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# Salting-in with a Salting-out Agent: Explaining the Cation Specific Effects on the Aqueous Solubility of Amino Acids

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**Abstract:**

Although the understanding of ion specific effects on the aqueous solubilities of biomolecules is crucial for the development of many areas of biochemistry and life sciences, a consensual and well-supported molecular picture of the phenomena has not yet been established. Mostly, the influence of cations and the nature of the molecular interactions responsible for the reversal of the Hofmeister trend in aqueous solutions of amino acids and proteins are still defectively understood. Aiming at contributing to the understanding of the molecular-level mechanisms governing the cation specific effects on the aqueous solubilities of biocompounds, experimental solubility measurements and classical molecular dynamics simulations were performed for aqueous solutions of three amino acids (alanine, valine and isoleucine), in the presence of a series of inorganic salts. The evidence gathered suggests that the mechanism by which salting-in inducing cations operate in aqueous solutions of amino acids is different from that of anions, and allows for a novel and consistent molecular description of the effect of the cation on the solubility based on specific interactions of the cations with the negatively charged moieties of the biomolecules.

**Keywords:** amino acids, solubility, salts, cation specific effects, molecular interactions, molecular simulation, Hofmeister series

## 1. Introduction

The solubility behavior of biomolecules in aqueous electrolyte solutions assumes a very important role in the life sciences and biotechnological developments. In spite of the large amount of work dedicated to this subject throughout the years, the molecular level description of the effects of the nature and concentration of ions in biological media is still not consensual and definitely established. The lack of a deep and well-supported molecular picture of the interactions which govern the biochemistry of vital processes is still one of the major critical issues in biochemistry and is actually limiting the development of medical and pharmaceutical solutions for diseases induced by biochemical disorders<sup>1-3</sup> and the improvement of the efficiency of biotechnological processes<sup>4,5</sup>.

While the rank of the relative influence of ions on the physicochemical behavior of aqueous systems, known as the Hofmeister series<sup>6</sup>, is well established and consensually recognized as general in a wide range of processes<sup>7-10</sup>, several, sometimes contradictory, molecular level interpretations of the phenomena have been proposed during the past century<sup>7,9,11-24</sup>. Lately, spearheaded by its biological, medical and biotechnological relevance, there has been a renewed interest in this area. As new data become available<sup>25-30</sup>, long-held classical ideas about changes in bulk water structure<sup>11-14</sup> are progressively being overturned, and newer theories, emphasizing the significant role of dispersion forces, ionic polarizabilities and the specific ion binding, have been proposed<sup>15,16,31,32</sup>. One of the most consistent theories was suggested by Zhang et al<sup>9,17,18</sup>, who described ion specific effects on the solubilities of macromolecules in terms of direct interactions of the ions with the solutes and with water molecules. This theory formed in fact a basis for the model that we have been developing and refining to interpret the solubility of charged molecules in aqueous solutions of inorganic salts or amino acids<sup>19-22,24</sup> and to explain the behavior of aqueous saline solutions of amino acids<sup>23</sup>, which is consistent with the most recent theories that underline the central role of ionic polarizabilities and of ion size in the interpretation of Hofmeister effects<sup>31,32</sup>. Despite a century of efforts, however, in an era characterized by profound technological and scientific advances, the knowledge of the molecular level interactions which govern the behavior of biomolecules in aqueous saline environments is still elusive. Therefore, further investigation on this subject, using alternative approaches and methods capable of providing reliable evidence, is required.

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As the simplest building blocks of more complex biomolecules, amino acids are ideal molecules to be studied as model compounds. The effect of ions on amino acid aqueous solubilities follows the Hofmeister series and is experimentally well documented and phenomenologically well established. Experimental measurements of the solubility of amino acids in (water+salt) mixtures<sup>33-42</sup> reveal that this property is affected by the nature and concentration of both the cation and the anion of the electrolyte, as well as by the structural characteristics of the biomolecules, the pH and temperature. In previous studies<sup>23</sup>, molecular dynamics (MD) simulation data were used by us in an attempt to interpret, at a molecular level, the experimentally observed solubility behavior of amino acids in aqueous saline solutions. The influence of the nature of the anion and of the amino acids, and the effect of salt concentration were considered, but there are still some questions concerning the molecular mechanism that remain unanswered and need to be clarified. Particularly, the specific effects of cations have been difficult to explain. Actually, although a few works provide some clues about the mechanisms responsible for the unexpected reversal of the Hofmeister trend observed for cations in aqueous solutions of proteins<sup>42-45</sup>, the exact nature of the molecular interactions is still defectively understood. In particular, a well supported molecular picture of the ion-specific nature of the interactions between sites of negative charge on proteins and cations has not yet been provided<sup>45</sup>. In order to further contribute to the understanding of the molecular mechanisms occurring in these systems, thermodynamic and MD simulation methods are used here to study the interactions between amino acids and salts in aqueous media and to evaluate their dependence on the physico-chemical characteristics of the cation. With that aim, experimental solubility measurements and MD simulations were performed for aqueous solutions of three amino acids - alanine (Ala), valine (Val) and isoleucine (Ile), all depicted in Figure 1 - in the presence of salts such as MgCl<sub>2</sub>, MgSO<sub>4</sub>, NH<sub>4</sub>Cl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, at  $T=298.15$  K. The ions were selected in order to assess the effect of the nature and charge of the cation on the amino acids aqueous solubilities. Moreover, because natural environments are very often neutral, the solubility measurements were carried out in a pH range close to the isoelectric point and therefore only the zwitterionic forms of the solutes were considered in the simulations.

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MD simulation methods have proved to be a valuable tool for the investigation of biochemical systems, including aqueous solutions and aqueous saline solutions of amino acids, peptides, proteins, lipid bilayers and hydrophobic solutes<sup>32,46-56</sup>, and we have previously used

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3 them with success to characterize the interactions behind the influence of salts on the behavior of  
4 amino acids and of other charged molecules such as ionic liquids (IL) in aqueous mixtures<sup>22-24</sup>.  
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6 Despite the existence of a large amount of work focusing on the solubility and stability of amino  
7 acids and proteins in the presence of salts<sup>31,33-43,47,57,58</sup>, the experimental solubility data available  
8 for the systems envisaged in the current study are very scarce<sup>40</sup>, not to mention the paucity of  
9 theoretical investigations. In this work, the analysis of the radial distribution functions (RDFs) of  
10 the various groups and moieties, estimated by MD, will provide an explanation for the solubility  
11 behavior experimentally observed for the aqueous solutions of amino acids and salts considered  
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## 20 21 **2.Experimental**

### 22 23 **2.1. Chemicals**

24 Ammonium sulfate and ammonium chloride 99.5% minimum purity were supplied by  
25 Merck, while magnesium chloride hexahydrate and magnesium sulfate heptahydrate both  
26 supplied by Panreac were 99% minimum purity. The amino acids dl-alanine and l-valine  
27 (Merck), and l-isoleucine (Fluka), were 99% minimum purity. All chemicals were used as  
28 received, and excluding the hydrated salts, the solids were kept in a dehydrator with silica gel to  
29 avoid water contamination. In all experiments double-ionized water was used.  
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### 35 36 **2.2. Experimental Procedure**

37 The solubility experiments were carried out using the analytical isothermal shake-flask  
38 method. Saturated solutions were prepared by mixing a small excess of solid solute with about  
39 80 cm<sup>3</sup> of solvent, already prepared by weighing ( $\pm 0.1$  mg) the appropriated amounts of salt and  
40 water. To reach equilibrium the solution was continuously stirred for 48 h and later the solution  
41 was allowed to settle at least 12 h before sampling. In this process, temperature was monitored  
42 with 4-wire platinum resistance probes (Pt-104, Pico-Technology) placed in direct contact with  
43 the solutions. This temperature measuring system was previously calibrated, ensuring that the  
44 solution temperature is within  $\pm 0.1$  K of the set temperature.  
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51 Samples (5 cm<sup>3</sup>) of the saturated liquid phase were subsequently collected using plastic  
52 syringes coupled with previously heated polypropylene filters (0.45  $\mu$ m) in order to avoid  
53 precipitation. Depending on the salt, two different methods were chosen for quantitative analysis.  
54 The gravimetric method was selected for the non-hydrated salts. Therefore, the samples were  
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3 placed into pre-weighed glass vessels and immediately weighed. Then, all the solvent was  
4 evaporated, and the crystals dried completely in a drying stove at 343.15 K for 3 days. Finally,  
5 the glass vessels were cooled in a dehydrator with silica gel for one day and weighed. The  
6 process was regularly repeated until a constant mass value was achieved. Each solubility value  
7 was an average of at least three different measurements.  
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11 For the solutions of hydrated salts, density measurements were performed instead, using a  
12 vibrating tube digital density meter (DMA 5000 M, Anton Paar) with a reproducibility within  
13  $\pm 3 \times 10^{-3}$  kg/m<sup>3</sup>. In this way, the samples were discharged into glass vessels containing a known  
14 weighed amount (between 12 to 15 g) of binary salt aqueous solution at the same salt molality as  
15 the ternary saturated solution. After mixing, densities were measured following standard  
16 procedures. For each solubility value, four independent density measurements were performed,  
17 which were converted into solubility after consideration of a linear calibration curve ( $r^2 > 0.999$ )  
18 relating the amino acid concentration (in g/kg of water) and the density. The accuracy of the  
19 implemented methodology was checked by measuring the densities of some ternary solutions of  
20 known concentration of amino acids. The maximum difference found between the real and  
21 calculated concentration was 0.6 g of amino acid per kg of water.  
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25 The pH was also measured (inoLab pH 720, WTW) at 298.15 K for some saturated  
26 amino acid solutions at the highest salt molality, showing a minor effect on solubility change as  
27 it varies from pH=4.81 in a saturated solution of isoleucine (in MgCl<sub>2</sub>) to pH=5.90 in a saturated  
28 alanine aqueous 2 molal NH<sub>4</sub>Cl solution.  
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### 32 33 34 35 36 37 38 39 40 41 42 **2.3. Computational Methods**

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44 MD calculations were performed for aqueous solutions of the zwitterionic forms of the  
45 amino acids (pH=7) at a concentration of approximately 0.35 mol dm<sup>-3</sup> in the presence of the  
46 salts. A concentration of 1.0 mol dm<sup>-3</sup> was selected for the salts. The simulations were carried  
47 out using the isothermal-isobaric  $NpT$  ( $T = 298.15$  K and  $p = 1$  bar) ensemble and the  
48 GROMACS 4.04 molecular dynamics package<sup>59</sup>. The equations of motion were integrated with  
49 the Verlet-Leapfrog algorithm<sup>60</sup> and a time step of 2 fs. The Nosé-Hoover thermostat<sup>61,62</sup> was  
50 used to fix the temperature while the Parrinello-Rahman barostat<sup>63</sup> was employed to fix the  
51 pressure. Starting configurations were generated in cubic boxes with lateral dimensions of 45 Å,  
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3 and periodic boundary conditions were applied in three dimensions. The systems were prepared  
4 by randomly placing amino acids, ions and water molecules in the simulation box. Six amino  
5 acid molecules were included in each box, solvated by 900 water molecules, and 17 cation-anion  
6 pairs were incorporated to obtain the 1.0 M salt concentration. Then, a 10000 step energy  
7 minimization was performed and followed by two simulations, the first one with 50000 steps for  
8 equilibration and the final one with 10 000000 steps for production (i.e., total production time of  
9 20 ns). After equilibration, the values of the box volume ranged between 27.1 and 30.2 nm<sup>3</sup>,  
10 depending on the particular combination of amino acids and ions. Equilibration was checked by  
11 ensuring that all observables (including the RDFs) fluctuated around their equilibrium values  
12 during the production stage.  
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21 The intermolecular interaction energy between pairs of neighboring atoms was calculated  
22 using the Lennard-Jones potential to describe dispersion/repulsion forces and the point-charge  
23 Coulomb potential for electrostatic interactions. Long-range electrostatic interactions were  
24 accounted for using the particle-mesh Ewald method<sup>64</sup>, with a cutoff of 1.0 nm for the real-space  
25 part of the interactions. A cutoff radius of 1.2 nm was used for the Lennard-Jones potential, and  
26 long-range dispersion corrections were added to both energy and pressure. All bond lengths were  
27 held rigid using the LINCS constraint algorithm<sup>65</sup>, while angle bending was modeled by a  
28 harmonic potential and dihedral torsion was described (where appropriate) by a Ryckaert-  
29 Bellemans function. Potentials available in the literature were taken for all the species considered  
30 in the simulations. Water was described by the rigid SPC/E model<sup>66</sup>, while the OPLS all-atom  
31 potential was used for the amino acids<sup>67</sup> and for the magnesium<sup>67</sup>, chloride<sup>68</sup> and ammonium  
32 <sup>69</sup>ions. For sulfate, the force field parameters of the second (std2) model proposed by Cannon et  
33 al.<sup>70</sup> were used.  
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44 One of the most critical issues when performing MD simulations is the choice of the  
45 force field, since it often has repercussions on the accuracy of the results obtained<sup>52,71-73</sup>. For  
46 instance, small changes in the effective pair potential between interacting ions can significantly  
47 affect solution thermodynamics and contact ion-pairing, and thus some force fields have failed to  
48 reproduce realistically some properties of aqueous ionic solutions<sup>74,75</sup>. The force fields selected  
49 for the ions in this work have provided accurate descriptions of aqueous saline solutions of  
50 amino acids<sup>23</sup> and it has been shown that, although absolute degrees of binding are somehow  
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3 affected by the choice of the model, relative changes along the Hofmeister series are unchanged  
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7 Radial distribution functions for several atomic pairs were sampled during the production  
8 stage using the `g_rdf` tool of GROMACS. Coordination numbers (CN) were calculated for the  
9 interactions between selected atoms. For that purpose, the function  $N(r)$  was obtained by  
10 integrating the corresponding RDFs ( $g(r)$ ):  
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$$N(r) = 4\pi\rho_B \int_0^r (r'^2 g(r')) dr' \quad (1)$$

