bonded interaction energy stems from a Lennard-Jones and a Coulombic term, as shown below:

\[ U_{\text{non-bonded}} = \sum_{a,b} f_{ab} \left[ 4\varepsilon_{ab} \left( \frac{\sigma_{ab}}{r_{ab}} \right)^{12} - \left( \frac{\sigma_{ab}}{r_{ab}} \right)^{6} \right] + \frac{1}{4\pi\varepsilon_0} \frac{q_a q_b}{r_{ab}} \]  (8)

where \( \varepsilon \) and \( \sigma \) denote Lennard-Jones parameters, \( q \) the partial charges, and \( \varepsilon_0 \) is the vacuum relative permittivity. The cross Lennard-Jones interaction parameters between \( a \) and \( b \) atoms (\( \varepsilon_{ab} \) and \( \sigma_{ab} \)) are computed in this force field through the geometrical mean of the corresponding Lennard-Jones
parameters for each site \( (e_a, e_b, \sigma_a, \sigma_b) \). The term \( f_{ab} \) in Eq.(8) is set to \( f_{ab} = 0.5 \) for intramolecular interactions between atoms separated exactly three bonds or \( f_{ab} = 1.0 \) in other cases.

The OPLS-AA force field is implemented in the GROMACS package [41]. Our atoms selection within the OPLS-AA force field is detailed in the Supplementary Material and was carried out according to the following considerations. Although OPLS-AA provides an atom definition for amino acids that take part in a peptidic chain as in the case of proteins, we did not choose it (the only exception was for arginine as explained below), since the free amino acids are independent molecules that do not form peptidic bonds. Therefore, we have selected the atoms according to the functional groups type that the amino acid presents. Thus, the atoms corresponding to the acid and the amino groups for all amino acids were selected from those defined for carboxylic acids and primary amines, respectively. This is the only consistent choice for all the amino acids, since other options would introduce a dependency on the residue, which is different in each case. Thus, residue atoms for cysteine were chosen as those for thiols, for glutamic acid as those for the carboxylic acids and alkanes, for glutamine as those for the amides and alkanes, for lysine as those for amines and alkanes, for methionine as those for sulfides and alkanes, and for threonine as those for alcohols and alkanes. The only exception is the arginine, for which residue atoms were selected as those provided by OPLS-AA for arginine, which corresponds to this amino acid taking part in a peptidic chain. The model parameters of equations (4-8) for our selection are also reported in the Supplementary Material within the topol.top GROMACS files.

Water molecule was modeled using the well-known TIP4P/2005 model of Vega and Abascal [36]. This is a rigid model with three interaction sites located in the three atoms of the molecule, respectively, and a four site (named M-site) located in the bisector of the Hydrogen-Oxygen-Hydrogen (HOH) angle. A Lennard-Jones center is located in the oxygen atom \( (\epsilon_O = 0.7749 \text{ kJ mol}^{-1}) \sigma_O = 0.31579 \text{ nm} \) whereas charges are placed in the hydrogens \( (q_H = 0.5564 \text{ e}) \) and in the M-site \( (q_M = -1.1128 \text{ e}) \). The model geometry is defined as follows: \( d_{OH} = 0.9572 \text{ nm}, d_{OM} = 0.1546 \text{ nm} \) and HOH angle is 104.52. The potential energy between water molecules and the amino acids is computed using the equation (8), together with geometric mixing rules for cross Lennard-Jones parameters.

Isothermal-isobaric (NpT) molecular dynamics simulations have been carried out using Gromacs package [41] version 2018.3. We considered samples of two thousand molecules in a cubic box under periodic boundary conditions, and in order to properly sample the internal degrees of freedom a short time step of a 0.5 fs has been used. Pressure and temperature control were achieved using
both a Parrinello-Raman barostat [42] and Nosé-Hoover thermostat [43-44], both with relaxation
times of 2 ps. A cutoff radius of 1 nm was used for the Lennard-Jones interaction. Coulombic
energy was computed using the Particle Mesh Ewald (PME) method [45] using the same cutoff
radius for the real part of the Ewald. Initial configurations correspond to non-overlapping random
packing of molecules. Specific atomic coordinates for each molecule were generated using the
Molden package [46]. The equilibration procedure involves two short simulation runs at $T = 298.15$
K, one in the NVT and the other in the NpT ensemble at 100 bar, followed for a third, 0.5 ns
simulation in the NpT ensemble at the working temperature and pressure. Production runs were 5 ns
long. Finally, for states close to the TMD for water and highly diluted aminoacids we have
performed a structural analysis in order to identify local structures corresponding to clathrate
hydrates, hexagonal and cubic ice and liquid-like configurations using the algorithm CHILL+ as
devised by Nguyen and Molinero [47].

