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Towards an understanding of the aqueous solubility of amino acids in the presence of salts: A Molecular Dynamics simulation study

Luciana I. N Tomé[‡], *Miguel Jorge*[ϒ], *José R. B. Gomes*[‡] *and João A. P. Coutinho*[‡]*

[‡]CICECO, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

[ϒ]LSRE/LCM – Laboratory of Separation and Reaction Engineering, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal

*Corresponding author

Tel: +351-234-370200; Fax: +351-234-370084; E-mail address: jcoutinho@ua.pt

Abstract:

Ion specific effects on the aqueous solubilities of biomolecules are relevant in many areas of biochemistry and life sciences. However, a general and well-supported molecular picture of the phenomena has not yet been established. In order to contribute to the understanding of the molecular-level interactions governing the behavior of biocompounds in aqueous saline environments, classical Molecular Dynamics simulations were performed for aqueous solutions of four amino acids (alanine, valine, isoleucine and 2-amino-decanoic acid), taken as model systems, in the presence of a series of inorganic salts. The MD results reported here provide support for a molecular picture of the salting-in/salting-out mechanism based on the presence/absence of interactions between the anions and the non-polar moieties of the aminoacids. These results are in good qualitative agreement with experimental solubilities and allow for a theoretical interpretation of the available data.

Keywords: amino acids, solubilities, Hofmeister series, molecular interactions, molecular dynamics

Introduction

Aqueous saline solutions are the natural environment of most biological molecules. The study of the effect of the nature and concentration of ions on the solubility of biomolecules is thus of utmost importance to understand the biochemistry of natural systems, develop medical and pharmaceutical responses to diseases induced by biochemical disorders [1-4], and improve the efficiency of biotechnological processes [5,6].

Specific ion effects on biomolecule solubility were first identified from the ability of certain salts to precipitate proteins in aqueous solution [7], and are now recognized to be general and relevant in a wide range of biochemical processes [8-13]. Although the rank of the relative influence of ions on the physico-chemical behavior of aqueous systems, known as the Hofmeister series [7], is well established, the underlying molecular mechanisms are far from being elucidated and consensual, in spite of the several explanations proposed during the last century [8,10,14-23]. The interpretation originally proposed was based on the ability of a particular ion to alter the hydrogen-bond network of water [14,16,17,23]. For some authors, there was little doubt that the main cause of the effect was how the bulk water structure was affected by ions that could be considered either “water structure makers” or “water structure breakers”. While salting-out inducing species, typically referred to as "kosmotropes", were believed to be able to "create" the bulk water structure, tending to precipitate proteins and prevent unfolding, salting-in inducing ions, classified as "chaotropes", would "destroy" it, leading to the solubilization and destabilization of folded macromolecules. Lately, the structure maker/breaker classical dogma has been severely questioned in face of new evidence [24-30] obtained in the past few years. Both experimental [24-27] and simulation studies [28-30] seem to indicate that the ions have little effect on the overall hydrogen bonding of water in bulk solution, and newer theories emphasizing the significant role of dispersion forces and involving the relative polarizabilities of the ions and the specific ion binding have been proposed [18,31]. One of the most consistent theories to understand the effect of salts on the aqueous solubility of molecules was suggested by Zhang et al. [10,15,19] to describe specific ion effects on the solubility of poly(N-isopropylacrylamide) in water. They claim that the

Hofmeister effects of salts on the solubilities of macromolecules depend on direct interactions of the ions with the solutes (macromolecules) and with water molecules in the first hydration shell of the solutes. This theory was recently successfully extended by us to the interpretation of the solubility of charged molecules in aqueous solutions of inorganic salts or amino acids [32-35].

The current interest in the Hofmeister series and its effects is evident from the explosion of publications on this subject [24-30]. Undoubtfully, long-held classical ideas about changes in bulk water structure are progressively being overturned as new data flurries. Nevertheless, the lack of a universal molecular picture to explain this phenomenon makes it consensual that further research on this matter is mandatory.

Besides their commercial and biochemical relevance, amino acids are the simplest building blocks of more complex biomolecules, such as peptides and proteins, and are therefore ideal molecules to be studied as model compounds. It is not surprising, thus, that much effort has been put into the study of their solubility properties with the aim of understanding the solubility, stability, activity and selectivity behavior of proteins and other biomolecules in aqueous saline media. The effect of salts on the aqueous solubilities of amino acids is experimentally well documented and phenomenologically well established. Experimental measurements of the solubility of amino acids in (water+salt) mixtures 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 [36-43]. The influence of different conditions, including pH [36,38,44] and temperature [40,42,43], have also been considered. As a general trend, ion effects on amino acid aqueous solubilities follow the Hofmeister series. Nevertheless, there are still some contradictory results [37,41], not to mention the lack of a consistent molecular description of the phenomenon. Clearly, alternative approaches and methods capable of providing evidence for the interactions that govern the influence of common salts on the aqueous solubilities of amino acids are required. Only then will it be possible to reach a solid and deep knowledge of the behavior of proteins and more complex biomolecules and, consequently, thoroughly understand some biochemical processes and control their biological implications [1], identify the causes and develop medical solutions

for so many diseases associated to protein disfunctions [2-4], and improve the efficiency of biotechnological processes [5,6].

In order to contribute to the understanding of the molecular mechanisms behind the effect of salts on the solubility of amino acids in aqueous solutions, molecular dynamics (MD) simulations were performed in this work for aqueous solutions of four amino acids - alanine (Ala), valine (Val), isoleucine (Ile), and a non-natural amino acid (2-amino-decanoic acid, Ada), all depicted in Figure 1 - in the presence of salts such as NaCl, KCl, $NaNO_3$, $NaClO_4$, and Na_2SO_4, at T = 298.15 K. The ions were selected in order to span the entire range of effects observed experimentally on amino acid aqueous solubility, from salting-in, to salting-out effects, passing through salts that have a negligible impact on the solubility. With the choice of these specific amino acids, we intend to simulate the effects of a different range of physical and chemical properties such as polarity and hydrophobicity. Moreover, because natural environments are very often neutral and, in addition, most of the experimental data available refers to aqueous saline solutions of amino acids at pH = 7, the simulations consider only the zwitterionic forms of the solutes.

MD simulation methods have proved to be a valuable tool for the investigation of biochemical systems [45-47], including studies on the interaction of common ions with proteins and peptides [47], and we have used them previously to understand the mechanisms behind the influence of salts on the behavior of other charged molecules such as ionic liquids [33]. This approach has, however, been seldom used to study aqueous saline solutions of amino acids [48]. In this work, the analysis of the radial distribution functions (RDFs) of the various groups and moieties, estimated by MD, and of the coordination numbers (C.N.) of ions around the amino acids, will give an insight into the preferential interactions between amino acids, ions and water, and provide support for a molecular mechanism behind the effects of salts on the behavior of amino acids in saline solutions. It is important to notice that the choice of force field employed in MD simulations has been shown to significantly affect some properties of aqueous ionic solutions, particularly as far as the pairing of simple ions with charged macromolecular surfaces or the pairing between small ions in water, considered key physical phenomena to explain many observed Hofmeister effects, is concerned [49-51]. Actually, small changes

in the effective pair potential between interacting ions can significantly affect solution thermodynamics and contact ion-pairing, and thus some force fields have failed to reproduce realistically the thermodynamics of electrolytes at certain concentrations [52,53]. For this reason, complementary data generated using different combinations of ion potentials will be additionally provided and discussed here. It will be shown that although absolute degrees of binding are somewhat affected by the choice of model, relative changes along the Hofmeister series are unchanged. Moreover, the present results are in qualitative agreement with experimental solubility data reported in the literature, which affords further consistency to our approach.