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16 where  $\rho_B$  is the number density of each atom in the bulk.  
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### 19 20 3. Results and Discussion

21 **3.1. Experimental.** The measured values for the solubilities of the amino acids in the aqueous  
22 solutions of the different salts, at various electrolyte concentrations and at  $T=298.15$  K, are  
23 presented in Tables 1 to 4, together with the standard deviation (in brackets). The maximum  
24 coefficient of variation is 0.80% for the ammonium solutions and 0.61% for the magnesium  
25 solutions. Figure 2 shows the relative solubility, expressed as the ratio between the solubility of  
26 the amino acid in the electrolyte solution to that in pure water, for the amino acids studied, in  
27 aqueous solutions of  $\text{NH}_4\text{Cl}$ ,  $\text{MgCl}_2$ ,  $(\text{NH}_4)_2\text{SO}_4$ , and  $\text{MgSO}_4$ .  
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34 Both increases (salting-in) and decreases (salting-out) of the amino acid aqueous  
35 solubilities are observed with the increase of the electrolyte concentration. As can be seen from  
36 Figure 2(a),  $\text{NH}_4\text{Cl}$  induces a salting-in effect on all the amino acids studied, with a magnitude  
37 dependent on the salt molality and on the nature of the biomolecule. For low  $\text{NH}_4\text{Cl}$   
38 concentrations ( $\leq 1$  molal), the importance of this effect increases in the order  $\text{Ala} < \text{Val} < \text{Ile}$ ,  
39 while for higher molalities of the salt (e.g. 2 molal) the opposite trend is observed. The relative  
40 aqueous solubility of Ala shows an almost linear dependence on the electrolyte concentration;  
41 for Val and Ile, we observe an initial increase in the relative solubility until it reaches a  
42 maximum, followed by a decrease, which is more pronounced for Ile than for Val. As shown in  
43 Figure 2(b),  $(\text{NH}_4)_2\text{SO}_4$  promotes salting-out of Val almost in the entire molality region  
44 (exception is the low molality regime), and this effect is more significant the higher the  
45 concentration of the salt.  
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55 The behavior of the magnesium salts is somewhat surprising. Given its extremely large  
56 energies of hydration<sup>76,77</sup>, presented in Table 5,  $\text{Mg}^{2+}$  should form hydration complexes<sup>78</sup> that  
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3 would contribute to a dehydration of the amino acids and their salting-out. This behavior is  
4 observed with other molecules such as ionic liquids<sup>79</sup> and proteins<sup>43,57</sup>. However, the solubility  
5 effects observed with the amino acids studied here are the opposite. The  $\text{Mg}^{2+}$  cation seems to be  
6 a strong salting-in agent, in good agreement with its “chaotropic” character described for other  
7 biomolecules such as polymers<sup>80</sup>, charged polypeptides<sup>45</sup> and some amino acids<sup>43</sup>. In fact,  
8  $\text{MgSO}_4$  and in particular  $\text{MgCl}_2$  have been found not only to have a rather unexpected behavior,  
9 but also to differ greatly from each other in their solubility effects on amino acids and proteins,  
10 and it has been inclusively suggested that the molecular-level mechanisms involving the  
11 interactions of these divalent cation salts with proteins are more complicated than those proposed  
12 for monovalent cations, such as Na, and are still not consensual<sup>43,45</sup>. As shown in Figure 2(a),  
13  $\text{MgCl}_2$  induces a pronounced salting-in of all the amino acids considered in this work, with a  
14 magnitude that increases with the molality of the salt. This increase is more significant and  
15 almost linear for Ala, while for Val and Ile it is smoother.  $\text{MgSO}_4$  (Figure 2(b)) also induces  
16 salting-in of all the amino acids, though less pronounced than that observed in aqueous solutions  
17 of magnesium chloride. For Ala, a significant initial increase in the relative solubility is followed  
18 by a decrease of the salting-in effect for higher salt concentrations. In the case of Ile, it was only  
19 possible to determine the solubility at 0.5  $\text{MgSO}_4$  molality, since experimental difficulties did  
20 not allow us to obtain the solubility of this amino acid at higher concentrations of this salt. The  
21 point obtained at 0.5  $\text{mol kg}^{-1}$  has, however, a high precision, and therefore it is possible to  
22 reliably state that magnesium sulfate induces salting-in of Ile at low concentrations.  
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39 Due to the scarcity of amino acid solubility data in aqueous electrolyte systems, no  
40 comparison is at the moment possible. However, the previous experience of the authors on these  
41 measurements<sup>38-40</sup>, the low standard deviations observed and a careful analysis of the solubility  
42 of the amino acids in water, all support the quality of the measured data. In fact, the solubilities  
43 in pure water of the three amino acids considered in this work compare well with results  
44 available in the literature. The solubility of dl-alanine is in high agreement with the value given  
45 by Ferreira et al.<sup>38</sup> and that of l-isoleucine is within the values presented in the detailed  
46 comparison published by Ferreira et al.<sup>39</sup> (see Table 2). Concerning l-valine, the solubility  
47 measured in this work, i.e., 58.45 g per kg of water, is in very good agreement with the average  
48 solubility from five independent sources<sup>81-83</sup>, which is 58.7 g/kg of water, supporting the quality  
49 of the data measured in this work.  
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3 **3.2. MD simulations.** To understand the specific effects of ions on the aqueous solubilities of the  
4 amino acids in terms of their molecular-level interactions, MD simulations of Ala, Val and Ile in  
5 water or in aqueous salt solutions were performed. Radial distribution functions (RDFs) were  
6 calculated for all the possible interactions involving the amino acid constituting groups (Figure  
7 1), the anions, the cations and water. These RDFs provide a quantitative description of  
8 enhancement (values larger than 1) or depletion (values smaller than 1) of densities of species  
9 around a selected moiety. The most relevant RDFs are presented in the main body of the paper,  
10 while additional plots are provided as Supporting Information.

11 **Solute-Water Interactions.** We start by analyzing the RDFs of water around the amino acid  
12 molecules, shown in Figure 3 for Ile (for which the effects are more evident) and in the  
13 Supporting Information for the other amino acids (Figures S1 and S2). The evidence obtained for  
14 the interaction pattern of the amino acids with water does not actually show significant  
15 differences in the hydration of the biomolecules among the different systems studied, but only  
16 minor dissimilarities which are likely to be related to a slight dehydration of the amino acids  
17 induced by the salts. As shown in Figure 3(a), there are no significant differences in the water  
18 distribution around the terminal carbon atoms of the amino acids due to the presence of the salts.  
19 Instead, only small, yet noticeable, decreases of the intensities of the peaks when going from the  
20 ammonium to the magnesium salts are observed around the apolar moieties of the biomolecules,  
21 suggesting that these are slightly more hydrated in the presence of the  $\text{NH}_4^+$  cation than in the  
22 presence of  $\text{Mg}^{2+}$ . These results are in good agreement with the hydration properties of the  
23 cations<sup>76,77</sup> reported in Table 5 and expected from the formation of hydration complexes that  
24 results from the strong hydration of magnesium ions in solution<sup>78</sup>. On the other hand, the  
25 charged amino group is more hydrated in systems comprising the chloride salts (Figure 3(b)),  
26 while the interactions of  $\text{COO}^-$  with water are decreased by the presence of all the electrolytes  
27 (Figure 3(c)). As discussed below, these differences in amino acid hydration are at least partly  
28 due to specific interactions between these molecules and the ions in solution.

29 **The Role of the Anion.** The patterns of interaction of the anions with the biomolecules are  
30 similar to those observed in a previous work<sup>23</sup> dealing with the solubility of amino acids in  
31 aqueous solutions of sodium-based salts. Actually, the RDFs displayed in Figure 4 for Ile do not  
32 reveal the presence of the sulfate ion in the first solvation layer around the non polar moieties of  
33 the amino acids, but show a clear and intense binding to their positively charged groups.

