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ORIGINAL PAPER

Solvation free energies of glutamate and its metal complexes: A computer simulation study

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Abstract Most biological ion channels demonstrate a high degree of selectivity for one type of ion more than others, and in many cases, how they control attaining this is still not clear. So we have studied on some metal ion compounds of glutamate. The Glutamate and its meal ion compounds (Ca^{2+}, Na^{+}, K^{+} and Li^{+}) were first modeled by ab initio calculations and then Monte Carlo simulation was used to calculate solvation free energies and also the complexes free energies for the related structures. The results indicated that Glutamate-Ca^{2+} have more stability in water than other metal ion. Also, it was found out that the more movement in ions; less stability of the structure would result. This trend can be seen both in gas and liquid phase.

Keywords Glutamate · Free energy perturbation · Monte Carlo simulation · Solvation

Introduction

Hydration of biomolecules is vitally important in molecular biology, since numerous biological processes involve a ligand binding to a nucleic acid or protein and thereby displacing the water hydration.

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Supposedly, computer simulations are powerful tools to study biomolecular systems and predict their properties through the use of techniques that consider small replications of the macroscopic system with manageable number of atoms. Molecular dynamics (MD) [1] or Monte Carlo (MC) [2] simulations generate representative configurations of these small replications in such a way that accurate values of structural and thermodynamic properties, such as energy, volume, pressure, enthalpy, entropy, and free energy, can be obtained with feasible amount of computation [3]. As for the energetics, experimental determination of different atomic interaction energies contributing to the total energy of a bimolecular system is almost impossible [4]. Obviously, from this view point, computer simulations and experimental measurements become complementary tools to study biomolecules. The first one provides a detailed atomic picture at a resolution in space, energy or time that is generally inaccessible by experimental means, whereas the second provides the necessary restriction of the configurational space that has to be sufficiently sampled in a simulation [5].

Admittedly, the participation of metal ions in biological processes is well known [6]. The acidic amino acid, glutamate, is considered the predominant excitatory neurotransmitter. Metal ions may play a direct role, as in oxidation reduction reactions, or may serve an indirect function such as ion channels. Potassium and calcium [7] channels play critical roles in many aspects of neuronal function. Ion channels are integral membrane proteins that form specific ion conduction pathways across cell membranes [8]. Channels may be gated by a variety of factors, including changes in transmembrane voltage and binding of ligands to receptor domains. Amongst the ligand-gated ion channels, ionotropic glutamate receptors mediate excitatory neurotransmission in the central nervous system of mammals.

Despite the wealth of information accumulated over the past two decades, there has been much controversy about some of the outstanding questions on how biological channels work. The first among these questions is the detailed dynamical processes underlying the permeation of ions across an open channel.

Channels generally discriminate anions from cations; some channels select sodium ions but reject potassium ions or vice versa. This selectivity mechanism needs to be understood in terms of the interactions of the permeating ions with the surrounding water and protein molecules [9].

Most ion channels demonstrate a high degree of selectivity for one type of ion over others, and yet in many cases, how they manage to achieve this is still not clear. It is generally accepted that the distribution of charges in ion channel proteins is responsible for their selectivity for either cations or anions. An issue that has been less well discussed is how channels select between ions with the same sign but different magnitude of charge. For example, why does a channel favor divalent ions over monovalent ions, and why does one type conduct while the other does not? The calcium channel, for example, conducts monovalent ions when no divalent ions are present in the surrounding solution, but the channel allows only divalent ions to pass as soon as they are present [10].

While there is a strong case that the selectivity for either divalent or monovalent ions arises directly from the electrostatic interaction with the partial charges in the channel walls [11–14], there are still some alternative theories in which both electrostatics and volume are important.

Nonner et al. [15] and Boda et al. [16] both proposed a model in which valence selectivity arises from competition between charge and available space. Corry et al. believed that electrostatic properties alone (i.e., without considering the limited volume) are enough to explain monovalent or divalent ion selectivity [17].