Computational Methods

MD calculations were performed for aqueous solutions of the zwitterionic forms of the amino acids in the presence of the salts. For KCl, three concentrations (0.25, 0.5 and 1.0 mol dm^{-3}) were selected. For the other salts, a concentration of 1.0 mol dm^{-3} was used. The simulations were carried out using the isothermal-isobaric *NpT* (T = 298.15 K and p = 1 bar) ensemble and the GROMACS 4.04 molecular dynamics package [54]. The equations of motion were integrated with the Verlet-Leapfrog algorithm [55] and a time step of 2 fs. The Nosé-Hoover thermostat [56,57] was used to fix the temperature while the Parrinello-Rahman barostat [58] was employed to fix the pressure. Starting configurations were generated in cubic boxes with lateral dimensions of 45 Å, and periodic boundary conditions were applied in three dimensions. The systems were prepared by randomly placing amino acids, ions and water molecules in the simulation box. Six amino acid molecules were included in each box, solvated by 900 water molecules. 17 cation-anion pairs were incorporated to obtain the 1.0 M concentration, except for KCl for which boxes with 4 and 9 cation-anion pairs were also used to obtain molarities of 0.25 M and 0.5 M, respectively. Then, a 10000 step energy minimization was performed and followed by two simulations, the first one with 50000 steps for equilibration and the final one with 5000000 steps for production. After equilibration, the values of the box volume ranged between 28.0 and 29.7 nm^3, depending on the particular combination of amino

acids and ions. Equilibration was checked by ensuring that all observables (including the RDFs) fluctuated around their equilibrium values during the production stage.

The intermolecular interaction energy between pairs of neighboring atoms was calculated using the Lennard-Jones potential to describe dispersion/repulsion forces and the point-charge Coulomb potential for electrostatic interactions. Long-range electrostatic interactions were accounted for using the particle-mesh Ewald method [59], with a cutoff of 1.0 nm for the real-space part of the interactions. A cutoff radius of 1.2 nm was used for the Lennard-Jones potential, and long-range dispersion corrections were added to both energy and pressure. All bond lengths were held rigid using the LINCS constraint algorithm [60], while angle bending was modeled by a harmonic potential and dihedral torsion was described (where appropriate) by a Ryckaert-Bellemans function. Potentials available in the literature were taken for all the species considered in the simulations. Water was described by the rigid SPC/E model [61], while the OPLS all-atom potential was used for the amino acids and for the sodium [62], potassium [62] and chloride [63] ions. For the perchlorate and nitrate ions, the models of Cadena and Maginn were used [64,65]. Finally, the force field parameters of the second model proposed by Cannon et al. were used for sulfate [66]. The effect of ion force field selection on the description of the systems was evaluated by performing additional MD simulations for Val/NaCl/water systems using ten selected combinations of five potentials for Na^+ and Cl^- ions (Smith-Dang [67], Dang [68], Weerasinghe-Smith [49], Aqvist [62] and Chandrasekhar [63] force fields).

Coordination numbers were calculated for the interactions between selected atoms in the saline solutions of Val and Ada. For that purpose, the function N(r) was obtained by integrating the corresponding RDFs (g(r)):

$$N(r) = 4.\pi.\rho_B.\int_0^r (r^2.g(r))dr \qquad (1)$$

where ρ_B is the number density of each atom in the bulk. The C.N. of a given ion (or water molecule) around a particular group of the amino acid may be calculated from several different RDFs (e.g., for ClO_4^- coordination around the NH_3^+ group, the C.N. can be obtained from N-Cl, N-O_{Cl}, H_N-Cl or H_N-O_{Cl} RDFs). In such cases, we verified the consistency of the calculations by checking that coordination numbers obtained from different RDFs were in close agreement. The values presented in this paper were obtained

from RDFs showing the most well-defined minima, thus enabling a more accurate truncation of the curve. The chosen RDFs (labels of selected atoms) are indicated in the corresponding tables, while the truncation radii are provided in Tables S2 and S3 of the Supporting Information.

Results and Discussion

In this work, MD simulation data is used to gather evidence for a molecular mechanism that can explain the experimental aqueous solubility of amino acids in the presence of salts available in the literature. From an analysis of the experimental data [36-43], it is clear that the type and magnitude of the solubility effects observed are dependent on the nature and concentration of the anion of the salt, as well as on the structural characteristics of the amino acids. In order to better rationalize and interpret these effects, they will be considered separately and discussed in the subsections below.

It is worth to note that the experimental data used in the discussion was obtained at T=298.15 K and pH=7. Under these conditions, all the amino acids are expected to be in their zwitterionic forms [69]. The structure of the amino acids considered in the MD simulations and the correspondent atom labeling are displayed in Figure 1.

Effect of the anion

To infer about the anion effect on the aqueous solubilities of aminoacids, the RDFs calculated from the MD simulations of Val in aqueous solutions of a series of sodium salts were considered. These RDFs provide a quantitative description of enhancement (values larger than 1) or depletion (values smaller than 1) of densities of ions or water around a selected part of the amino acid molecule.

The conventional kosmotropic/chaotropic model would interpret the observed effects of the salts on the solubility of the amino acids as resulting from a modification of

the water structure, and thus on the solvation of the amino acids by water in the presence of salts. The RDFs presented in Figure 2 show, however, an entirely different picture. Not only are no significant differences observed on the water distribution around the amino acids due to the presence of salts, but actually, contrary to what the previous model would suggest, a small but noticeable decrease of the intensity of the RDF peak on going from Na_2SO_4 to NaCl/$NaNO_3$ to $NaClO_4$, is observed around the apolar moieties of the amino acids. This would suggest that the amino acid is actually more solvated by water in the presence of SO_4^{2-}, a kosmotropic ion, than in the presence of ClO_4^-, a chaotropic ion that induces salting-in. This observation is confirmed by analysis of the water coordination numbers around the terminal carbon atoms (C_t) of Val and Ada, presented in Table 1 – the water C.N. is much smaller for ClO_4^- than for the other ions. In Ada, this dehydration effect is accompanied by a non-negligible aggregation of non-polar chain ends, as evidenced by the C_t-C_t coordination number (Table 1), which is largest in the presence of ClO_4^-. Nevertheless, it is important to notice that such chain aggregation was only observed for Ada (which possesses a rather long alkyl chain), while a stronger hydration of the terminal carbons in SO_4^{2-} solutions than in ClO_4^- mixtures was verified for all the systems under study.