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3 Although the interaction of  $\text{SO}_4^{2-}$  with the carboxylate group is also significant, this affinity is a  
4 consequence of the presence of the cations around  $\text{COO}^-$  in the aqueous solutions, which shield  
5 unfavorable interactions of the anions with the negatively charged parts of the amino acids and  
6 promote the indirect binding observed in the RDFs. In fact, the strong peak observed for  $\text{MgSO}_4$   
7 in Figure 4(c), as well as the very strong first peak for this ion pair observed in Figure 9(b), are  
8 evidence for a cation-mediated interaction between the  $\text{SO}_4^{2-}$  and  $\text{COO}^-$  anions. In order to better  
9 visualize the molecular picture described, a snapshot from a simulation of (Ile+ $\text{MgSO}_4$ +water)  
10 mixtures showing the relative positions of the ions around the amino acid is displayed in Figure  
11 5, and the spatial distribution functions (SDF) calculated for this system are provided in Figure 6.  
12 As it can be seen in Figure 6(a), the SDF region for the  $\text{Mg}^{2+}$  cation is between the region  
13 comprehended by the carboxyl group of Ile and the sulfate ion, supporting the idea of a  
14  $\text{COO}\cdots\text{Mg}^{2+}\cdots\text{SO}_4^{2-}$  configuration. Moreover, as shown in Figure 6(b), the SDF regions for  $\text{SO}_4^{2-}$   
15 are mainly located at one of the sides of the amino acid, near the  $\text{NH}_3^+$  group, suggesting both a  
16 preferential presence of the anion near the positively charged moiety of Ile and a less favorable  
17 presence in the vicinity of  $\text{COO}^-$ .  
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30 As far as  $\text{Cl}^-$  is concerned, the simulation results do not show any important association  
31 of the anion to the non polar moieties of the amino acids, but only interactions with the  $\text{NH}_3^+$   
32 group (albeit significantly weaker than for  $\text{SO}_4^{2-}$ ). The patterns of the interactions described for  
33 Ile are also observed for Val (Figure S4, *cf.* SI). In the case of Ala (Figure S3, *cf.* SI), an apparent  
34 appreciable affinity for  $\text{C}_t$  is, however, a reflection of the interaction of the anions with the  
35 charged moieties of the amino acid due to the small size of the alkyl chain of the amino acid (all  
36 its carbon atoms are close to its charged moieties). These results support the salting-in/salting-out  
37 mechanism based on the presence/absence of interactions between the low/high charge density  
38 anions and the hydrophobic moieties of the amino acids proposed before<sup>23</sup> and are consistent  
39 with the lower impact of chloride salts observed experimentally and with the strong salting-out  
40 influence of the sulfate ion on the aqueous solubilities of amino acids<sup>33,34,38</sup>. It has been shown<sup>43</sup>  
41 that regardless of the cationic species used,  $\text{SO}_4^{2-}$  salts promote salting-out effects of amino  
42 acids, proteins and other macromolecules, consistently with the behavior expected from the  
43 Hofmeister series of anions.  
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54 **The Role of the Cation.** Although the molecular interpretation of the influence of anions on the  
55 aqueous solubilities of biomolecules has generated much debate over the years, the molecular  
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phenomena behind the cation-specific effects have been more difficult to explain and are still  
poorly understood<sup>42-45</sup>. To evaluate the role of the cation, we begin by considering the results  
obtained for  $\text{NH}_4\text{Cl}$ , a salt composed of weakly hydrated ions (Table 5). As shown in Figure  
7(a),  $\text{NH}_4^+$  is strongly associated to the carboxylate group of the amino acids, as suggested by the  
intense peaks corresponding to the contact pair  $\text{O}(\text{COO}^-)\cdots\text{N}(\text{NH}_4\text{Cl})$ . The RDF peaks displayed  
in Figure 7(b) for the distribution of the N atom of  $\text{NH}_4^+$  around  $\text{C}_\alpha$  of the amino acids indicate  
that the ammonium is present only at large distances. It seems thus that, contrarily to the salting-  
in anions, the weak binding of  $\text{NH}_4^+$  to the non polar moieties of the amino acids is not very  
significant, as observed in the case of sodium and potassium ions<sup>23</sup>. To better support this result,  
the data obtained in this work for  $\text{NH}_4\text{Cl}$  were compared to experimental<sup>34,38</sup> and to simulation<sup>23</sup>  
data obtained previously for other salts comprising the same anion,  $\text{KCl}$  and  $\text{NaCl}$ . As can be  
seen in Figure 8 for Ile and Figure S5 (*cf.* Supp. Inf.) for Ala and Val, the simulation results do  
not reveal the presence of significant interactions of  $\text{NH}_4^+$ ,  $\text{K}^+$  and  $\text{Na}^+$  with the hydrophobic  
groups of the amino acids, since the peaks observed in the RDFs of these cations around the  $\text{C}_\alpha$   
atoms of Ala, Val and Ile occur at large distances. The interactions of the ammonium ion are  
nevertheless somewhat stronger than those of  $\text{Na}^+$  and  $\text{K}^+$ , which are practically absent from the  
vicinity of the non polar parts of the biomolecules.

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According to the MD results, the low charge density  $\text{NH}_4^+$  interacts weakly with the  
hydrophobic regions of Ala, Val and Ile through a combination of ion-induced dipole and  
dispersion interactions, promoting a slender stabilization of the amino acids in water and  
therefore a slight salting-in effect. On the other hand, almost no interactions occur in sodium and  
potassium aqueous solutions, and therefore even less pronounced impacts on the solubility are  
expected. These results are in good agreement with the experimental data available for the  
behavior of the systems under study, systematized in Figures S6 and S7 (*cf.* Supp. Inf.). Indeed,  
as shown in Figure S6, while  $\text{NH}_4\text{Cl}$  induces small increases in the solubility of Ala in water,  
 $\text{NaCl}$  promotes a slight salting-out effect. For  $\text{KCl}$  there are two contradictory experimental  
results, but the simulation data reported in this work does not support a salting-in influence of  
this salt – instead, based on the observed absence of interactions between  $\text{K}^+$  and the non-polar  
groups of Ala, a salting-out effect of  $\text{KCl}$  is expected, with a magnitude greater than that induced  
by  $\text{NaCl}$ , as observed experimentally by some authors<sup>38</sup>. Since the intensities of the RDF peaks  
referring to the  $\text{C}_\alpha$ -cation contact pairs in Ile, Ala and Val mixtures (Figure 8 and Figure S5)

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3 decrease in the same order, the rank of the relative influence of the ions is likely to be similar  
4 among the systems considered. In fact, as presented in Figure S7,  $\text{NH}_4\text{Cl}$  and  $\text{NaCl}$  induce,  
5 respectively, an increase and a slight decrease in the aqueous solubility of Val, while,  
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decrease in the same order, the rank of the relative influence of the ions is likely to be similar among the systems considered. In fact, as presented in Figure S7,  $\text{NH}_4\text{Cl}$  and  $\text{NaCl}$  induce, respectively, an increase and a slight decrease in the aqueous solubility of Val, while, inconsistently, the experimental data available for  $\text{KCl}$  is indicative of a salting-in effect. For Ile, the experimentally observed influence of  $\text{NH}_4\text{Cl}$  (this work) is salting-in, but there is no solubility data available for the other two salts. It is worth to notice, however, that some differences observed in the RDFs of Figures 8 and S5 when comparing the different amino acids might help explain dissimilarities in the magnitude and/or direction of the effects promoted by the salts. This issue, related to the influence of the structural characteristics of the biomolecules, will be discussed below.

If, as previously shown<sup>23</sup> and reinforced by the results reported above, there is no significant binding of  $\text{Cl}^-$  with non-polar regions, both in  $\text{KCl}$  and  $\text{NaCl}$  systems<sup>23</sup> and in  $\text{NH}_4\text{Cl}$  solutions, then the role of the cation-amino acid association will be dominant in determining the direction of the solubility effects induced by the salt. However, the evidence gathered for  $\text{NH}_4\text{Cl}$  solutions suggests that the molecular mechanisms governing the cation-specific effects are different from those proposed for anions, according to which “chaotropic” species positioned in the extreme of the Hofmeister series, like  $\text{ClO}_4^-$ , establish favorable interactions with the non polar groups of the biomolecules, inducing, as a consequence, remarkable increases of their aqueous solubilities<sup>23</sup>.

To acquire additional knowledge about the mechanism of action of the cations, we analyzed in detail the results obtained for the divalent magnesium ion. The RDFs depicted in Figures 8 and S5 reveal the absence of direct interactions of  $\text{Mg}^{2+}$  with the non polar moieties of the amino acids, being the solvation layer occupied by water molecules as shown in Figure 3. On the other hand, the association of the cation to the charged groups, especially to the negatively charged carboxylate group, is exceptionally strong. Indeed, the RDF peaks corresponding to the contact pairs  $\text{Mg}^{2+} \cdots \text{O}(\text{COO}^-)$ , displayed in Figure 9(b) for Ile and in Figures S8 and S9 for Ala and Val (*cf.* Supp. Inf.), are remarkably intense and occur at very short distances, indicating the presence of an extremely significant  $\text{Mg}^{2+}$  structuring around  $\text{COO}^-$ . This suggests the formation of a complex of the cation with the amino acids, which would explain its strong salting-in influence. An issue that comes up in this context and which is worth to notice is that sometimes amino acid-salt RDFs cannot, *per se*, be directly related to the experimental solubility data.

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Actually, despite the presence of ion-amino acid short-range correlations, the presence of the salt may cause enhanced amino acid-water correlations which can lead to salting-out (as opposed to salting-in which might be anticipated by analyzing the amino acid-salt RDF alone). For instance, it has been shown for aqueous sodium salt solutions of amides<sup>84</sup> that, despite the observed short-range peak corresponding to the interaction of Na<sup>+</sup> with the carbonyl oxygen, this group is preferentially hydrated, resulting in salting-out by NaCl and not salting-in. Ion specific effects will not be therefore correctly interpreted unless the relative affinities of amino acid-salt and water-amino acid are compared. As it can be seen from the comparison of the relative intensities of the RDF peaks displayed in Figure 3(c) and Figure 9(b), not only the Mg<sup>2+</sup>-O(COO<sup>-</sup>) interaction is very strong, but also it is stronger than the H(H<sub>2</sub>O)-O(COO<sup>-</sup>) association. This result provides support to the salting-in influence observed and explained for the magnesium salts.

A tridimensional picture of the interactions between the magnesium ion and the amino acids can be obtained from the spatial distribution functions (same isovalue as in Figure 6) calculated for the magnesium chloride salt around isoleucine, depicted in Figure 10. As shown in Figure 10(a), the SDF regions for the Mg<sup>2+</sup> ions are clearly located around the carboxyl group of the amino acid, as also observed in the case of MgSO<sub>4</sub> mixtures (Figure 6). A few dissimilarities between the two magnesium salt systems considered are, however, worth to notice. First, the configuration of the type COO<sup>-</sup>···cation···anion observed in the case of the magnesium sulfate aqueous solutions (Figure 6) does not exist in the magnesium chloride mixtures (Figure 10(a)). Furthermore, while, as discussed above, the sulfate anion is preferentially positioned at one side of the amino acid, near the positively charged group of Ile and avoiding the vicinity of COO<sup>-</sup> (Figure 6(b)), Cl<sup>-</sup>, though also preferentially found close to NH<sub>3</sub><sup>+</sup>, is located at both sides of the amino acid (Figure 10(b)).

Divalent cations have well recognized specific binding to proteins, negatively charged polyelectrolytes, peptides, nucleic acids and fatty acid headgroups, and are known to stabilize a variety of protein structures<sup>44,45,85-88</sup>, but information on the exact nature of the interactions between those ions and the anionic groups of the biomolecules is vague and scarce. It is well known that magnesium cations can form complexes with amino acids and proteins in aqueous solutions<sup>89</sup> and molecular models of those species have been proposed<sup>78,86</sup>. Divalent magnesium ions seem to prefer ligands of low polarizability, with oxygen being the favorite coordinating

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3 atom, and have been found to bind directly to the polar hydrophilic protein residues, surrounded  
4 by a shell of non polar hydrophobic groups. In negatively charged structures,  $Mg^{2+}$  is directly  
5 bound to the negatively charged residues, and it has been suggested that the association is a  
6 stepwise process where some of its inner-shell water molecules are replaced by negatively  
7 charged side-chains<sup>86</sup>. Tian et al.<sup>78</sup> suggested a molecular structure for complexes between  
8 amino acids and magnesium ion. According to the authors, the cation is bound to four oxygen  
9 atoms, in a cyclic [amino acid-Mg-amino acid] complex. As discussed above (Figure 9), the  
10 simulation results obtained in this work suggest instead the possibility of the formation of a  
11 complex between the amino acids and  $Mg^{2+}$ , which would be charged and consequently more  
12 soluble, justifying the pronounced salting-in effect induced by  $MgCl_2$ . Since the intensity of the  
13 interactions between the carboxylate groups of the amino acids (Figure S10, *cf.* Supp. Inf.) is  
14 decreased in the presence of  $MgCl_2$ , our results do not support the presence of complexes of the  
15 type proposed by Tian et al.<sup>78</sup>. Though a clear description of the structure of amino acid-  
16 magnesium complexes cannot be found in literature and the data obtained in this work does not  
17 either allow to properly infer the molecular nature of those species, further support for the  
18 existence of such complexes can be provided by several studies. In fact, chelation of magnesium  
19 ions by carboxylic acids and amino acids in aqueous solutions has been considered before and  
20 the stability constants for such systems have been reported<sup>90-92</sup>. Moreover, the participation of  
21 the carboxyl group in the chelating reaction has been confirmed by spectroscopic methods<sup>92</sup>.  
22 Other pharmaceutical and medical reports describe as well the use of preparations of  
23 magnesium-amino acid chelates as commercial products to treat human magnesium deficiency  
24 related diseases<sup>93,94</sup>. Those chelate forms are very soluble and are known to be one of the most  
25 bioavailable forms of magnesium<sup>94</sup>.  
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Further evidence for the mechanism under study can be obtained from the analysis of the RDFs corresponding to the interactions between the  $NH_4^+$ ,  $Mg^{2+}$ ,  $Na^+$  and  $K^+$  cations and the carboxylate group, displayed in Figure 11 for Ile and Figure S11 (*cf.* Supp. Inf.) for Ala and Val. The comparison of the MD data reveals that the association of  $Mg^{2+}$  to  $COO^-$  is indeed much more remarkable than that of the other cations, which can be understood in terms of the charge of these cations. The RDFs also suggest that the binding of  $NH_4^+$  to the carboxylate group is somewhat more important than that of  $Na^+$ . Almost no interactions are observed for  $K^+$  as indicated by the depletion of the peaks corresponding to the contact pair  $K^+ \cdots O(COO^-)$ . These