4. Results and discussion.

4.1. Experiments

Values for the densities, $\rho$, were determined each 0.5 K in the temperature interval (273.65-283.15)
K at atmospheric pressure for the diluted aqueous solutions of the seven amino acids, and are
collected in Tables B1-B7 of the Supplementary Material. The $\rho$ vs $T$ experimental data for each
concentration were fitted to a second degree polynomial ($\rho = A_0 + A_1 T + A_2 T^2$ ), that was used for
the computation of the TMD, using the expression $T_{TMD} = -A_1/(2A_2)$. Figure 2 illustrates the
behavior of the density $\rho$ as a function of temperature, $T$ (experimental data and fitting curve) for
concentrations selected to cover the whole working composition interval for six of the seven amino
acids. As it can be seen, the polynomial fit follows closely the experimental data, providing a good
estimate of the TMD, which is also shown in Figure 2.

The differences between the TMD of a mixture from that of the pure water, $\Delta T = TMD$
(mixture) – TMD (pure water), are collected in Table 2 and plotted in Figure 3 as a function of mole
fraction, $x$, (left panel) and mass concentration, $c$, (right panel). In all cases, $\Delta T$ decreases with
concentration, indicating that amino acids behave as “structure breakers” (in the more restrictive
version of this concept). The slight $\Delta T$ variation against mole fraction for cysteine (mass molar =
121 g·mol$^{-1}$) and threonine (119 g·mol$^{-1}$) contrasts with the stronger decrease observed for arginine
(174 g·mol$^{-1}$), glutamic acid (147 g·mol$^{-1}$), glutamine (146 g·mol$^{-1}$), and methionine (149 g·mol$^{-1}$);
the results for lysine (146 g·mol⁻¹) are between both groups. Therefore, certain correlation between the amino acid mass molar and ΔT-x data is found (the lower mass molar the slighter variation of ΔT versus x). This point is best illustrated if ΔT is plotted against mass concentration c since the mass molar effect is eliminated. As it can be seen in the right panel of Figure 3, the ΔT data are much closer compared with the previous ΔT-x plot; it seems that eliminating the mass molar dependence, all amino acids show similar behaviour. In any case, some small differences still appear. The variation of ΔT against mass concentration c for arginine, cysteine, lysine, and threonine is almost identical and ΔT data for glutamine and glutamic acid show a stronger variation with c; results for methionine are between both groups. In order to give a quantitative support to this discussion, the slopes of the ΔT-x and ΔT-c data were obtained by fitting of the experimental data to the well-known Despretz equation [48],

\[ ΔT = K_y y \]

(9)

where K is the Despretz constant, and y can be either the mole fraction, x, or the mass concentration, c. Results are given in Table 3. \( K_c \) data are almost identical for arginine, cysteine, lysine, and threonine, significantly larger in magnitude for glutamic acid and glutamine, and \( K_c \) takes an intermediate value for methionine. It is interesting to compare these numbers with those previously obtained by us [31] for tyrosine (-0.0917 K·mg⁻¹·g), tryptophan (-0.08052 K·mg⁻¹·g), histidine (-0.1027 K·mg⁻¹·g), phenylalanine (-0.0839 K·mg⁻¹·g), and proline (-0.0855 K·mg⁻¹·g). Thus, seven of the twelve amino acids studied by us take approximately the same value (around -0.082 K·mg⁻¹·g), three take a lower value (around -0.10 K·mg⁻¹·g) and two an intermediate value (-0.091 K·mg⁻¹·g). The effect on the TMD is, in any case, very similar and no influence of the chemical nature of residue can be detected. This implies that there seems to be no correlation between the character of the amino acids (polar, hydrophilic, amphipathic, or charged) and their “structure-breaker” behavior in solution, which is rather puzzling. Finally, it is important to highlight that the \( K_c \) values obtained previously for two proteins [27], α-chymotrypsin and bovine serum albumin, in the folded (-0.0299, -0.0312 mg⁻¹·g) and the degraded form (-0.0479, -0.0414 mg⁻¹·g) are significantly higher than all the values obtained for the amino acids, i.e., at least these proteins do not act as strong “structure-breakers”. This result is very interesting, since it shows that change in the TMD of protein solutions does not result from a simple addition of the behavior of their individual amino acids. Other factors must be taken into account, such as the influence of the peptidic bond, or conformational effects.