The origin of this dehydration was analyzed by performing two additional simulations with a single Ada monomer in perchlorate and sulfate solutions, thus eliminating aggregation effects. In this case, the calculated coordination numbers for the interaction between the terminal carbon atom and water show an increase of ~3 water molecules when going from perchlorate (C.N.=17.48) to sulfate (C.N.=20.01), Table 2, which is similar to the difference found in the simulations with 6 Ada molecules on perchlorate (C.N.=11.44) and on sulfate (C.N.=14.33) solutions (Table 1). Additionally, the coordination numbers calculated for the interaction between C_t and the anions are ~1 in the presence of ClO_4^- and 0 in the case of SO_4^{2-} solutions. This shows clearly that perchlorate anions appear near the hydrophobic chains, contrasting with the behavior found for solutions containing sulfate anions. Therefore, it is possible to suggest that, due to the preferential binding of the perchlorate anion to the non polar moieties of Ada, the interactions between the Ada tails are enhanced and, thus, it is more likely that chain aggregation occurs as a consequence of dehydration, rather than the opposite. We suggest

a mechanism responsible for approaching the Ada tails similar to that described very recently by some of us for the role of silicate anions on the growing mechanism of surfactant micelles in the early stages of the synthesis of periodic mesoporous silica.[70,71]

In Figures 3(a-e) the RDFs of the anions around C_t and C_B atoms (representative of the non-polar part of Val), around the O and C atoms of the carboxylate group, and around the N atom of the amino group (representatives of the charged moieties of Val), are displayed. The first remarkable result is that two anions present opposite extreme behaviors. While ClO_4^- exhibits an appreciable affinity for C_t and C_B, as suggested by the intense first peak of the RDFs of Figures 3(a) and 3(b), the distribution for SO_4^{2-} around C_t does not reveal the presence of this ion in the first solvation layer (Figure 3(a)). In contrast, the RDFs of Figure 3(e) show a clear and intense association of the anions, in particular of SO_4^{2-}, with the positively charged amino group of Val. The higher intensity of the SO_4^{2-} peak in the RDF may result from it being a divalent anion. All the other monovalent anions have RDF peaks of similar intensities; however ClO_4^- seems to be more distant from the nitrogen atom than the other anions. The interaction of anions with the negatively charged carboxyl group (Figures 3(c) and 3(d)) is small, as expected due to the electrostatic repulsion, being significant around the oxygen atoms only for SO_4^{2-}. The highest affinity of the latter to the carboxyl group is a consequence of the presence of a higher number of Na^+ cations around COO^- moieties in SO_4^{2-} solutions. This will shield unfavorable interactions of SO_4^{2-} anions with the negatively charged parts of the amino acid and promote the indirect binding observed in the RDF.

As far as NO_3^- and Cl^- are concerned, the RDFs suggest that they present a somewhat intermediate behavior between those two extremes. They show relatively weak interactions with C_B and, while the RDFs suggest the presence of some structuring for these anions around C_t, no significant association to C_t is observed. The binding of Cl^- to the apolar moieties is somewhat weaker than that of NO_3^-, as demonstrated by the more intense peak shown in the RDFs of the latter (Figures 3(a) and 3(b)). On the other hand, Cl^- is closer than NO_3^- to the charged groups of Val (Figure 3(e)). Nevertheless, with the exception of the interaction anion$\cdots$N(NH_3) atom, the interactions disclosed by the RDFs are weak for both species.

The calculated RDFs provide interesting qualitative insight about the differences in water/salt/amino acid interactions. Quantitative evidence can be obtained from the analysis of the coordination numbers determined for the interactions between selected atoms in aqueous saline solutions of Val. From the results displayed in Table 1, it can be seen that the concentration profiles of the various ions and water molecules around the amino acids are actually consistent with the molecular interpretation obtained above from the structural data. Comparing the C.N. obtained for the interactions of the C_t atom of Val with the different anions, it is observed that the highest values occur for ClO_4^- (0.50) and the lowest for SO_4^{2-} (0.13). In contrast, the number of SO_4^{2-} ions surrounding each NH_3^+ group is the highest relative to the other ionic species. Furthermore, the intermediate values calculated for the C.N. for the interactions in NaCl, $NaNO_3$ and KCl are consistent with the less significant impact of these salts on the aqueous solubility of the biomolecules. Nevertheless, Cl^- and NO_3^- ions show distinct behavior – while Cl^- interacts weakly with both polar and apolar regions, NO_3^- shows appreciable interactions with both apolar and polar moieties. In the latter case, the two effects cancel each other and the net result is a small impact on solubility.

The picture that emerges from the MD simulation results is that the water structure and the solvation of the aminoacids by water is not significantly affected by the presence of salts. Concerning the interactions of the salts with the amino acid, it is observed that while salting-out inducing anions, such as SO_4^{2-}, are highly bonded to the amino acid charged moieties, they do not interact with its apolar parts, particularly with the terminal carbons, C_t. On the contrary, salting-in inducing anions, such as ClO_4^-, interact favorably with the amino acid apolar moieties, while their interaction with the charged part is weaker than observed for salting-out inducing salts. These results are in agreement with what was previously observed concerning the effect of salts on the solubility of ionic liquids [32-35] and, even more important, they are consistent with the experimental observations reported for the aqueous solubility of Val in presence of inorganic salts [39,40].

According to the simulation results, the interaction of SO_4^{2-}, an ion possessing high charge-density, with a hydrophobic moiety of the amino acid is a highly unfavorable process, so this ion excludes itself from the vicinity of the apolar groups of Val due to its

preferential hydration and preferential binding to the charged parts of the amino acid. As a result, the solubility of the amino acid in water decreases, as observed experimentally [40]. In contrast, the large and weakly hydrated ClO_4^- interacts directly with the apolar moieties of Val through a combination of ion-induced dipole and dispersion interactions, promoting the stabilization of the amino acid in water and therefore a salting-in effect. According to the simulation results, the interactions of Cl^- and NO_3^- are milder than those of the two other ions, and intermediate impacts on the solubility are expected. The Cl^- anion will have a negligible effect on the amino acid's aqueous solubility or at least a slight salting-out effect since it avoids interacting with the apolar parts. The NO_3^-, however, seems to be able to establish interactions with the hydrophobic moieties of the solute and thus to increase its solubility. These observations are in good agreement with the experimental results found in the literature for the behavior of Val in aqueous solutions of $NaNO_3$ and NaCl [39].

Explanations for the salt effect on the solubility of biomolecules based on interactions of the ions and water molecules with the hydrocarbon backbones and the charged amino and carboxyl groups of peptide fragments are not entirely novel and were used by Khoshkbarchi and Vera [37], although without any evidence, to interpret the experimental solubility data obtained for (amino acid+water+salt) systems. Recently, neutron diffraction experiments carried out on aqueous mixtures containing denaturant ions have provided evidence for the role of the hydration strength of the latter on protein stability in aqueous solutions [72] and strongly support that a major contribution to the denaturant effect is the preferential interaction of the ions with the protein surface. Strongly hydrated ions are preferentially retained in the bulk solvent and excluded from the protein surface, whereas a weak hydration leads to a preferential interaction of the ion with the protein surface. Other works concerning the study of aqueous saline solutions of proteins by MD simulation techniques [46] have shown that ion effects on protein association and solubility are the result of a balance between direct binding of small ions with charged amino acid moieties and interaction of large ions with non-polar surface patches. MD simulation studies on the effect of ions on the interaction between hydrophobic surfaces [30] suggest a strong correlation between the strength of that interaction and the degree of preferential binding/exclusion of the ions relative to the

surfaces. An increased interaction between hydrophobic surfaces (referred to as salting-out) is associated with high charge density ions which exhibit preferential exclusion by forming strong hydration complexes away from the hydrophobic surfaces. A decreased interaction (referred to as salting-in) is associated with low charge density ions that exhibit preferential binding. The results obtained here are in line with the conclusions of these works, stressing the importance of the presence/absence of interactions between the ions and the non-polar moieties of the biomolecules on the promotion of salting-in/salting-out effects, instead of an indirect effect mediated by the water structure.