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3 observations are quantitatively corroborated by the values of the CN calculated for the  
4 interactions of the cations with the carboxyl group of the amino acids (Table 6) which decrease  
5 in the order  $Mg^{2+} > NH_4^+ > Na^+ > K^+$ . These results suggest that because of its high charge density,  
6  $Mg^{2+}$  is able to form charged complexes with the amino acids, which are very soluble and will  
7 have a positive effect on the strong salting-in influence of this ion. The association of  $NH_4^+$  to  
8 the carboxylate group is much less intense than that involving the  $Mg^{2+}$  cation. Nevertheless this  
9 interaction may anticipate the formation of weak single charged positive complexes which are  
10 less soluble than the double charged ones formed in the case of aqueous solutions containing  
11 magnesium salts. Therefore less pronounced salting-in effects are promoted by  $NH_4Cl$  as  
12 observed experimentally in this work (Tables 1 and 3, Figure 2(a)).  $Na^+$  and particularly  $K^+$  are  
13 not able to establish such interactions, probably because their solvation by the water molecules is  
14 more favorable (Table 5), and their solubility effects are then governed by a distinct mechanism  
15 as discussed above. Previous works on this subject provide further support to this interpretation.  
16 In fact, it has been argued that ammonium and sodium ions cannot form cyclo-complexes with  
17 amino acids in the same way as magnesium and other divalent cations such as  $Ca^{2+}$ <sup>78</sup>, and  $Na^+$   
18 and  $K^+$  are often viewed as roughly neutral<sup>95</sup>. Density functional theory calculations of the  
19 relative stability of gas-phase complexes between metal ions and amino acids<sup>96-98</sup> have shown  
20 that the Gibbs energies of (ion-amino acid) systems are much less positive in the case of divalent  
21 ions than of monovalent ions. For instance, for (ion-arginine) complexes<sup>96</sup>, the values of the gas-  
22 phase Gibbs energies follow the order  $Ni^{2+}(-1544 \text{ kJ mol}^{-1}) < Cu^{2+}(-1404 \text{ kJ mol}^{-1}) < Zn^{2+}(-$   
23  $1280 \text{ kJ mol}^{-1}) < Mg^{2+}(-1028 \text{ kJ mol}^{-1}) < Ca^{2+}(-660 \text{ kJ mol}^{-1}) < Li^+(-328 \text{ kJ mol}^{-1}) < Na^+(-225$   
24  $\text{ kJ mol}^{-1}) < K^+(-152 \text{ kJ mol}^{-1})$ , at  $T=298.15 \text{ K}$ .

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26 All evidence suggests that the mechanism by which salting-in inducing cations act in  
27 aqueous solutions of amino acids is different from that of anions. Contrarily to these, salting-in  
28 cations do not establish important interactions with the non polar moieties of the biomolecules,  
29 but instead with their charged parts, especially with the carboxylate group. The type and strength  
30 of that association will determine the differences found in the magnitude of the solubility effects  
31 promoted. Divalent cations such as  $Mg^{2+}$  are able to form charged and very soluble complexes  
32 with the amino acids, therefore acting as strong salting-in agents. For monovalent cations like  
33  $NH_4^+$ , the binding to  $COO^-$  still occurs but is less favorable, and therefore the ammonium ion  
34 promotes only a slight increase in the aqueous solubilities of the amino acids. When the solvation  
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3 by water of some cations is more favorable than the interaction with the carboxylate, it leads to a  
4 decrease in the aqueous solubility of the biomolecules and the underlying molecular mechanism  
5 is thus similar to that observed for the salting-out effects of anions. Interestingly, this reversal in  
6 the Hofmeister series for cations is observed in systems containing sites of negative charge<sup>45,80</sup>,  
7 but not in systems comprising neutral biocompounds or other types of molecules<sup>57,79</sup>. For  
8 instance, in IL-based aqueous two-phase systems, the magnesium behaves as a salting-out  
9 inducing ion<sup>79</sup> since it is readily solvated by water and thus does not interact with the low charge  
10 density IL anion.  
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12 **The cation-anion competition.** While the data obtained in this work for the chloride salts,  
13 comprising different cations with a weakly hydrated anion, enables the clarification of the  
14 mechanism by which salting-in inducing cations operate in aqueous solutions of amino acids, the  
15 analysis of the results for the sulfate salts, containing a strongly hydrated anion, allows the  
16 elucidation of the competing effects of the cation and anion on these systems.  
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18 A brief comparison of the experimental and simulation data obtained for  $\text{NH}_4\text{Cl}$  vs  
19  $(\text{NH}_4)_2\text{SO}_4$  or  $\text{MgCl}_2$  vs  $\text{MgSO}_4$  aqueous solutions of Val provides further insight into the  
20 molecular interaction mechanism proposed here. According to the results obtained in this work  
21 (Tables 1 to 4, Figure 2) the presence of a salting-out anion leads to a decrease of the magnitude  
22 of the salting-in induced by the cation and, in the case of  $(\text{NH}_4)_2\text{SO}_4$ , even to a change in  
23 direction of the solubility effect. It is therefore reasonable to conjecture that when the anion is  
24 sulfate, the interactions of the cations with the amino acids are overcome by the interactions  
25 occurring between the anion and the other species of the system.  
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27 As shown in Figure S4 and Figure 12 for Val,  $\text{SO}_4^{2-}$  is significantly associated to the  
28 charged groups of the amino acid, but shows no binding to its non polar moieties and has a very  
29 favorable hydration. Its action is thus ruled by the mechanism previously proposed for the  
30 salting-out anions<sup>23</sup>. In systems containing cations such as  $\text{NH}_4^+$ , with a relatively small affinity  
31 to the amino acid, the sulfate ion, behaving as a strong salting-out inducing ion, controls the  
32 solubility effect promoted by the salt. As reported in this work (Table 2 and Figure 2), a decrease  
33 in the aqueous solubility of Val in the presence of ammonium sulfate is observed. On the other  
34 hand, when  $\text{SO}_4^{2-}$  is the counterion of  $\text{Mg}^{2+}$ , a cation that strongly associates to the amino acid, a  
35 complex interplay of interactions will take place and their balance will determine the final effect  
36 observed. The less pronounced increases in the solubility of Val in aqueous solutions of  
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3 magnesium sulfate relatively to the magnesium chloride systems can therefore be explained in  
4 terms of a competition between the interactions established, in which the salting-out formation of  
5 hydration complexes by  $\text{SO}_4^{2-}$  is balanced by the salting-in effect of the strong  $\text{Mg}^{2+}/\text{COO}^-$   
6 interactions.  
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10 The conclusions derived from the analysis of the results obtained for the aqueous  
11 solutions of Val gain further support when Ala and Ile solutions are considered. In fact, similar  
12 interaction patterns are observed for these amino acids (Figures S3 and S4, *cf.* Supp. Inf.),  
13 enabling to interpret the more pronounced increase of the solubility of these amino acids in the  
14 presence of  $\text{MgCl}_2$  than in mixtures containing  $\text{MgSO}_4$  (Tables 3 and 4, Figure 2(b)), and the  
15 salting-out of Ile occurring in concentrated solutions of  $(\text{NH}_4)_2\text{SO}_4$ <sup>40</sup>.  
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21 In summary, when the salts comprise an anion with low hydration energy, the direction  
22 and magnitude of the solubility influence observed will be almost only governed by the nature of  
23 the cation. When the anion has a significantly favorable hydration, both the cation and the anion  
24 will have a role on the solubility impact of the salt, and a complex balance of competitive  
25 interactions will determine its influence.  
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### 33 Conclusions

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35 Experimental solubility measurements and MD simulations were performed to obtain  
36 further insight into the molecular-level interactions that control the solubility behavior of amino  
37 acids in aqueous saline environments. The results obtained enable to clarify the role of the cation  
38 and of the anion on the mechanisms by which salts operate in aqueous solutions of amino acids.  
39 The evidence gathered shows that the molecular phenomena governing the action of salting-in  
40 inducing cations is different from those behind the effects of anions. Contrary to the latter,  
41 salting-in inducing cations do not establish important interactions with the non polar moieties of  
42 the biomolecules, but instead with their charged parts, especially with the carboxylate group. The  
43 type and strength of that association is determined by the charge properties of the cation and will  
44 be responsible for the differences found in the magnitude of the solubility increases promoted.  
45 When the anion has a favorable hydration, both the cation and the anion will have a predominant  
46 role on the solubility impact of the salt, and a complex balance of competitive interactions will  
47 rule its influence.  
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Since amino acids can be taken as model systems, the molecular-level mechanism reported here can be helpful for understanding the solubility and stability behavior of other more complex biomolecules in aqueous electrolyte solutions, and thus be relevant to develop many areas of biotechnology and life sciences.