The experimental density can be used to compute the partial molar volume \( v_1 \) of the amino acids as follows

\[ v_1 = v + (1 - x) \left( \frac{\partial v}{\partial x} \right) \]

(10)
where \( v \) is the molar volume of the mixture. Taking the limit at infinite dilution \((x \to 0)\) one can find the relation

\[
v_1^\infty = v_2^{%} + \left( \frac{\partial v}{\partial x} \right)_{\partial x = 0},
\]

\( (11) \)

where \( v_2^{%} \) is the molar volume of pure water. This equation was used to obtain \( v_1^\infty \) for all the systems of this work. These data are presented in Table B8 in the Supplementary Material and they are plotted as a function of temperature in Figure 4. \( v_1^\infty \) increases with temperature as expected from the \( \Delta T - x \) decrement (a mathematical relation between both slopes is introduced below). Partial molar volumes at infinite dilution in water for these amino acids were previously measured by Kharakoz et al. [49], Millero et al. [50], and Mishra et al. [51]. Kharakoz et al. [49] made measurements from 288.15 K to 328.15 K for cysteine, glutamic acid, glutamine, and methionine. Millero et al. [50] reported values at 298.15 K for arginine, cysteine, glutamic acid, and methionine. Finally, Mishra et al. [51] determined \( v_1^\infty \) at 298.15 K for arginine, glutamic acid, glutamine, methionine, and threonine. These numbers are compared with those of this work in the right panel of Figure 4. There is a reasonable agreement between our values and literature data. The main deviations occur for arginine (our values lie somewhere between those of Millero et al. [50] and Mishra et al. [51]) and for glutamic acid, for which our date disagree somewhat with those of Millero et al. [50].

A thermodynamic consistency check is possible using \( \Delta T - x \) and \( v_1^\infty - T \) data through the following equation

\[
\left( \frac{\partial v_1^\infty}{\partial T} \right)_{T=277.13} = -\left( \frac{\partial \Delta T}{\partial x} \right)_{T=277.13} 2\alpha_2 v_2^{%} 277.13. \quad (12)
\]

where the parameters of the fitting equation (3) are used. Equation (12) clearly shows the relation between the \( v_1^\infty - T \) and \( \Delta T - x \) slopes commented before. Consistency check is carried out by comparing the temperature derivative of the partial molar volume at infinite dilution obtained directly from experimental \( v_1^\infty \) data with that obtained indirectly from equation (12) using the mole fraction derivative of \( \Delta T - x \) data. In order to compute the first quantity, \( v_1^\infty - T \) data were fitted to a second order polynomial in temperature

\[
v_1^\infty = B_0 + B_1 T + B_2 T^2, \quad (13)
\]
where \( B_0, B_1, B_2 \) are the fitting parameters, presented in Table 4 together with their corresponding standard deviations, \( s \). The quantities involved in the consistency check are shown in Table 5, and their fairly good agreement can be readily appreciated. These results confirm the accuracy of the experimental measurements as well as the reliability of Equation (12).

4.2. MD simulations

NpT molecular dynamics simulations in the temperature interval (238.15-308.15) K at atmospheric pressure for pure water and aqueous solutions of the seven amino acids at mole fractions, \( x = 0.0025, 0.0050, 0.0075, \) and 0.0100 were performed using the models described in Section 3. Density was computed during the production run. Figure 5 shows the evolution of density as a function of temperature for pure water and for aqueous solutions of six amino acids (the corresponding curve for threonine aqueous solutions is separately presented in the Supplementary Material). The \( \rho-T \) curve corresponding to the TIP4P/2005 model of water (plotted in Figure 5) provides a very accurate description of the experimental behavior of real water [36]; its temperature of maximum density is estimated to be 275.7 K, very close to the experimental value of 277.13 K. Addition of an amino acid tends to increase the density values over the whole temperature range, but to a greater extent at lower temperatures. This implies a shift of the temperature of maximum density towards lower values as the amino acid concentration increases.