A question that comes up in this context is the importance of contact ion-pair interactions between oppositely charged moieties, as well as their sensitivity to the force field model used. Indeed, ion pairing is a physical phenomenon that has been very often considered in explanations of the observed Hofmeister effects, and whose importance is commonly recognized in the understanding of the structure and stability of biological systems. The effect of ion-pairing can be assessed from the RDFs of the interactions between the salt cations and the central atoms of the anions for different valine/salt/water systems obtained for the different salts (*cf.* Supporting Information) and from the results displayed in Table 1 for the C.N. calculated for the $C^+\cdots A^-$ interactions. According to these results, ion-pairing is significant in the case of NaCl, but less evident for the other salts considered. This is consistent with the weaker interactions observed between this salt and both polar and apolar regions of the amino acids. However, this result brings up an (apparent) controversy – if the SO_4^{2-} affinity to the COO^- moiety is promoted, as discussed above, via $Na^+\cdots COO^-$ interactions, one would expect a stronger Cl^- affinity to the COO^- moiety, inconsistent with the strong Na^+/Cl^- pairing observed. Such non-local effects highlight the limitations of analyzing the interactions only in terms of pair RDFs. A more complete explanation would require the calculation of the full Ornstein-Zernike equations [70], but this is outside the scope of this work. Nevertheless, one can take into account the following considerations. Indeed, while in the case of Na_2SO_4 the Na^+ cation interacts preferentially with the COO^- group, promoting an indirect binding of the SO_4^{2-} anions to the latter, in NaCl systems Na^+ established more favorable interactions with Cl^- in bulk solution than with COO^-. As a consequence, a higher number of SO_4^{2-} anions (compared to Cl^-) will be found in the vicinity of the carboxyl group, as supported by the

C.N. calculated for the interaction $Na^+\cdots COO^-$ (0.576 for Na_2SO_4 against 0.284 for NaCl).

It is well documented that solution thermodynamics and contact ion-pairing are rather sensitive to small changes in the effective pair potential between the interacting ions and therefore, the accuracy of the simulated properties, especially solubility, may depend on the particular description of ion-ion interactions [49-53]. As a matter of fact, in previous works it has been shown that the formation of ion pairs in, for instance, aqueous NaCl solutions [49,50] or aqueous solutions with carboxylate-based anions [51], can be significantly overestimated and are strongly dependent on the force field model used. To take this issue into account, additional MD simulations for Val/NaCl/water systems using ten selected combinations of five potentials (Smith-Dang [67], Dang [68], Weerasinghe [49], Aqvist [62] and Chandrasekhar [63] force fields) for Na^+ and Cl^- ions were performed. Nevertheless, it is worth to note that the values obtained for the $Na^+\cdots Cl^-$ C.N. with the OPLS potential agree with the corresponding C.N. calculated using the recently developed Weerasinghe-Smith potential [49] for Na^+ and for Cl^- (please see entries OPLS/OPLS and WS/WS in Table S1) It has been shown that the latest model reproduces many of the known properties of sodium chloride solutions including density, isothermal compressibility, ion diffusion constants, relative permittivity, and heat of mixing [49], a fact that supports the reliability of the results provided in the present work. One should, however, be aware of some of the limitations associated to the use of the OPLS potential, namely in the description of the ion-pairing. Actually, based on the activity coefficients of the ions [69], COO^- should pair more than Cl^- (i.e., C.N. ($COO^-\cdots Na^+$) > C.N. ($Na^+\cdots Cl^-$)). Only some of the results presented in Table S1 confirm this trend. As previously shown by Hess et al [51], the OPLS model does not correctly describe the thermodynamic behavior of sodium salt solutions. Nevertheless, since the OPLS model was used for Na^+ in all the calculations performed in this work, the ion potential will not have influence on the relative comparison and interpretation of the results obtained with different anions.

The general patterns of interaction of Val with ions which induce pronounced solubility effects, such as SO_4^{2-} and ClO_4^-, are qualitatively similar to those observed for the other amino acids studied in this work. However, as can be seen from the RDFs of the various anions with the polar and apolar moieties of Ile and Ada (*cf.* Supporting

Information), there are a few differences which might justify, for these larger aminoacids, the difference observed in the magnitude of the effects promoted. For the ions placed in the middle of the rank of the Hofmeister series, such as NO_3^- and, to a certain extent, Cl^-, those dissimilarities are more detectable and may become marked enough in order to justify not only differences in the magnitude of the solubility effects induced, but also in their direction as discussed below.

Effect of the amino acid side chain

The structural characteristics of each amino acid are a determining factor in the magnitude and direction of the solubility effects promoted by salts. To evaluate the influence of the amino acid side chain, the effect of a salting-out inducing ion positioned at the extreme of the Hofmeister series - SO_4^{2-} - and a salting-in ion positioned at the opposite extreme - ClO_4^- - were studied.

The RDFs calculated for the S atom of SO_4^{2-} around the C_t atom of Val, Ile and Ada, presented in Figure 4(a), show that the highest peak corresponding to S····C_t interactions shifts from about 0.65 to 0.85 nm when moving from Val to Ile, implying a closer approach of the anions to the C_t atoms of Val. In both cases, the intensity of the first peak is rather similar and a minor presence of SO_4^{2-} is observed in the second solvation layer. The observed shift in the peaks correlates well with the increase in distance between C_t and the polar moieties of the amino acids from Val to Ile, which seems to imply that the anion····C_t interaction is simply a side-effect of the strong attraction between the ion and the polar groups. Consistently, for Ada, in which C_t is quite far from the polar moieties, the RDFs show that the anion concentration around C_t is below the average density, indicating that there is no interaction between the SO_4^{2-} anions and the C_t atom of Ada.

To clarify how the SO_4^{2-} interactions decrease with the increasing size of the non-polar moiety of the amino acid, the RDFs of the anion around each of the carbon atoms of Ada's side chain are presented in Figure 5(a). They show that the intensity of the distribution of the anions around the alkyl chain decreases and their position shifts away

from the chain as one progresses from the carbon atoms closer to the charged moieties of the amino acids to carbon atoms positioned in the terminal part of the alkyl chain. Actually, as indicated by the position and intensity of the peaks of the RDFs, the SO_4^{2-} is still found in the first solvation layer around C_B, it moves to more external layers on C_2 and C_3, and it is absent in the last carbons of the chain. Clearly, the SO_4^{2-} anions interact with charged moieties of the amino acids but seem unable to establish an interaction with non-polar moieties of the amino acid. The calculated C.N. values depicted in Table 1 provide quantitative further evidence for this behavior. Actually, there are almost no SO_4^{2-} anions around the C_t atom of Ada (C.N. for $C_t \cdots A^-$ interaction is 0.02), but their presence is still observed around the apolar groups of Val (C.N. for $C_t \cdots A^-$ interaction is 0.13). Conversely, the C.N. for the $NH_3^+ \cdots A^-$ interaction is very significant both in Val and Ada aqueous solutions. These qualitative and quantitative patterns suggest that as the non-polar moiety of the amino acid increases, the interactions with the anion become weaker and an increasingly stronger salting-out effect will be observed. This is in good agreement with the salting-out effects observed experimentally induced by the SO_4^{2-} anions on amino acids [41].

For very small amino acids like glycine (Gly), dominated by the charged moiety, the interactions with the SO_4^{2-} anions will be favorable and a salting-in effect could be expected. This is what is observed experimentally by Ferreira et al. [41], explaining the apparently surprising observation of a salting-out inducing salt being able to induce salting-in. Going from Gly to Ala, the increase in the non-polar part of the amino acid would reduce the favorable interactions with the SO_4^{2-} anions and explain why this salting-out inducing anion has essentially no effect on the Ala solubility [41], and has a salting-out effect for other amino acids with a larger non-polar moiety.