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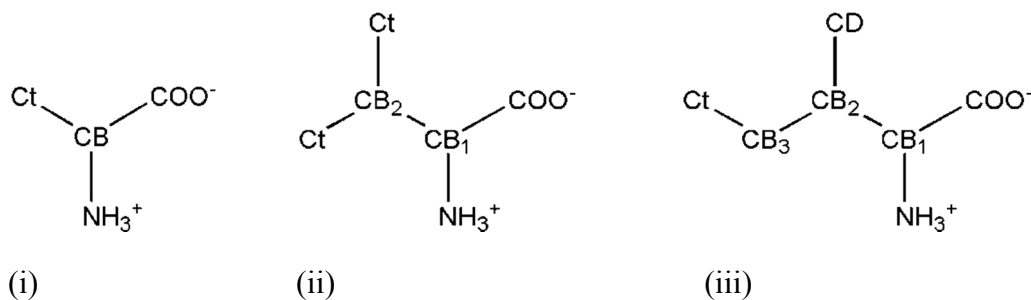
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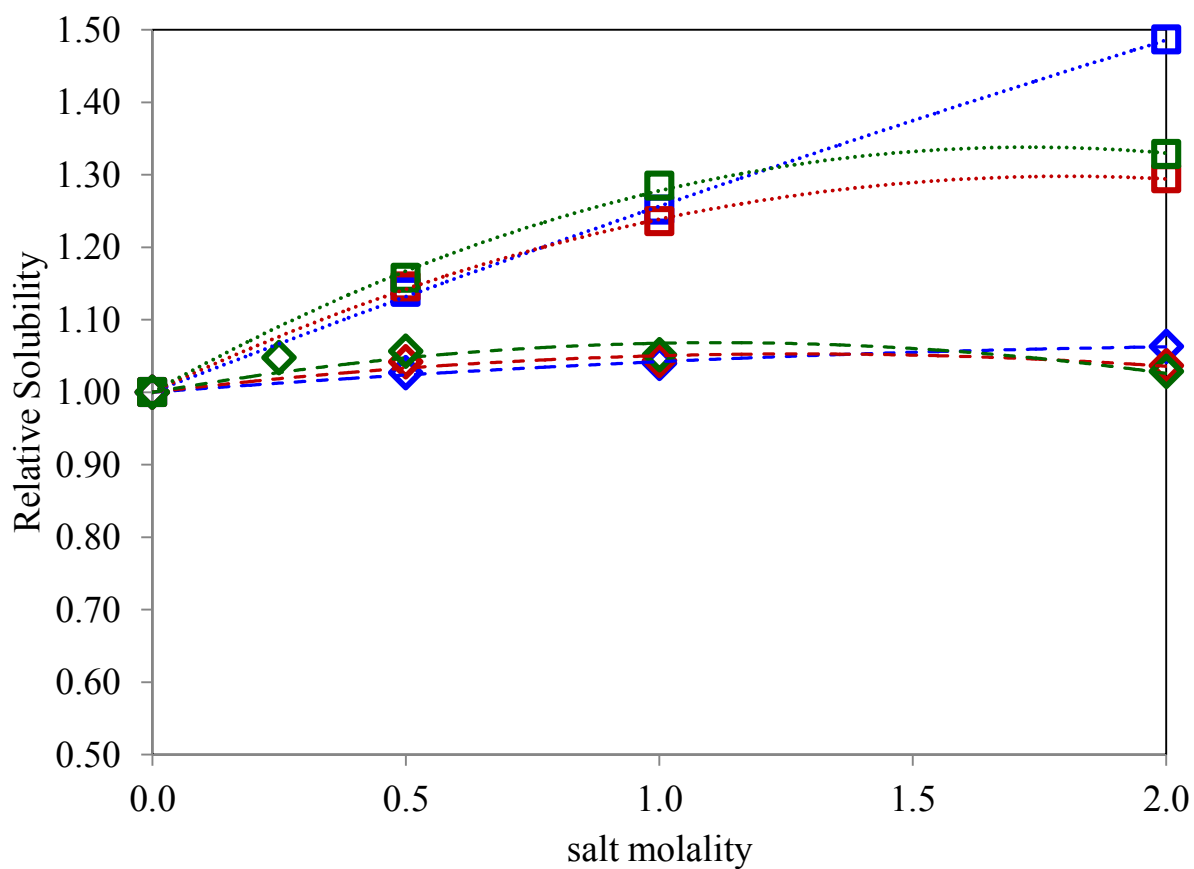
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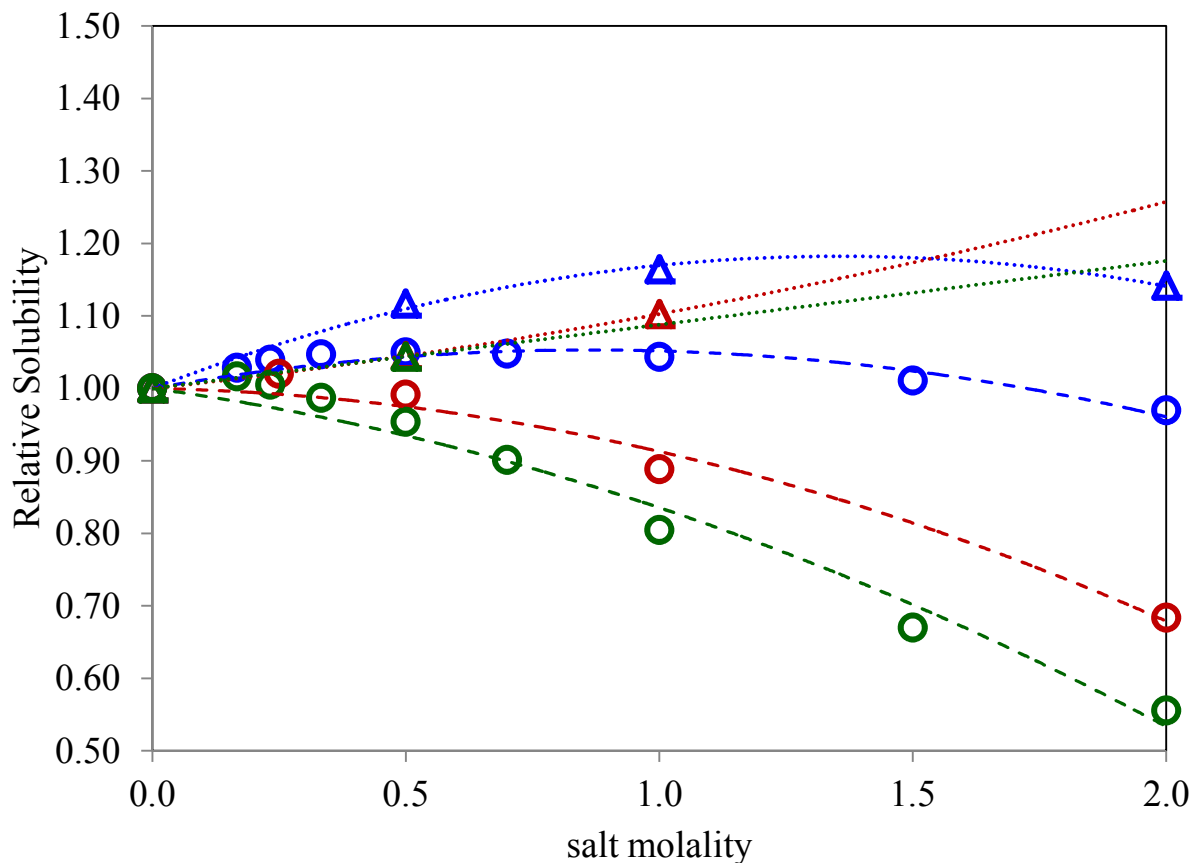
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**Figure 1.** Structure and atom labeling of the amino acids studied in this work: (i) alanine (Ala); (ii) valine (Val) and (iii) isoleucine (Ile).



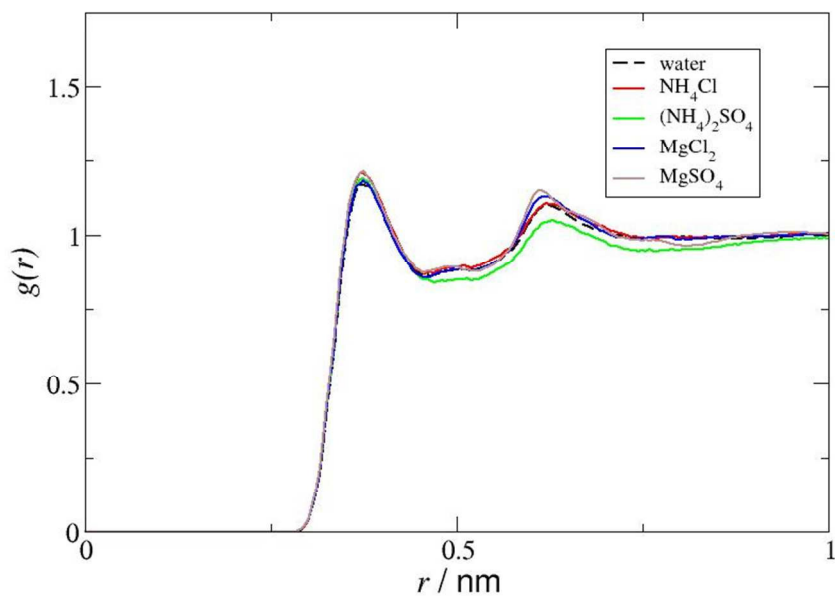
(a)



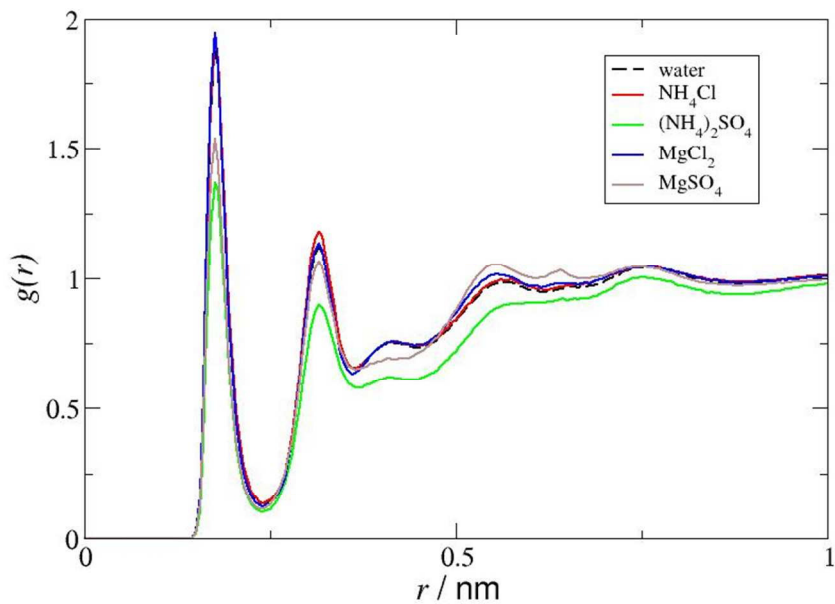


(b)

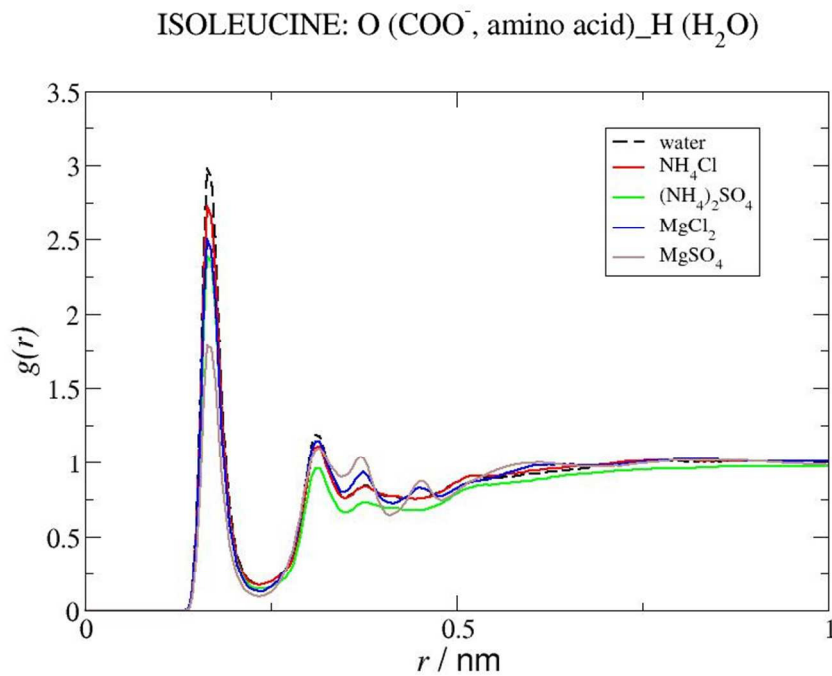
**Figure 2.** Relative solubility of alanine (blue), valine (red) and isoleucine (green) in (a)  $\diamond$ ,  $\text{NH}_4\text{Cl}$ ;  $\square$ ,  $\text{MgCl}_2$ ; and in (b)  $\text{O}$ ,  $(\text{NH}_4)_2\text{SO}_4$ ;  $\Delta$ ,  $\text{MgSO}_4$  aqueous solutions, at 298.15 K. Lines are guides to the eyes: dashed lines,  $\text{NH}_4\text{Cl}$  and  $(\text{NH}_4)_2\text{SO}_4$ ; dotted lines,  $\text{MgCl}_2$  and  $\text{MgSO}_4$ . The values of the solubility of alanine and isoleucine in aqueous  $(\text{NH}_4)_2\text{SO}_4$  solutions were taken from ref 40.