In order to provide a quantitative description of this feature, TMDs were computed in the same way as in the experiments, \( i.e. \) by fitting the \( \rho-T \) simulation data to a second order polynomial and evaluating the TMD from the fits. Figure 6 shows \( \Delta T \) as a function of mole fraction, \( x \), (left panel) and mass concentration, \( c \), (right panel). Simulation data exhibit the same slight correlation with the molar mass of the amino acids, as found in the analysis of the experimental results; cysteine and threonine have the smallest slope (as found in the experiments) compared with those amino acids of higher molar mass. Moreover, when \( \Delta T \) is plotted vs mass concentration, \( c \), the apparent influence of the molar mass vanishes, as can be seen in the right panel of Figure 6. In other words, simulation data have the same qualitative trends as the experimental results. The \( \Delta T - x \) and \( \Delta T - c \) curves were also fitted to the Desprezt equation (9), and the calculated Desprezt constants are presented and compared with experimental results in Table 3. Simulation constants are always below the experimental ones. On the average, simulated Desprezt constants, \( K_c \), are 0.02 K·g·mg\(^{-1}\) below their experimental counterparts. This result has been also found in a previous work on the change in the TMD of methanol solutions [22]. We found that for all intermolecular potential models tested [22] \( \Delta T \) simulation values were substantially below the experimental data for all
alcohol concentrations. Moreover, in [22] it was found that even when the potential parameters were adjusted so as to reproduce the excess properties of the mixture, no improvement on the $\Delta T$ description was achieved. Interestingly, aqueous solutions of amino acids, being significantly more complex than diluted aqueous methanol, simulated models seem to be affected by the same shortcomings.

After performing a local structure analysis using the CHILL+ algorithm on trajectories of 2000 particle samples containing 1 and 10 amino acid molecules (arginine and threonine), we have found that local ice-like or interface-ice like structures are not promoted by the presence of these solvents. This implies that these amino acids will not act as "structure makers” in the sense of the Iceberg model. In contrast, clathrate-like (both bulk and interface) structures increase by a factor of 3 and 6 per cent respectively. These structures present distorted tetrahedral coordinations different from that of ice, which seems to underlie the fact that the anomalous thermodynamic and structural behavior of the amino acid solution is less pronounced than that of water.

The partial molar volume at infinite dilution $v_1^{\infty}$ has also been estimated from the simulation runs. Figure 7 shows the simulated $v_1^{\infty}$ data plotted as a function of temperature for all the amino acids. In all cases $v_1^{\infty}$ increases with temperature which is completely consistent with the decrease of $\Delta T$ amino acid as concentration grows. In the right panel of Figure 7, a comparison of simulation results with experimental data is shown for a reduced temperature range (closer to the experimental working temperature interval). As it can be seen, the agreement between both sets of data is, not surprisingly, rather poor. A straightforward explanation could be ascribed to the limit transferability of the model parameters. These are usually determined with the aim of describing the properties of pure substances. There is in principle no warranty that they can be successfully transferred to mixture problems. Moreover, the choice of Lennard-Jones cross-parameters between amino acids and water is also somewhat arbitrary. In this work, we use the geometric mean as it is usual in the OPLS-AA force field model. One might in principle use other mixing rules, or, following previous experience, fit the cross interactions to the experimental excess properties over the whole concentration range [22]. Given the results for the $\Delta T$ of methanol solutions found in [22] it is unlikely this strategy would perform better in the present instance.
Conclusions.