To study the effects at the other extreme of the Hofmeister series, ClO_4^-, a typically salting-in inducing ion, was used. The RDFs calculated for the Cl atom of the anion around the C_t of the amino acids, presented in Figure 4(b), show a completely different scenario from the case of SO_4^{2-}. In fact, a comparison of the position and intensities of the peaks of the RDFs of Figure 4(b) with those of Figure 4(a) confirms that ClO_4^- has a direct interaction with C_t in the first solvation layer that was absent for SO_4^{2-}. The intensity of the peaks of the $Cl \cdots C_t$ contact pairs decreases from Val to Ada, but

there is still a clear interaction of ClO_4^- with the C_t atom of Ada, which was totally absent in the case of SO_4^{2-}. These structural data are consistent with the trend observed in the concentration profile of the anions. In fact, the C.N. for the $C_t \cdots A^-$ interactions in ClO_4^- systems decrease from Val to Ada and are much higher than those calculated for the corresponding SO_4^{2-} systems.

The interactions of the anion with the increase in size of the non-polar moiety of the amino acid can be evaluated by the RDFs of the Cl atom of ClO_4^- around the carbon atoms of Ada, depicted in Figure 5(b). They are remarkably different from the RDFs presented in Figure 5(a), suggesting that, unlike what happened with SO_4^{2-}, ClO_4^- is able to bind, although weakly, to the methylene groups of the alkyl chain of the 2-amino-decanoic acid. It is also worth noticing that while the intensity and the positions of the RDFs peaks for C_i-S (*i*=B, 2, 3, ..., t) change monotonically with increasing distance to the polar region, indicating that SO_4^{2-} actually avoids the most apolar moieties of the amino acid, the RDFs of $C_i \cdots$Cl contact pairs show a non monotonic behavior, with a decrease of peak intensity from C_B to C_6 and then an increase back to C_t. This trend is likely to be related to the more pronounced aggregation of Ada's hydrophobic chains observed in ClO_4^- aqueous solutions, which is responsible for a weaker binding of these anions to the carbon atoms positioned in the middle of the side chain of the amino acid.

The interactions of the salting-in inducing ions, such as ClO_4^-, are thus completely different from those observed for the salting-out inducing ions such as SO_4^{2-}. The ClO_4^- anion not only interacts less with the charged moieties of the amino acids, but also presents an interaction with their non-polar moieties through an ion-induced dipole interaction that is responsible for the salting-in effects induced by this anion, as previously observed for other charged molecules [33]. Although no experimental data is available for this anion, the salting-in of amino acids promoted by anions is well established in the literature for NO_3^-, which sits close to ClO_4^- in the Hofmeister series [7,8].

The results of the RDFs described constitute also a strong argument against the classical "structure maker/breaker" model and support a molecular model according to which the influence of salts on the solubility of amino acids in water is not the result of effects on water structure, but on the ability of the salt ions to act, or not, as cosolutes promoting the solvation of the amino acid.

As far as ions such as NO_3^- and Cl^-, positioned in the middle of the Hofmeister series, are concerned, some conclusions can also be drawn. As can be observed from the RDFs of NO_3^- and Cl^- around C_t of Val, Ile and Ada (see Figure 3(a) and Figures S1 and S2 in the Supporting Information), some structuring of these ions is observed around the terminal methyl group of the amino acids. As the non-polar moiety of the molecule increases, the interaction with NO_3^- becomes more important, and around the C_t atom of Ada the RDF of NO_3^- is similar to that of ClO_4^-. This is in contrast to what is observed for Cl^-, since these ions are totally absent from the vicinity of the terminal groups. When interactions with C_B are considered, upon moving from Val to Ile, the binding of NO_3^- to C_B is slightly strengthened, both in the first and in the second solvation layers. The peaks referring to Cl-C_B contact pairs, however, continue to be absent. These qualitative observations are entirely supported by the coordination numbers of NO_3^- and Cl^- around C_t atoms, shown in Table 1.

The picture that emerges from the RDFs follows the trend of the Hofmeister series. Like ClO_4^-, NO_3^- is more strongly bound to the apolar part of the amino acids than Cl^- and is therefore able to promote salting-in. As the capability to interact with the non-polar moiety of the amino acid decreases, observed upon moving towards Cl^- and SO_4^{2-}, the salting-in inducing ability disappears and the salts become increasingly salting-out inductors. This observations closely follow the observed experimental effects on the amino acid solubility in aqueous salt solutions [37,39,41]. In general terms, thus, both the RDFs and the C.N. results suggest that the affinity of the ions to the non polar moieties of the amino acids is a key factor to determine the solubility effects of salts on aqueous solutions of the latest.

Effect of the concentration of the salt

The experimental data available for the solubility of Ala in aqueous solutions of KCl at several salt concentrations presents a peculiar behavior [41]. A salting-in effect appears to occur at very low salt concentration, while at higher concentrations, the solubility of the amino acid has an inverse dependence with the salt concentration [41].

To assess the effect of salt concentration on the aqueous solubility of amino acids, MD calculations for aqueous solutions of Ala in the presence of KCl at three different salt concentrations were performed. The RDFs obtained are displayed in Figure 6. They show that the interactions both with the charged and with the non-polar moieties of the amino acid decrease with the concentration. As discussed above, the results obtained here and those previously reported for the effect of salts on the solubility of ionic liquids [32-35] indicate that the salting in/out effects are controlled by the presence/absence of interactions with the non-polar moieties of the aminoacids. If for low salt concentrations the interactions with Ct are strong enough to induce salting-in, the decrease in the intensity of these interactions with increasing concentration, shown by the RDFs, follows a pattern similar to what is observed as one moves from salting-in towards salting-out – i.e., ions show progressively weaker interactions with the non-polar parts of the amino acid. The same conclusions can be drawn from the concentration profiles of the Cl anion around polar and apolar moieties of the aminoacid, shown in Supporting Information (Figure S4). The changes in the profiles with concentration are thus coherent with a transition in behavior from salting-in to salting-out, as observed experimentally by Ferreira et al.[41].

Conclusion

Molecular Dynamics simulations have been performed in an attempt to understand, at the molecular level, the experimentally observed solubility behavior of amino acids in aqueous saline solutions. The RDFs and C.N. show clear signs of important interactions of ClO_4^- with the apolar part of the amino acids, while strong association with the charged groups and absence of interaction with the hydrophobic moieties are observed for the high charge-density sulfate ion. As the chain of the amino acid increases, the preference of ClO_4^- for the apolar moieties and the lyophobicity of SO_4^{2-} become more pronounced, resulting in stronger salting-in and salting-out effects induced, respectively, by these ions. The interactions established by NO_3^- are comparatively less intense, but there is still a clear preference for apolar groups, that becomes more prominent as the alkyl chain of the amino acid increases. The least

significant interactions were observed for the Cl^- ion and, accordingly, its impact on the amino acid solubilities is the least important. These results support a mechanism of salting-in based on the direct interaction of the anions with the non-polar moiety of the amino acids. They suggest that the salt effect is not related with the changes in the water structure but instead result from the type and intensity of interactions that are established between the salt ions, the water molecules and the amino acids and, thus, their magnitude and direction are dependent on the nature and concentration of the cations, anions and amino acids that are present in a given system.