ISOLEUCINE: C<sub>t</sub> (amino acid)\_O (H<sub>2</sub>O)

(a)

ISOLEUCINE: H (NH<sub>3</sub><sup>+</sup>, amino acid)\_O (H<sub>2</sub>O)

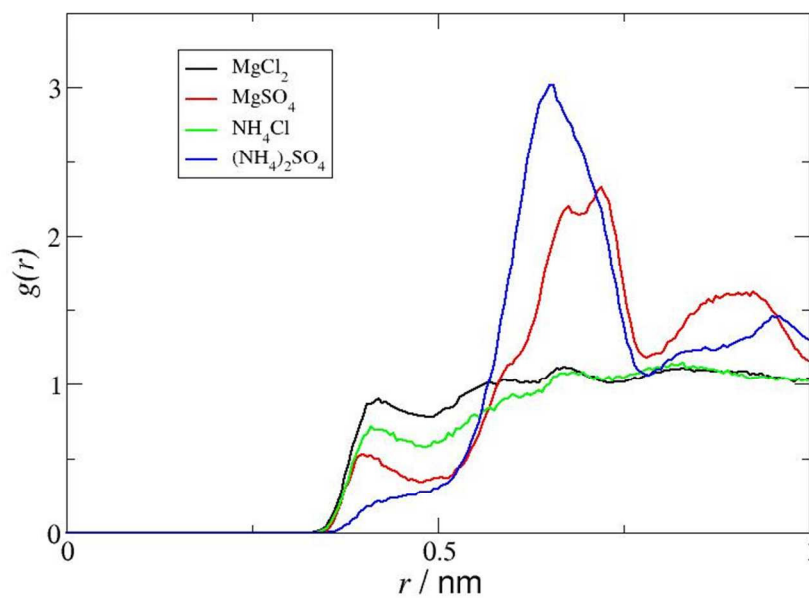
(b)



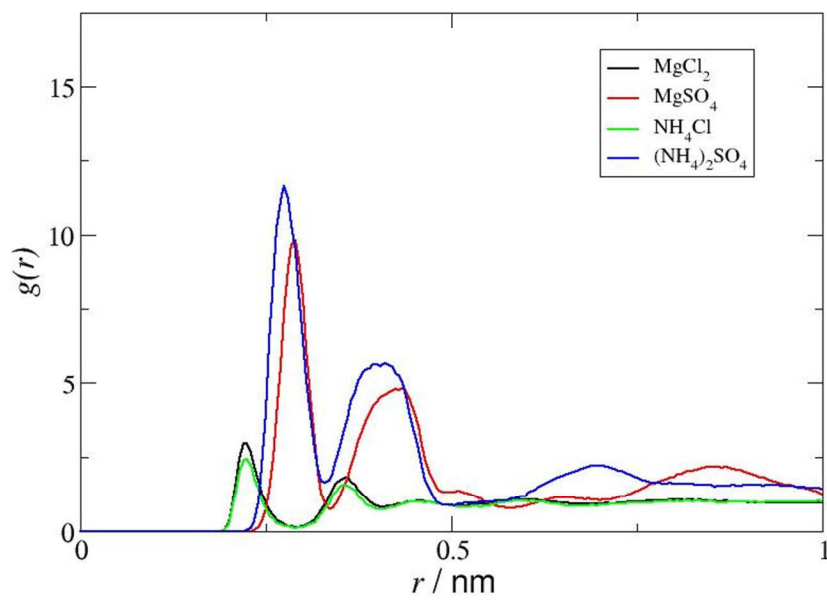
(c)

**Figure 3.** Radial distribution functions of the oxygen and hydrogen atoms of water around selected groups of Isoleucine.

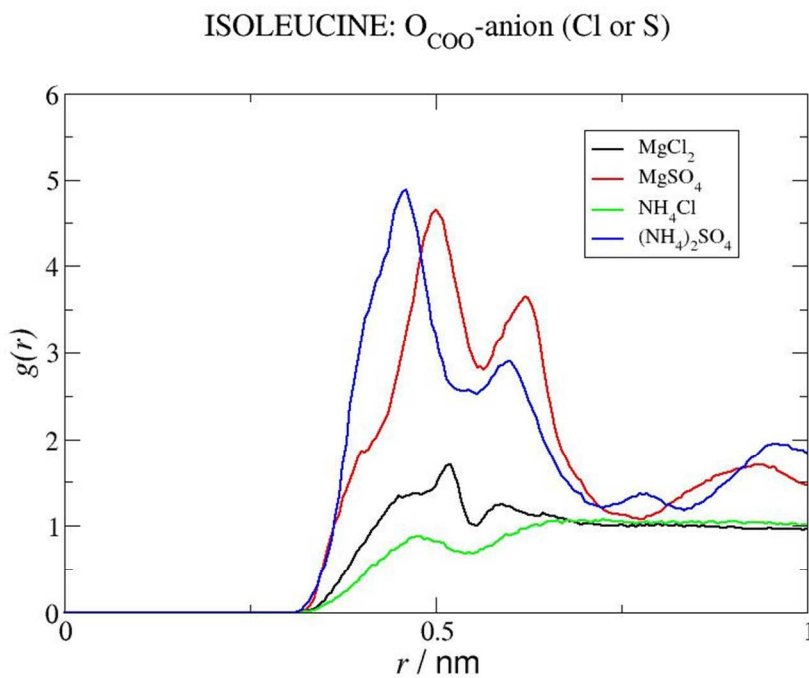
## ISOLEUCINE: Ct-anion (Cl or S)



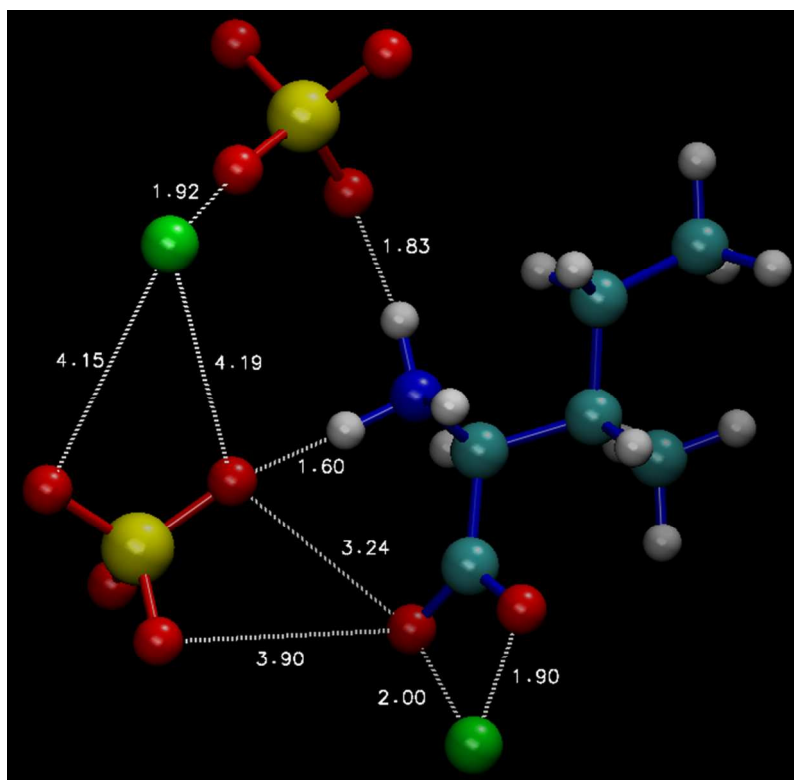
(a)

ISOLEUCINE:  $\text{H}_{\text{NH}_3}$ -anion (Cl or S)

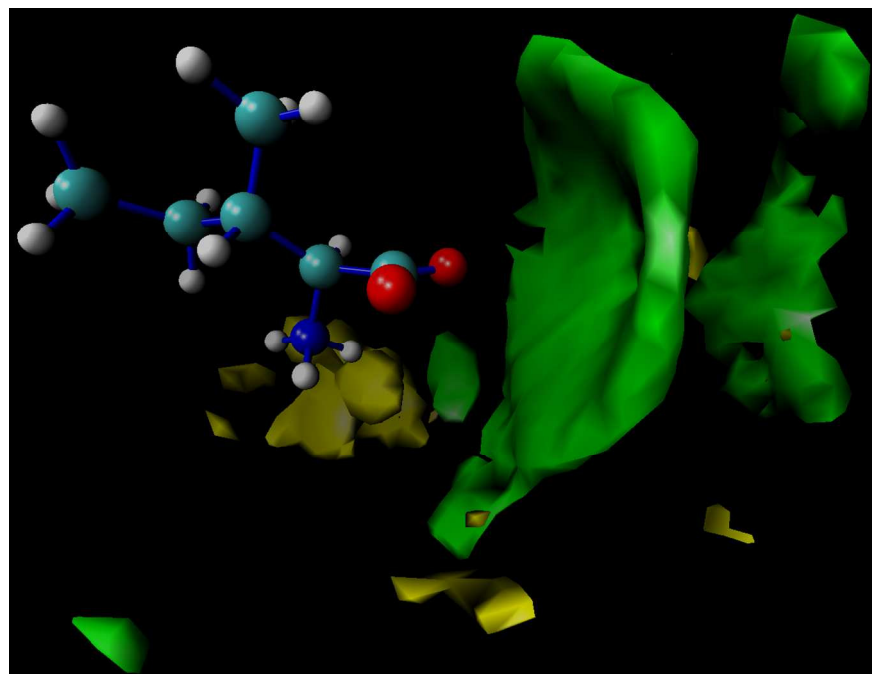
(b)



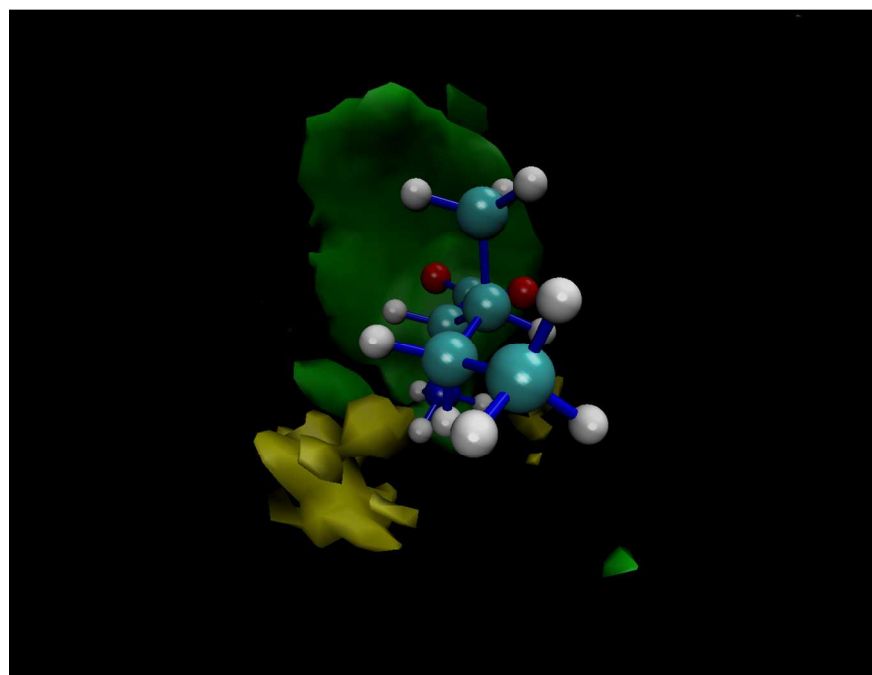
**Figure 4.** Radial distribution functions between different molecular regions of Ile and the central atom (Cl or S) of the anion.



**Figure 5.** Snapshot from a simulation of (Ile+MgSO<sub>4</sub>+water) mixtures, showing the distances (Å) between selected atoms. The water molecules are omitted for clarity. Light blue spheres represent carbon atoms, dark blue spheres are nitrogen atoms, red spheres are oxygens, white spheres are hydrogens, yellow spheres are sulfur, and green spheres are magnesium.