During the past several years, there have been enormous strides in our understanding of the structure–function relationships in biological ion channels by use of some generally accepted theoretical tools for studying ion channels, such as Ohm's law, Fick's laws , Nernst–Planck equation and PNP theory and MD simulations [9].

Ohm's law, simple as it may be, can provide us with useful insights about the permeation mechanisms across a transmembrane pore. Fick's law provides a similar relationship between the flux of ions and the concentration gradient across a channel. In general, there could be both a potential and a concentration gradient driving the ions across an ion channel. This situation is described by the Nernst–Planck equation that combines Ohm's and Fick's laws.

In the Poisson-Nernst-Planck (PNP) theory, as the name implies, Poisson's equation is coupled to the Nernst–Planck equation, and the two equations are solved simultaneously, yielding the potential, concentration, and flux of ions in the system. The two coupled equations are notoriously difficult to solve analytically, except for a few special cases (see Refs. [18] and [19]). For an excellent review on the applications and limitations of the methods mentioned above, please see [9].

Recently, Miloshevsky and Jordan developed [20] the new computational method Monte Carlo Ion channel proteins (MCICP) which was used to simulate water-protein, ion-protein, and protein-protein interactions, for application to the study of permeation and gating in ion and water channels [21].

Surprisingly, most of these methods have studied the mechanisms of permeation with little consideration of the interaction details between the structure of channel and the ion which is passing through the channel. Therefore, unfortunately, these methods cannot explain why Ba2+, for example, conducts through calcium channels at a greater rate than Ca^{2+} or why it is blocked by Zn2+. Such phenomena are probably a result of the detailed interaction between these ions, the protein atoms and surrounding molecules and are more likely to be elucidated using classical or ab initio molecular dynamics studies [17]. In the present study, our goal is to answer to the fundamental and at the same time important questions about the nature of interactions between the glutamate residue, as the active residue in ion attraction in calcium channels, and some metal ions in different mediums through computational techniques. The outcomes can reveal the attraction of ions from an atomistic view point.

For the calcium channel, mutation experiments have suggested that four glutamate residues line the selectivity filter in close proximity to form a single binding site capable of holding two ions [17, 22–25], and play a crucial role in determining the permeation characteristics of the channel.

We know that the ion channels are the complicated protein systems, and the selectivity of ion channels may arise from many different factors, but we have used those site of channels that react with cations. Therefore we considered the reaction of glutamate with metal ions and comparison of these metal-ligand systems is the base of our study. By these Glu-cation model systems we can compare the selectivity of ion channels qualitatively and also the CPU time of our calculations have been reduced. In fact for comparison the systems, the best way is that consider the section of molecules that takes part in reactions. In our research the main reactant is the glutamate section and use of whole structure of channels does not effect the qualitative results and only increases the amount of computations.

Therefore in this research study we present quantitative results of the ab initio interactions between the active part of a calcium channel in absorbing ions (glutamate residues which form the pore) and some metal ion compounds of it, which is in the next part followed by the Monte Carlo simulations of solvation free energies of glutamate and the interested compounds. In addition we have computed formation free energies of the metal complexes of glutamate.

Methodology

The ability to accurately calculate solvation free energies of molecules using thermodynamic perturbation (TP) is one of the important developments in computational chemistry [26]. These methods have wide applicability not only in studies of absolute solvation free energies but also in studies of binding free energies and protein and nucleic acid stability. It is now possible, in some cases, to calculate relative and absolute solvation free energies within "chemical accuracy". While it is important that these methods reproduce the experimental results, the real aim is to use these theoretical methods in a predictive manner and in cases where the experiments cannot be performed.

Solvation free energies of amino acids are inaccessible experimentally due to problems of low volatility. In this case, the application of theoretical methods may be our best hope to gain physical insights into the effects of solvation.