We have tested several combinations of different force field models for ions. Although quantitative values, such as coordination numbers, are somewhat sensitive to details of the model, the qualitative insight obtained is independent of the choice of force field. It is worth noticing, however, that the molecular interpretations given in this work are based on results derived from MD simulations which do not include explicit polarization. Although the importance of the inclusion of polarization effects on the description of the solution thermodynamics and of contact ion pairing has been demonstrated, indicating that interactions with hydrophobic regions will in general increase (depending on the polarizability of the species and on the hydrophobic character of the interaction site) while those with polar moieties will in general decrease (depending on the species nature) [47,48,71,72], the use of a polarizable potential is still computationally too demanding. Even though the reported results have proved to be reliable and perfectly valid for the models investigated, polarizable simulations of the systems under study should be considered in future work, as they would be useful to verify the hypothesis and interpretations here proposed.

The systems studied here, involving interactions between ions, amino acids and solvent, are fairly complex, both from a qualitative and quantitative perspective. The use of the Kirkwood-Buff theory, however, has proved to be very promising in the quantification of MD simulation results for complex systems [75] and should be therefore considered in future projects. Another suggestion for future work is the explicit calculation of solvation free energies of aminoacids in the presence of salts. Although they are computationally demanding, and may present some technical difficulties to

account for the presence of ions in the solvent medium, they should provide results that can be more directly related to experimental solubilities.

The molecular-level mechanism reported here for the solubility of amino acids in the presence of salts can be helpful for understanding the solubility and stability behavior of proteins and more complex biomolecules in saline environments, and thus be relevant for development and further research in the domains of biochemistry and life sciences.

Acknowledgments

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Supporting Information

Radial distribution functions calculated for ions around isoleucine, ions around 2-amino-decanoic acid, and ion pairing in different valine/salt/water solutions, as well as coordination numbers calculated for Val/NaCl/water systems using different combinations of Na+ and Cl- potentials. This information is available free of charge via the Internet at http://pubs.acs.org/.

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Figures

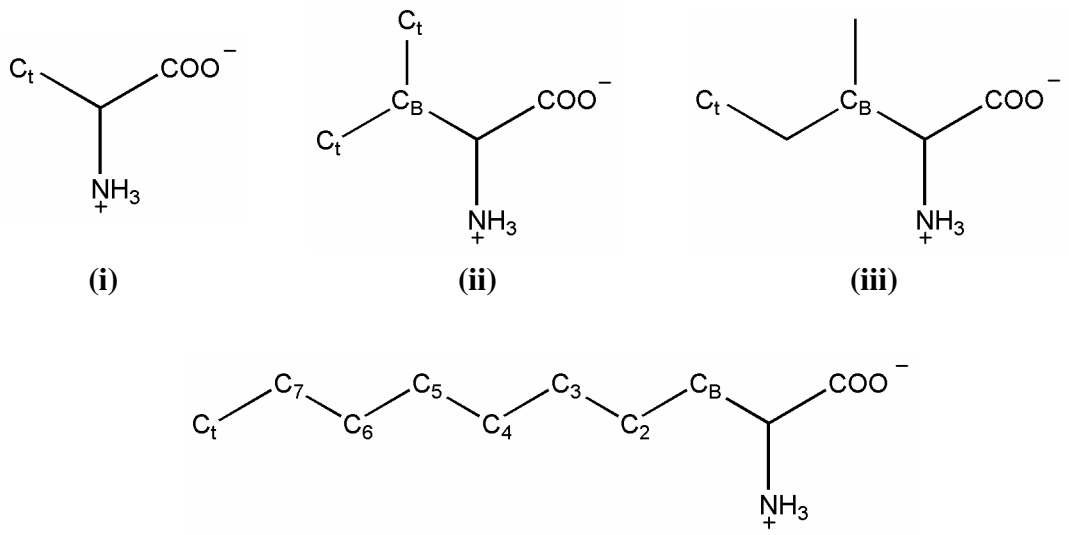


Figure 1. Structure of the amino acids studied in this work – (i) alanine (Ala); (ii) valine (Val); (iii) isoleucine (Ile); (iv) 2-amino-decanoic acid (Ada) – and corresponding atom labeling. C_t stands for the terminal carbon atom of the amino acid side chain while C_B is used to denote the first carbon atom of the amino acid side chain.

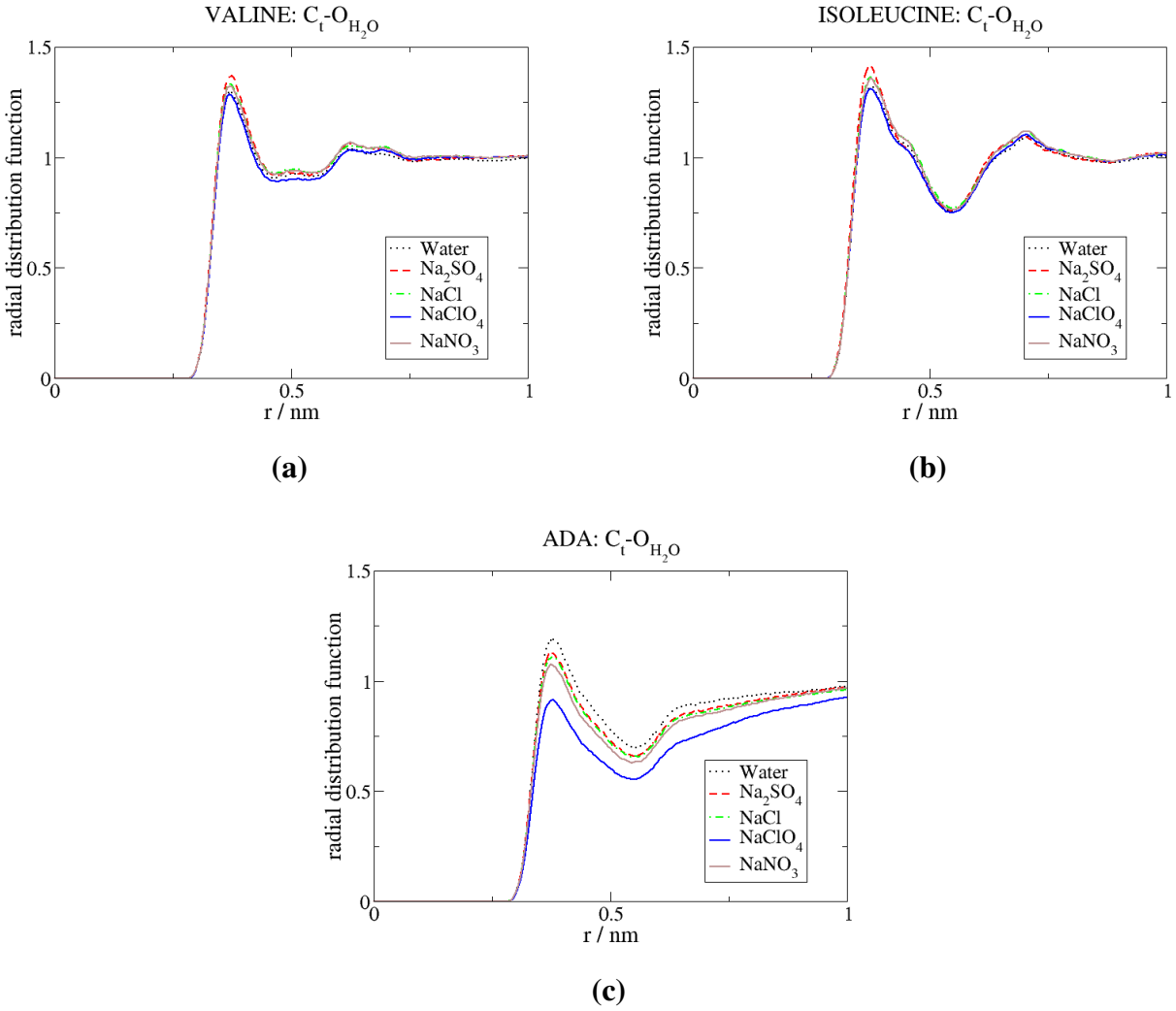
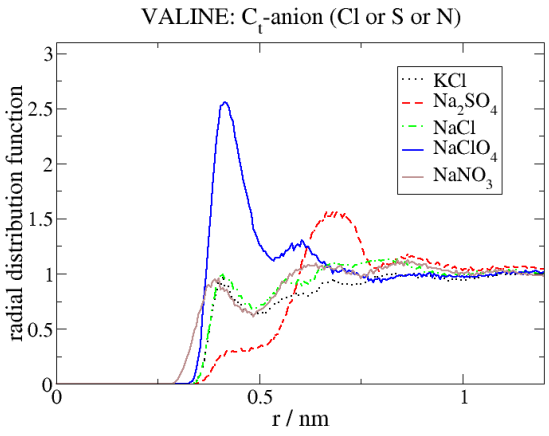
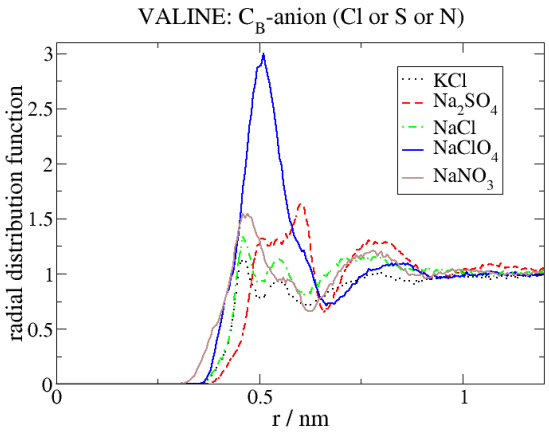