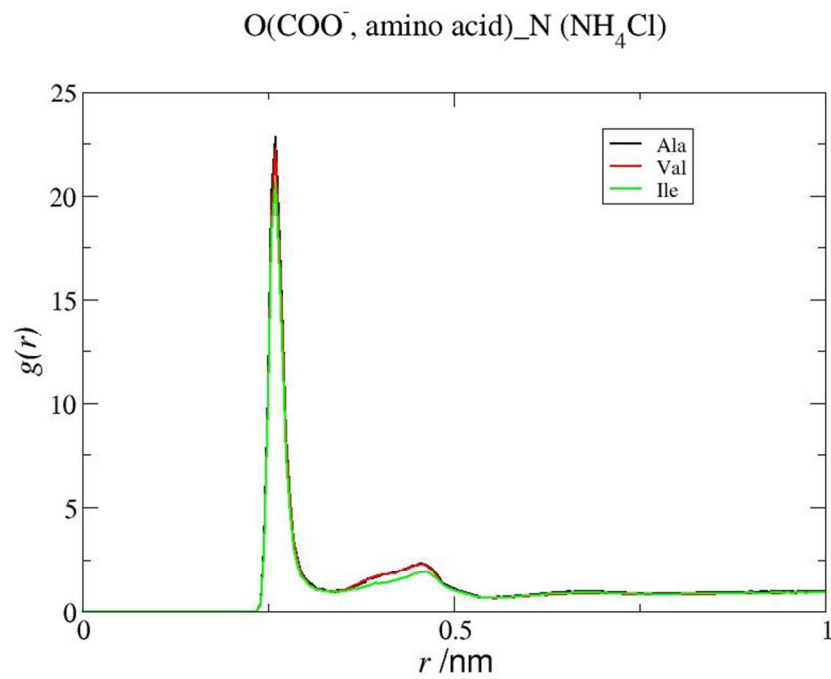


(a)

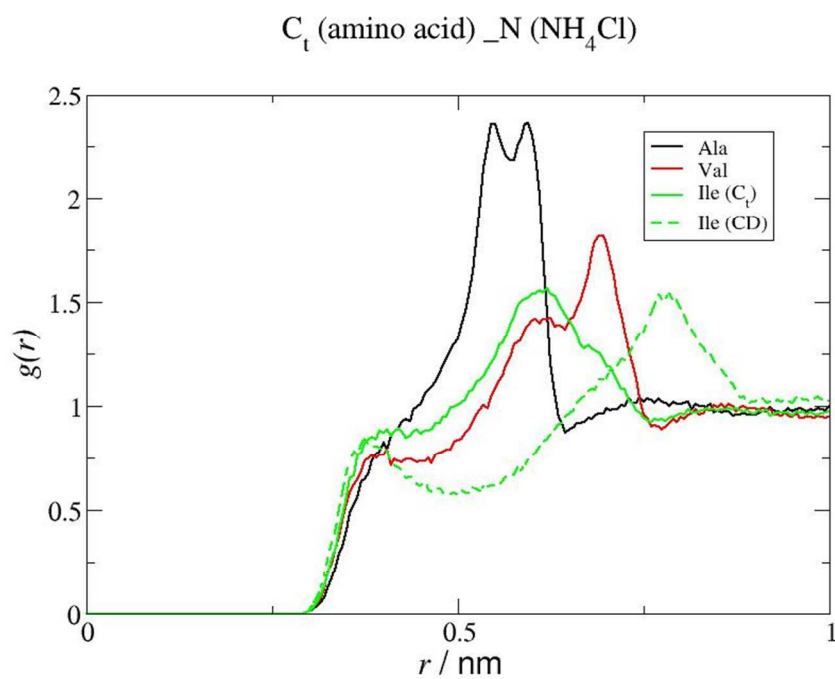


(b)

**Figure 6.** Spatial distribution functions (SDF) for the magnesium (green) and sulfate (yellow) ions around Ile in (Ile+MgSO<sub>4</sub>+water) mixtures. Color code for the explicitly represented atoms is the same as in Figure 8.



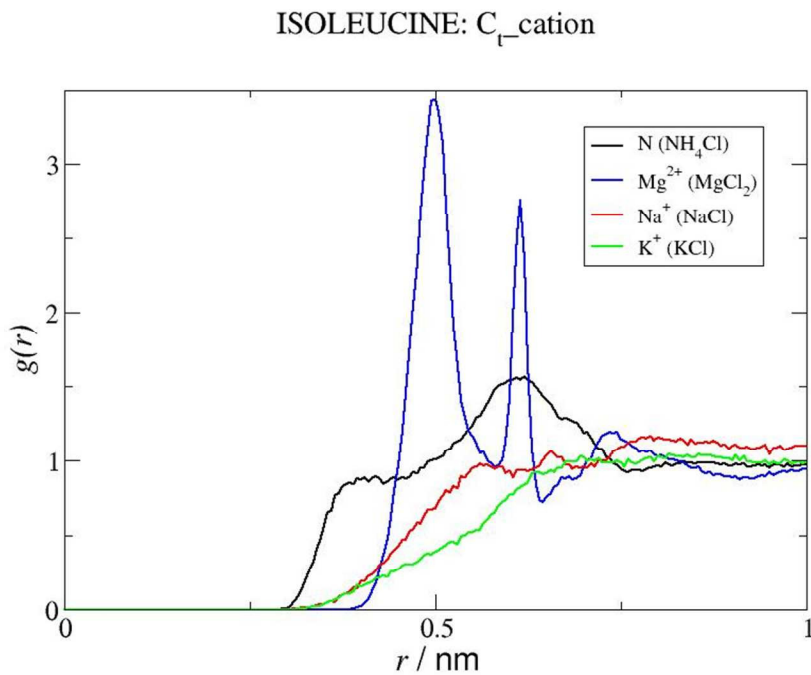
(a)



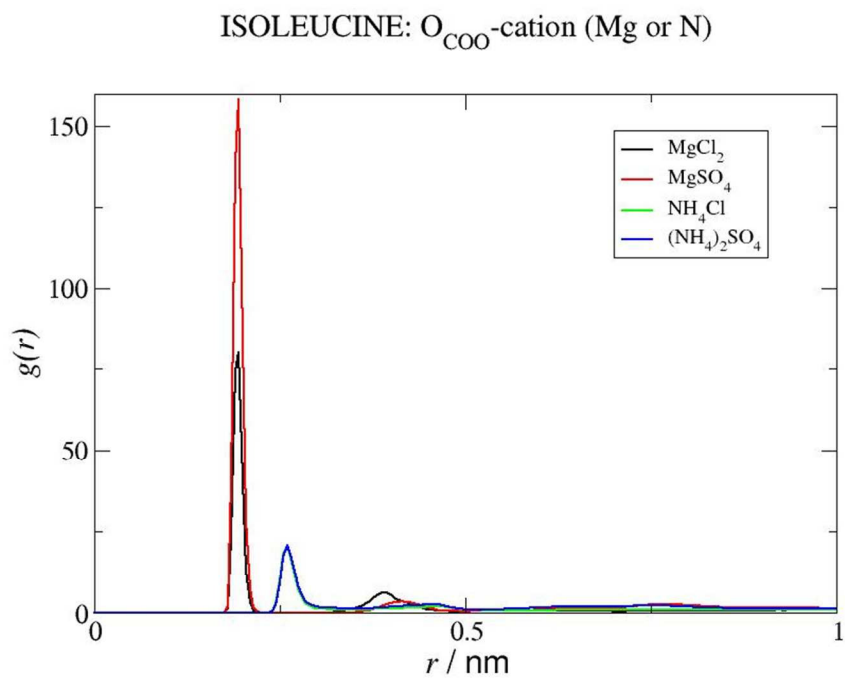
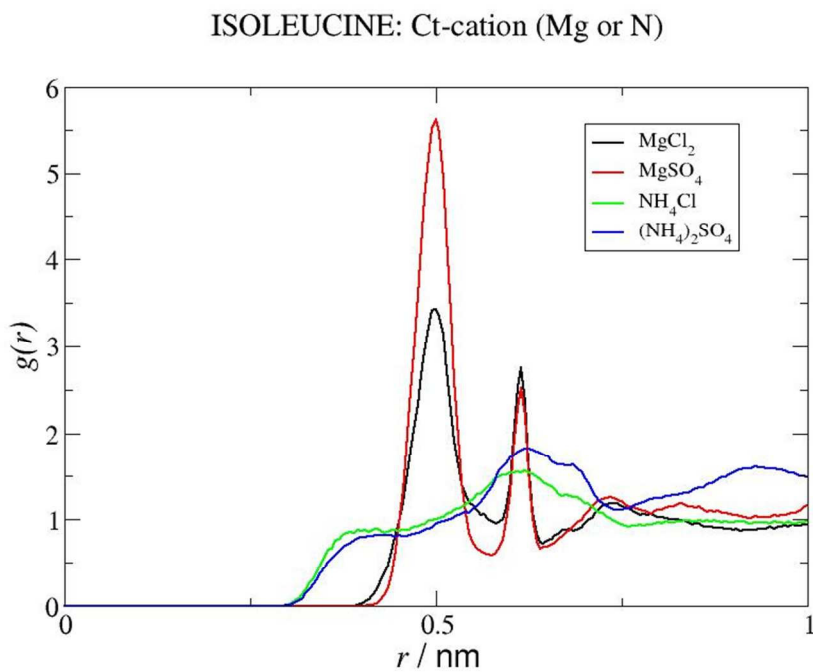
(b)

**Figure 7.** Radial distribution functions for the interactions between different molecular regions of the amino acids and the central atom (N) of the cation of NH<sub>4</sub>Cl.



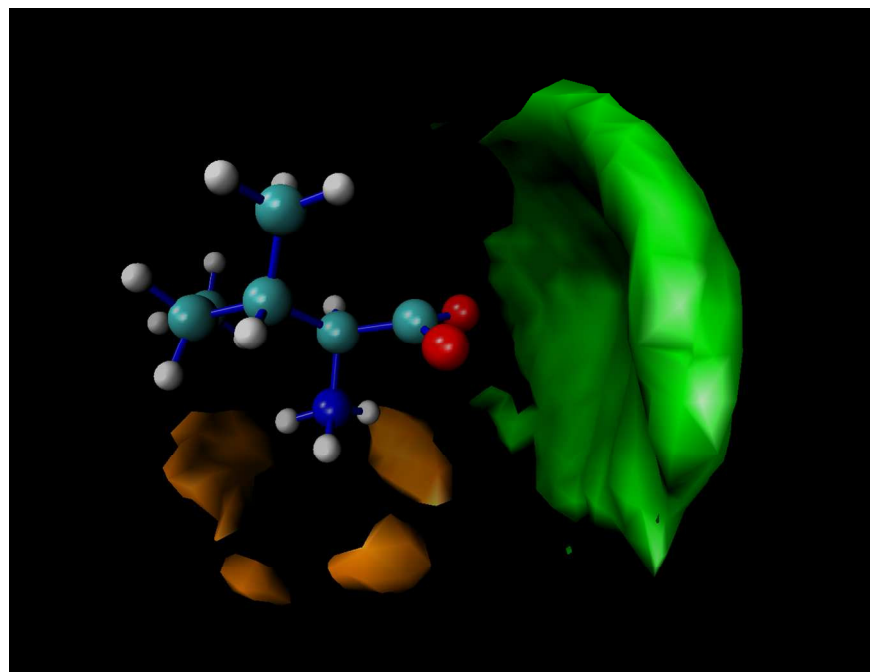


**Figure 8.** Radial distribution functions for the interactions between the cations of NH<sub>4</sub>Cl (this work), MgCl<sub>2</sub> (this work), NaCl (ref 23) and KCl (ref 23) and the terminal carbon atom (C<sub>1</sub>) of Ile.

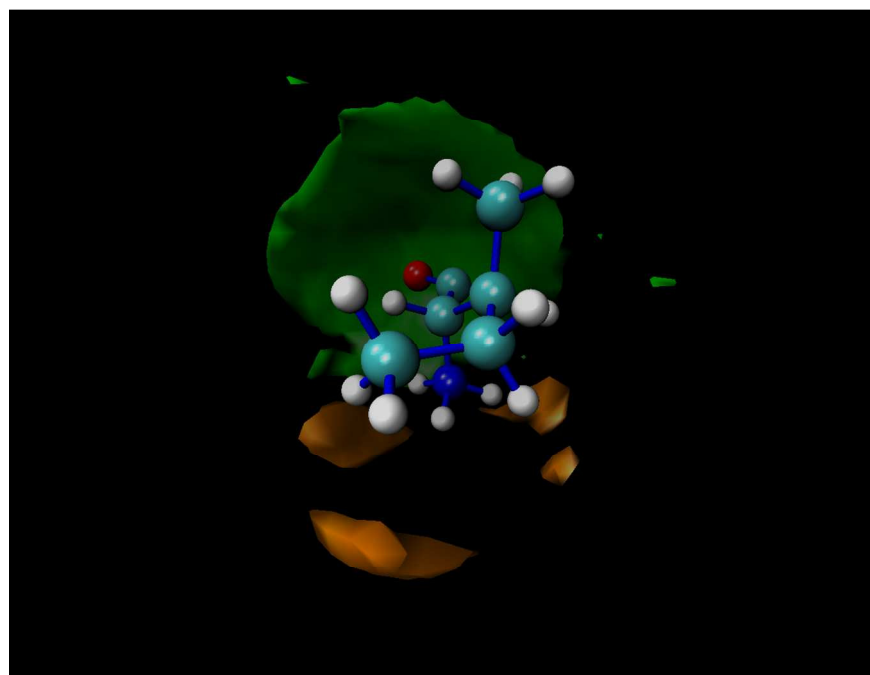


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**Figure 9.** Radial distribution functions between different molecular regions of Ile and the central atom (Mg or N) of the cation.