Experimental measurements of the density for aqueous solutions of seven amino acids in the diluted region were made using tube vibration densimetry at atmospheric pressure around the temperature of maximum density of water. From these data, the variation of the TMD induced by the addition of the amino acid was computed and analysed as a function of the solute concentration. $\Delta T$ versus $x$ dependence is shown to be strongly influenced by the molar mass of the amino acid (the lower the molar mass the smaller the change in of $\Delta T$ with $x$). This dependence is no longer visible when $\Delta T$ is plotted as a function of the mass concentration, $c$; all $\Delta T-c$ curves look very similar for the solutes analyzed herein. From a quantitative point of view, the Despretz constant $K_c$ takes values in the interval (-0.100, -0.080) K·g·mg$^{-1}$, in accordance with the $K_c$ values for other amino acids, previously measured by us [31]. These numbers contrast strongly with those obtained for aqueous solutions of two proteins, $\alpha$-chymotrypsin and bovine serum albumin, [27] both lying in the range (-0.04, -0.02) K·g·mg$^{-1}$. This significant difference indicates that the change in $\Delta T$ for protein solutions does not result from the simple combination of effects stemming from the behavior of pure amino acids in solution. In this context, further studies on the influence of peptides or polypeptides on the TMD are clearly needed. Everything seems to indicate that peptidic bonds play a major role in connection with the reduction of the “structure breaker” character of proteins when compared with amino acids. Finally, partial molar volumes at infinite dilution $v_1^{\infty}$ were also computed and found to be in close agreement with literature data. The $v_1^{\infty}$ was seen to increase with temperature, in agreement with the decrease of $\Delta T$ with solute concentration. The consistency check based on ideas of Armitage et al. [14] quantitatively supports this qualitative analysis.

For all the systems studied experimentally, molecular dynamics simulations were also performed. This also provided an assessment of OPLS-AA and TIP4P/2005 force fields to account for the experimental behavior. The $\Delta T$ variation in terms of concentration from the simulation results follows the same qualitative trends as the experiment, but $\Delta T$ falls more rapidly with amino acid concentration. The simulated Despretz constants, $K_c$, are on the average 0.020 K·g·mg$^{-1}$ lower than the experimental ones. This result is consistent with previous findings for aqueous solutions of methanol. A structural analysis of water molecules in solution along the MD runs showed no increment of ice-like water molecules, thus confirming the “structure breaker character” of aminoacids. The partial molar volume at infinite dilution was also computed and also departs considerably from the experimental values. It is unlikely that this is the reason behind the poor performance of the simulation to reproduce the experimental $K_c$. Our previous experience with methanol solutions indicates that even an accurate model for the excess properties over the whole
compositions range is not enough to guarantee the correct account of the concentration
dependence of the change of the TMD. An essentially different approach is needed, perhaps in the
direction of incorporation polarizabilities and a better description of the hydrogen bond both in the
potential model for pure substances and for cross-interactions. Basically non-pairwise additive
components must be at play in this apparently simple problem.

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Europeo de Desarrollo Regional (FEDER) under grant No. FIS2017-89361-C3. The authors are
indebted to Dr. E.G. Noya for kindly providing a CHILL+ structural analysis code.
References


[34] CHARMM. Chemistry at HARvard Macromolecular Mechanics. https://www.charmm.org
[46] G.Schaftenaar, CMBI, the Netherlands, MOLDEN a pre- and post processing program of molecular and electronic structure, http://cheminf.cmbi.ru.nl/molden/
Table 1. CAS number, supplier mass fraction purity ($w$), and source of the samples.

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>CAS number</th>
<th>$w$</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>74-79-3</td>
<td>&gt;0.98</td>
<td>Alfa-Aesar</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>52-90-4</td>
<td>&gt;0.99</td>
<td>Acros Organics</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>56-86-0</td>
<td>&gt;0.99</td>
<td>Acros Organics</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>56-85-9</td>
<td>&gt;0.98</td>
<td>Alfa Aesar</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>56-87-1</td>
<td>&gt;0.98</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>63-68-3</td>
<td>&gt;0.98</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>72-19-5</td>
<td>&gt;0.98</td>
<td>Acros Organics</td>
</tr>
</tbody>
</table>
Table 2. Temperature of maximum density at pressure $p=101$ kPa of the amino acid aqueous solutions of this work referred to that of pure water, $\Delta T$, as a function of mass concentration $c$ (milligrams of amino acid per water gram).a