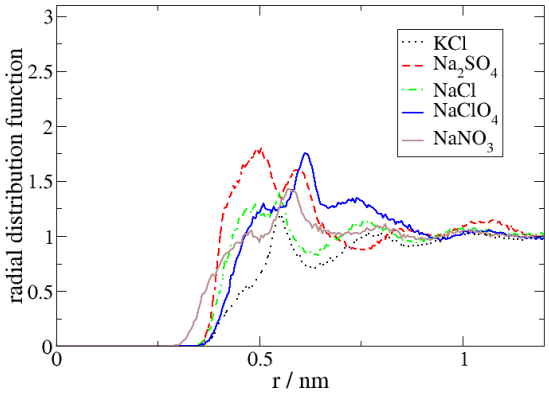
Figure 2. Radial distribution functions of the water oxygens around the terminal carbon atom (C_t) of (a) Val, (b) Ile and (c) Ada, in pure water and in the presence of the different inorganic salts.


VALINE: C_t-anion (Cl or S or N)
radial distribution function
r / nm
KCl
Na_2SO_4
NaCl
$NaClO_4$
$NaNO_3$

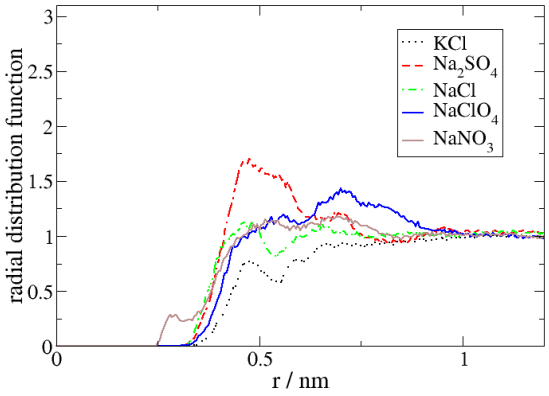
(a)


VALINE: C_B-anion (Cl or S or N)
radial distribution function
r / nm
KCl
Na_2SO_4
NaCl
$NaClO_4$
$NaNO_3$

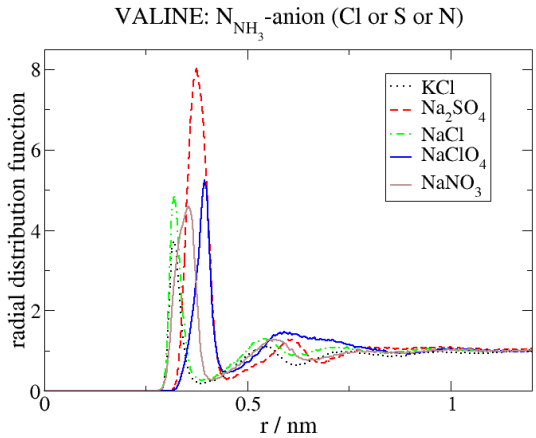
(b)


VALINE: C_{COO}-anion (Cl or S or N)
radial distribution function
r / nm
KCl
Na_2SO_4
NaCl
$NaClO_4$
$NaNO_3$

(c)


VALINE: O_{COO}-anion (Cl or S or N)
radial distribution function
r / nm
KCl
Na_2SO_4
NaCl
$NaClO_4$
$NaNO_3$

(d)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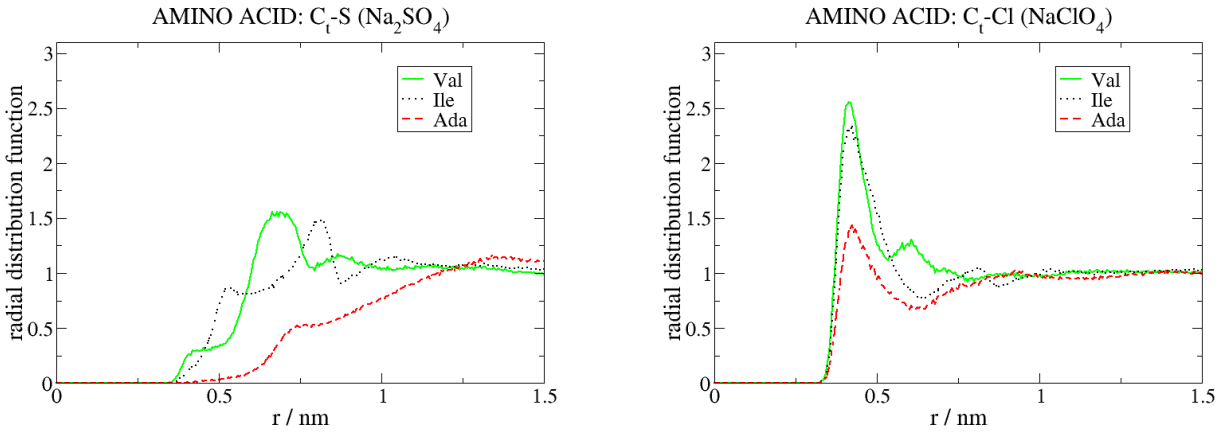
radial distribution function
r / nm
KCl
Na_2SO_4
NaCl
$NaClO_4$
$NaNO_3$

(e)

Figure 3. Radial distribution functions between different molecular regions of Val and the central atom of the anions (Cl, N or S): (a) and (b) C_t and C_B atoms of the side chain; (c) and (d) C and O atoms of the carboxylate group; (e) N atom of the amino group.