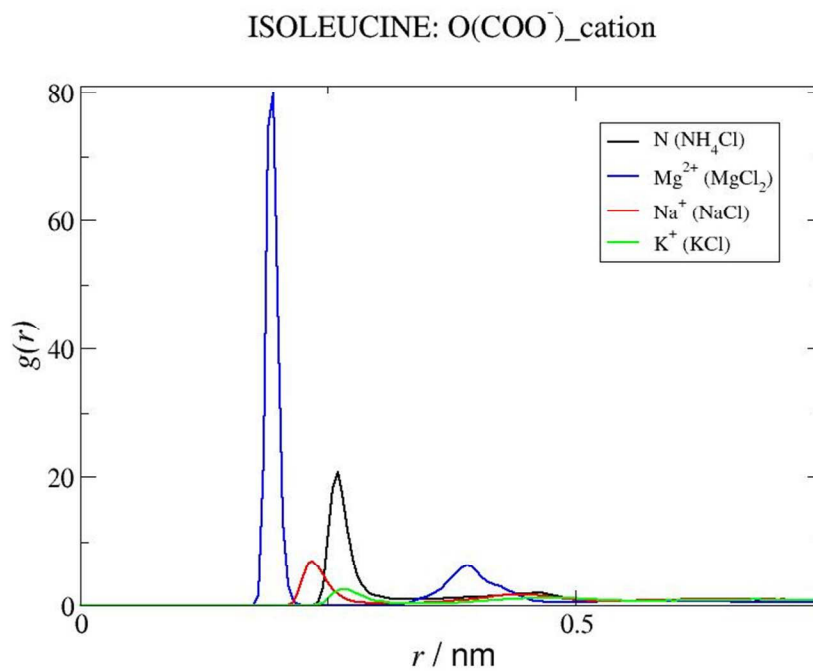


(a)



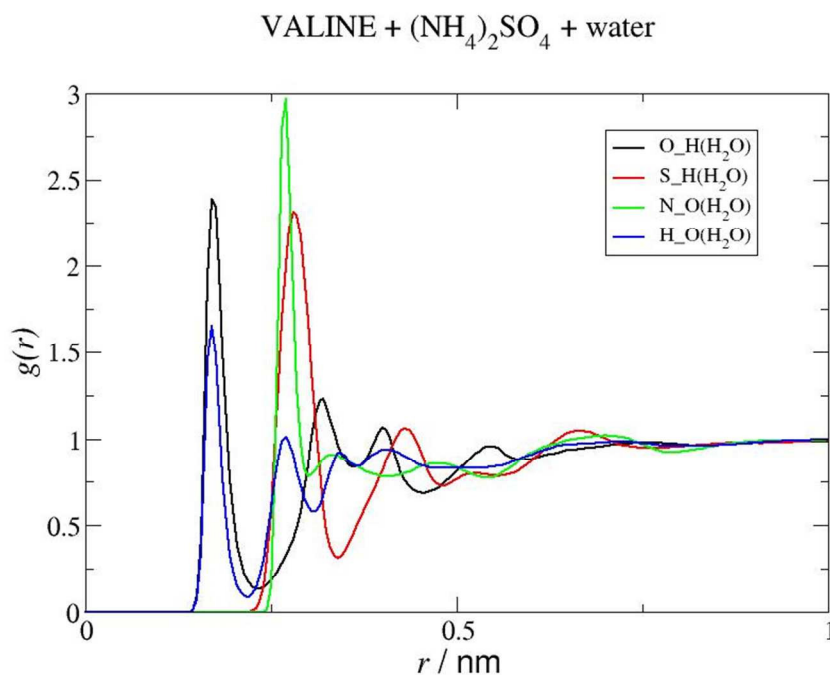
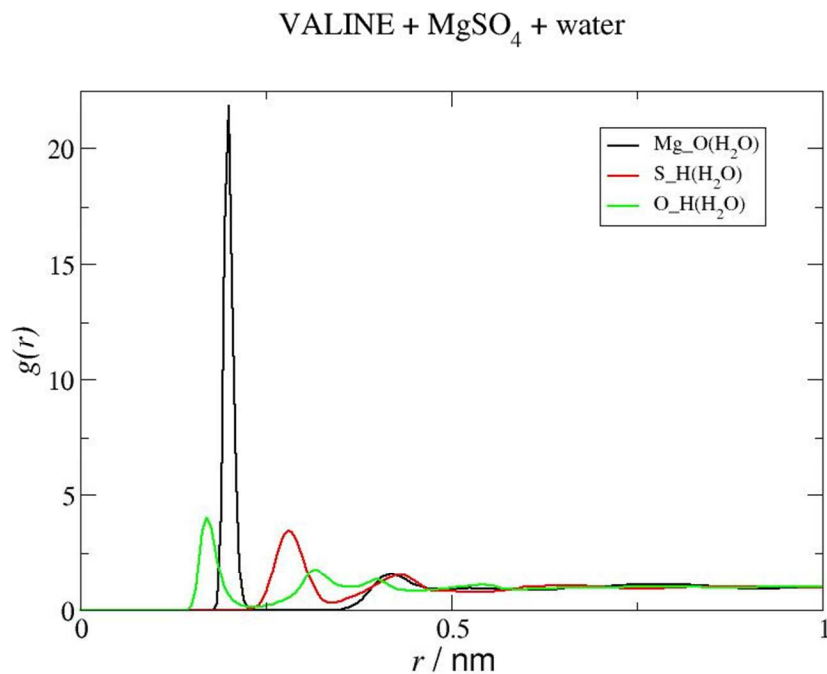
(b)

**Figure 10.** Spatial distribution functions (SDF) for the magnesium (green) and chloride (orange) ions around Ile in (Ile+MgCl<sub>2</sub>+water) mixtures. Color code for the explicitly represented atoms is the same as in Figure 8.



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**Figure 11.** Radial distribution functions for the interactions between the cations of NH<sub>4</sub>Cl (this work), MgCl<sub>2</sub> (this work), NaCl (ref 23) and KCl (ref 23) and the carboxyl group of isoleucine.



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**Figure 12.** Radial distribution functions for the interactions between water molecules and the ions in aqueous solutions of Val in the presence of MgSO<sub>4</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

**Table 1.** Amino acid solubility (g/kg of water) at  $T= 298.15$  K and different  $\text{NH}_4\text{Cl}$  molalities.

$\text{NH}_4\text{Cl}$ Molality	dl-Alanine	l-Isoleucine	l-Valine
0.00	165.44 (0.20)	34.756 (0.048)	58.449 (0.236)
0.25	-	36.419 (0.077)	-
0.50	169.90 (0.11)	36.722 (0.083)	60.910 (0.078)
1.00	172.04 (0.05)	36.554 (0.250)	61.010 (0.237)
2.00	175.88 (0.07)	35.764 (0.232)	60.608 (0.445)

**Table 2.** Amino acid solubility (g/kg of water) at  $T=298.15$  K and different  $(\text{NH}_4)_2\text{SO}_4$  molalities.

$(\text{NH}_4)_2\text{SO}_4$ Molality	dl-Alanine	l-Isoleucine	l-Valine
0.00	165.44 (0.20) <sup>(a)</sup>	34.756 (0.048) <sup>(a)</sup>	58.449 (0.236) <sup>(a)</sup>
0.17	165.77 <sup>(b)</sup>	33.59 <sup>(b)</sup>	-
0.23	170.50 <sup>(b)</sup>	34.15 <sup>(b)</sup>	-
0.25	-	-	59.629 (0.240) <sup>(a)</sup>
0.33	173.57 <sup>(b)</sup>	33.15 <sup>(b)</sup>	-
0.50	173.99 <sup>(b)</sup>	32.04 <sup>(b)</sup>	57.941 (0.240) <sup>(a)</sup>
0.70	173.71 <sup>(b)</sup>	30.28 <sup>(b)</sup>	-
1.00	172.95 <sup>(b)</sup>	27.03 <sup>(b)</sup>	51.921 (0.109) <sup>(a)</sup>
1.50	167.56 <sup>(b)</sup>	22.51 <sup>(b)</sup>	-
2.00	160.74 <sup>(b)</sup>	18.67 <sup>(b)</sup>	39.947 (0.321) <sup>(a)</sup>

<sup>(b)</sup> Ref 40<sup>(a)</sup> This work

**Table 3.** Amino acid solubility (g/kg of water) at  $T=298.15$  K and different  $\text{MgCl}_2$  molalities.

$\text{MgCl}_2$ Molality	dl-Alanine	l-Isoleucine	l-Valine
0.00	165.44 (0.20)	34.756 (0.048)	58.449 (0.236)
0.50	188.35 (0.05)	40.247 (0.245)	66.983 (0.158)
1.00	207.08 (0.14)	44.652 (0.021)	72.230 (0.279)
2.00	245.95 (0.10)	46.182 (0.236)	75.686 (0.230)



**Table 4.** Amino acid solubility (g/kg of water) at  $T=298.15$  K and different  $\text{MgSO}_4$  molalities.

$\text{MgSO}_4$ Molality	dl-Alanine	l-Isoleucine	l-Valine
0.00	165.44 (0.20)	34.756 (0.048)	58.449 (0.236)
0.50	184.82 (0.15)	36.284 (0.128)	61.067 (0.050)
1.00	192.61 (0.20)		64.447 (0.370)
2.00	188.91 (0.17)		

**Table 5.** Molar Entropy of Hydration,  $\Delta_{\text{hyd}}S$ , and Gibbs Free Energy of Hydration,  $\Delta_{\text{hyd}}G$ , at 298.15 K for the ions studied in this work<sup>76,77</sup>.

Ion	$\Delta_{\text{hyd}}S / (\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1})$	$\Delta_{\text{hyd}}G / (\text{kJ}\cdot\text{mol}^{-1})$
$\text{Cl}^-$	-75	-340
$\text{SO}_4^{2-}$	-200	-1080
$\text{NH}_4^+$		-285
$\text{Mg}^{2+}$	-331	-1830
$\text{Na}^+$	-111	-365
$\text{K}^+$	-74	-295

**Table 6.** Calculated Coordination Numbers (CN) for the interactions of the cations with the carboxyl group of the amino acids in salt/water/amino acid ternary systems.

Interaction	Ala	Val	Ile
$\text{Mg}^{2+}(\text{MgCl}_2)\text{-O}(\text{COO}^-)^a$	0.18	0.12	0.12
$\text{N}(\text{NH}_4\text{Cl})\text{-O}(\text{COO}^-)^b$	0.12	0.11	0.10
$\text{Na}^+(\text{NaCl})\text{-O}(\text{COO}^-)^c$	0.03	0.05	0.03
$\text{K}^+(\text{KCl})\text{-O}(\text{COO}^-)^d$	0.02	0.01	0.02
$\text{Mg}^{2+}(\text{MgSO}_4)\text{-O}(\text{COO}^-)^e$	0.12	0.12	0.24

<sup>a</sup> Calculated from the  $\text{Mg}^{2+}(\text{MgCl}_2)\text{-O}_{\text{COO}^-}$  RDF. <sup>b</sup> Calculated from the  $\text{N}(\text{NH}_4\text{Cl})\text{-O}_{\text{COO}^-}$  RDF. <sup>c</sup> Calculated from the  $\text{Na}^+(\text{NaCl})\text{-O}_{\text{COO}^-}$  RDF. <sup>d</sup> Calculated from the  $\text{K}^+(\text{KCl})\text{-O}_{\text{COO}^-}$  RDF. <sup>e</sup> Calculated from the  $\text{Mg}^{2+}(\text{MgSO}_4)\text{-O}_{\text{COO}^-}$  RDF.

## TOC