<table>
<thead>
<tr>
<th></th>
<th>L-Arginine</th>
<th>L-Cysteine</th>
<th>L-Glutamic acid</th>
<th>L-Glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c$ (mg·g⁻¹)</td>
<td>$\Delta T$ (K)</td>
<td>$c$ (mg·g⁻¹)</td>
<td>$\Delta T$ (K)</td>
<td>$c$ (mg·g⁻¹)</td>
</tr>
<tr>
<td>1.261</td>
<td>-0.112</td>
<td>1.853</td>
<td>-0.171</td>
<td>0.531</td>
</tr>
<tr>
<td>2.383</td>
<td>-0.199</td>
<td>3.100</td>
<td>-0.261</td>
<td>1.575</td>
</tr>
<tr>
<td>3.870</td>
<td>-0.338</td>
<td>4.495</td>
<td>-0.377</td>
<td>2.218</td>
</tr>
<tr>
<td>5.283</td>
<td>-0.452</td>
<td>5.392</td>
<td>-0.439</td>
<td>2.886</td>
</tr>
<tr>
<td>8.145</td>
<td>-0.710</td>
<td>7.310</td>
<td>-0.611</td>
<td>3.573</td>
</tr>
<tr>
<td>9.969</td>
<td>-0.851</td>
<td>8.750</td>
<td>-0.759</td>
<td>4.394</td>
</tr>
<tr>
<td>11.693</td>
<td>-1.006</td>
<td>11.353</td>
<td>-0.972</td>
<td>5.037</td>
</tr>
<tr>
<td>13.247</td>
<td>-1.127</td>
<td>14.497</td>
<td>-1.264</td>
<td>6.125</td>
</tr>
<tr>
<td>15.792</td>
<td>-1.333</td>
<td>17.111</td>
<td>-1.455</td>
<td>6.985</td>
</tr>
<tr>
<td>15.825</td>
<td>-1.334</td>
<td>18.192</td>
<td>-1.541</td>
<td>7.887</td>
</tr>
<tr>
<td>17.666</td>
<td>-1.493</td>
<td>21.362</td>
<td>-1.863</td>
<td>8.904</td>
</tr>
<tr>
<td>25.904</td>
<td>-2.225</td>
<td>10.166</td>
<td>-1.104</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.670</td>
<td>-1.266</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>L-Lysine</th>
<th>L-Methionine</th>
<th>L-Threonine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c$ (mg·g⁻¹)</td>
<td>$\Delta T$ (K)</td>
<td>$c$ (mg·g⁻¹)</td>
<td>$\Delta T$ (K)</td>
</tr>
<tr>
<td>0.944</td>
<td>-0.061</td>
<td>0.816</td>
<td>-0.046</td>
</tr>
<tr>
<td>2.428</td>
<td>-0.208</td>
<td>2.016</td>
<td>-0.156</td>
</tr>
<tr>
<td>3.698</td>
<td>-0.306</td>
<td>4.010</td>
<td>-0.346</td>
</tr>
<tr>
<td>5.173</td>
<td>-0.441</td>
<td>6.158</td>
<td>-0.541</td>
</tr>
<tr>
<td>8.225</td>
<td>-0.682</td>
<td>8.315</td>
<td>-0.735</td>
</tr>
<tr>
<td>11.365</td>
<td>-0.946</td>
<td>9.851</td>
<td>-0.898</td>
</tr>
<tr>
<td>13.231</td>
<td>-1.126</td>
<td>12.248</td>
<td>-1.112</td>
</tr>
<tr>
<td>16.891</td>
<td>-1.381</td>
<td>14.482</td>
<td>-1.350</td>
</tr>
<tr>
<td>17.714</td>
<td>-1.435</td>
<td>18.115</td>
<td>-1.690</td>
</tr>
<tr>
<td>22.698</td>
<td>-1.885</td>
<td>24.874</td>
<td>-2.359</td>
</tr>
<tr>
<td>25.242</td>
<td>-2.081</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aStandard uncertainties: $u(c) = 0.05$ mg·g⁻¹; $u(\Delta T) = 0.003$ K, $u(p)= 1$ kPa.
**Table 3.** Despretz constant, $K_x$ and $K_c$, and their standard uncertainties $u(K_x)$ and $u(K_c)$ of the amino acids of this work obtained from experiments and simulation.