(a) **(b)**

Figure 4. Radial distribution functions of the S atom of Na_2SO_4 (a) and of the Cl atom of $NaClO_4$ (b) around C_t atoms of Val, Ile and Ada.

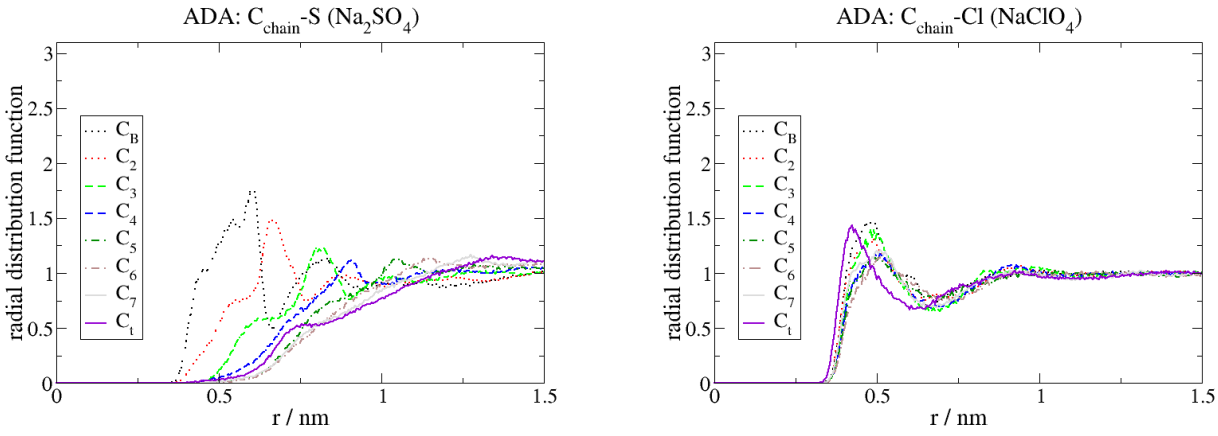
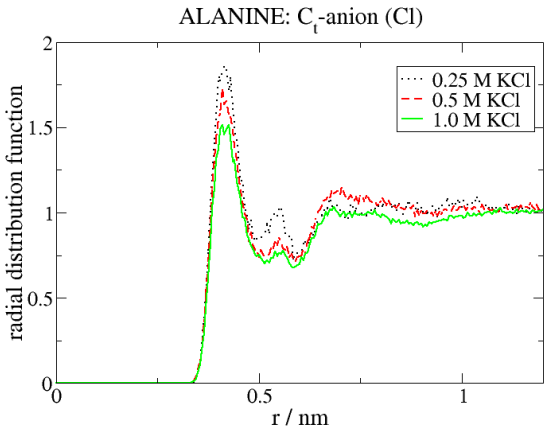
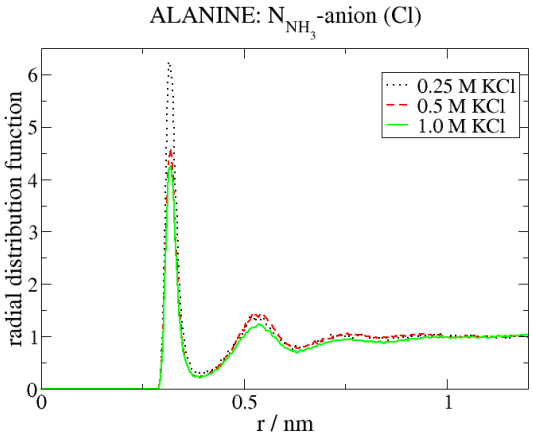


Figure 5. Radial distribution functions of the S atom of Na_2SO_4 (a) and of the Cl atom of $NaClO_4$ (b) around the carbon atoms of Ada's side chain.



(a)



(b)

Figure 6. Radial distribution functions of the Cl atom of KCl around (a) C_t atom and (b) N atom of the amino group of Ala, at three different concentrations.

Table 1. Calculated coordination numbers for the interactions between selected atoms in aqueous saline solutions of valine (Val) and 2-amino-decanoic acid (Ada).[a,b]

Salt	NH_3^+····A^-		NH_3^+····H_2O[e]		COO^-···C^{+f}		COO^-···H_2O[g]		C_t····A^-[h]		C_t····H_2O[i]		C^+····A^-[j]		C_t····C_t
	Val	Ada	Val	Ada	Val	Ada	Val	Ada	Val	Ada	Val	Ada	Val	Ada	Ada
$NaClO_4$	0.25[c]	0.21[c]	2.48	2.24	0.24	0.12	5.02	4.65	**0.50**	**0.42**	10.90	11.44	0.19	0.18	**0.81**
Na_2SO_4	**0.50**[c]	**0.60**[c]	2.37	2.14	**0.58**	**0.30**	4.92	4.66	0.13	0.02	**11.88**	14.33	0.26	0.33	0.45
NaCl	0.13[d]	0.16[d]	**2.75**	2.43	0.28	0.26	5.22	4.73	0.15	0.11	11.63	14.01	**0.51**	0.48	0.65
$NaNO_3$	0.25[c]	0.36[c]	2.67	2.46	0.23	0.22	5.33	5.11	0.19	0.26	11.68	13.65	0.18	0.18	0.46
KCl	0.10[d]	0.12[d]	2.70	**2.62**	0.08	0.12	**5.34**	**5.16**	0.14	0.11	11.57	**14.85**	0.46	**0.50**	0.36

[a] NH_3^+ refers to the cationic amine group of the amino acid; A^- refers to the salt anion; COO^- refers to the carboxyl group of the amino acid; C^+ refers to the salt cation; C_t refers to the terminal C atoms in the apolar chain of the amino acid. Largest quantity for each pair appears in bold case.
[b] The values of *r* at which the RDFs used for the calculation of the coordination numbers were truncated are presented in Table S2.
[c] Calculated from the H_N-O_{anion} RDF
[d] Calculated from the H_N-Cl RDF
[e] Calculated from the H_N-O_{water} RDF
[f] Calculated from the O_{COO}-Cation RDF
[g] Calculated from the O_{COO}-H_{water} RDF
[h] Calculated from the C_t-Anion Center RDF
[i] Calculated from the C_t-O_{water} RDF
[j] Calculated from the Cation-Anion Center RDF

Table 2- Calculated coordination numbers (C.N.) for the interactions between selected atoms in aqueous saline solutions containing a single 2-amino-decanoic acid species.[a]

Salt	C_t····A^-[b]		C_t····H_2O[c]	
	r[d]	C.N.	*r*[d]	C.N.
$NaClO_4$	0.66	0.96	0.55	17.48
Na_2SO_4	N.P.[e]	-	0.56	20.01

[a] A^- refers to the salt anion; C_t refers to the terminal C atoms in the apolar chain of the amino acid.
[b] Calculated from the C_t-Anion Center RDF
[c] Calculated from the C_t-O_{water} RDF
[d] Values of *r* (nm) at which the RDF was truncated
[e] RDF does not present any peak. C. N. calculated at *r*=0.66 nm (end of first peak for $NaClO_4$) is 0.05 only.

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