<table>
<thead>
<tr>
<th></th>
<th>$K_x \pm u(K_x)$ (K)</th>
<th>$K_c \pm u(K_c)$ (K·mg⁻¹·g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp.</td>
<td>Sim.</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>-827.9 ± 2.4</td>
<td>-1048 ± 12</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>-578.0 ± 2.5</td>
<td>-686 ± 23</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>-875.9 ± 5.4</td>
<td>-825 ± 42</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>-839.1 ± 2.8</td>
<td>-785 ± 46</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>-671.8 ± 2.6</td>
<td>-977 ± 23</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>-773.3 ± 5.6</td>
<td>-1083 ± 36</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>-552.9 ± 4.2</td>
<td>-664 ± 30</td>
</tr>
</tbody>
</table>
Table 4. Fitting coefficients of the partial molar volume of amino acids at infinite dilution against temperature following equation (13) as well as standard deviation of the fit $s$.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>$B_0$ ($10^{-6}$m$^3$·mol$^{-1}$)</th>
<th>$B_1$ ($10^{-6}$m$^3$·mol$^{-1}$·K$^{-1}$)</th>
<th>$B_2$ ($10^{-6}$m$^3$·mol$^{-1}$·K$^{-2}$)</th>
<th>$s$ ($10^{-6}$m$^3$·mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>-165.137</td>
<td>1.886</td>
<td>-0.00306</td>
<td>0.0025</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>-150.684</td>
<td>1.452</td>
<td>-0.00235</td>
<td>0.0026</td>
</tr>
<tr>
<td>L-Glutamic Acid</td>
<td>-256.534</td>
<td>2.235</td>
<td>-0.00361</td>
<td>0.0081</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>-246.716</td>
<td>2.220</td>
<td>-0.00362</td>
<td>0.0036</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>-174.966</td>
<td>1.877</td>
<td>-0.00308</td>
<td>0.0040</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>-190.564</td>
<td>1.911</td>
<td>-0.00309</td>
<td>0.0047</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>-193.362</td>
<td>1.792</td>
<td>-0.00298</td>
<td>0.0039</td>
</tr>
</tbody>
</table>
Table 5. Temperature derivative of the partial molar volume at infinite dilution of the amino acid aqueous solutions obtained directly, \((\partial \nu_1^\infty / \partial T)^d\), and indirectly through equation (12), \((\partial \nu_1^\infty / \partial T)^l\).

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>((\partial \nu_1^\infty / \partial T)^d) ((10^{-6}\text{m}^3\cdot\text{mol}^{-1}\cdot\text{K}^{-1}))</th>
<th>((\partial \nu_1^\infty / \partial T)^l) ((10^{-6}\text{m}^3\cdot\text{mol}^{-1}\cdot\text{K}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>0.192</td>
<td>0.208</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.149</td>
<td>0.145</td>
</tr>
<tr>
<td>L-Glutamic Acid</td>
<td>0.237</td>
<td>0.220</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>0.215</td>
<td>0.210</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.172</td>
<td>0.168</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>0.202</td>
<td>0.194</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.143</td>
<td>0.139</td>
</tr>
</tbody>
</table>
Figure 1. Molecular graphics of the amino acids of this work made using Molden package [46]
Figure 2. Density $\rho$ of selected mixtures of amino acid in water of mass concentration $c$ (in parenthesis and in mg·g$^{-1}$) plotted against temperature $T$. Points are experimental data and full lines a second order polynomial fit. Dotted line indicates the position of the TMD of the mixture and the asterisk that of pure water.
Figure 3. Experimental temperature of maximum density of aqueous solutions of amino acids referred to that of pure water, $\Delta T$, plotted as a function of mole fraction $x$ (left) and mass concentration $c$ (right).
Figure 4. Experimental partial molar volume at infinite dilution of the aminoacids in water $v_T^\infty$ plotted as a function of temperature $T$. Left panel (this work) and right panel (this work versus literature)
Figure 5. Simulation densities $\rho$ of selected mixtures of amino acid in water of mole fraction $x$ plotted against temperature $T$. Points are simulation data at mole fraction $x = 0.0$ (black), 0.0025 (blue), 0.0050 (green), 0.0075 (yellow), and 0.010 (red).
Figure 6. Simulation temperature of maximum density for the aqueous solutions of the amino acids referred to that of pure water, $\Delta T$, plotted as a function of mole fraction $x$ (left) and mass concentration $c$ (right).
Figure 7. Simulation partial molar volume at infinite dilution $\nu_1^\infty$ of the amino acids in water plotted as a function of temperature $T$. Left panel (simulation) and right panel (simulation versus experiments).