To calculate the solvation free energies of molecules we focus here on applying thermodynamic perturbation method to the computation. We have taken appropriate steps to obtain the most accurate results possible with a molecular mechanical based approach. We have utilized appropriate force field and the Monte Carlo simulation.

Potential energy functions

The computational efficiency with which the energy can be calculated using a given model is often an important factor as there are a very large number of water molecules present, together with a solute. A wide range of force fields have been proposed. Moreover many properties can be easily determined using computer simulation methods and so readily compared with experiment. It is also one of the most challenging systems to model accurately.

Total potential energy of a chemical system, E_{tot}, includes internal potential energy (E_{int}) and external potential energy (E_{ext}) terms:

$$E_{tot} = E_{int} + E_{ext} \tag{1}$$

Table 1 TIP3 parameters for water molecule

Site	q	$10^{-3}A^2$ KcalÅ^{12}/mol	C^2 KcalÅ^6/mol
O	−0,834	582	595
H	0,417	0	0

In our program, internal potential function has been disregarded and only external or intermolecular potential function has been considered.

The monomers were represented by interaction sites usually located on nuclei. The interaction energy between two molecules, A and B, were expressed by pair wise sum of interaction contributions:

$$E_{AB} = \sum_{i}^{onA} \sum_{j}^{onB} E_{ij}^{AB} \tag{2}$$

We used transferable intermolecular potential functions [27, 28] (TIP3) for water molecules (solvent) and optimized potential for liquid simulations [29] (OPLS) for solute. For both models, the pair potential function E_{ij} was represented by Coulombic and Lennard-Jones terms between sites centered on nuclei.

$$E_{ij}^{AB} = \frac{q_i q_j e^2}{r_{ij}} + \frac{A_{ij}}{r_{ij}^{12}} - \frac{C_{ij}}{r_{ij}^{6}} \tag{3}$$

A_{ij} and B_{ij} are the repulsion and attraction coefficients for the non bonded interactions; r_{ij}, q_i, and q_j are the interatomic distance between atoms i and j and the atomic charges on atoms i and j; and ε is the effective dielectric constant. Each type of site has three parameters, a charge in electron, q, A and C. The TIP3 model uses a total of the three sites for the electrostatic interactions. The partial positive charges on the hydrogen atoms are exactly balanced by an appropriate negative charge located on the oxygen atom. The TIP3 parameters for water [30] have been included in Table 1.

The OPLS model is a modified form of TIPS that has parameters fitted to liquid state properties, and so is more suitable for studies of liquids. The model works well for a

Table 2 OPLS Lennard–Jones parameters for amino acids

Atom	ε,Kcal/mol	σ,Å
O (C=O)	0,210	2,960
C (C=O)	0,105	3,750
N	0,170	3,250
Other C	0,060	3,500
H on N	0,000	0,000
H on C	0,030	2,500

Table 3 OPLS Lennard–Jones parameters for metal ion [44]

Metal ion	ε,Kcal/mol	σ,Å
Li	0,0183	2,1264
Na	0,0028	3,3304
K	0,0003	4,9346
Ca	0,4497	2,4120

variety of alcohols, amines, aliphatic and aromatic hydro carbons, sulfur compounds, ether, amino acids, and nucleic acid bases. It has the form of Eq. 3. The OPLS Lennard–Jones parameters for amino acids [31–35] are included in Table 2. Table 3 provides some information about the OPLS parameters for metal ions.

Free energy

The free energy difference between two states A and B, of a system may be derived from classical statistical mechanics [36] allowing us to express this difference as Eq. 4 is the free energy perturbation (FEP) master equation.

$$\Delta G = G_B - G_A = -RT \ln\langle \exp -(E_B - E_A)/RT \rangle \qquad (4)$$

E_B–E_A is the potential energy difference (ΔG) between states A and B of the system. R is the molar gas constant, T is the absolute temperature, and the symbol $\langle\rangle$ indicates an ensemble average taken using the potential for state A.

In practice, one introduces a coupling parameter (λ) into the potential function which allows us to calculate the free

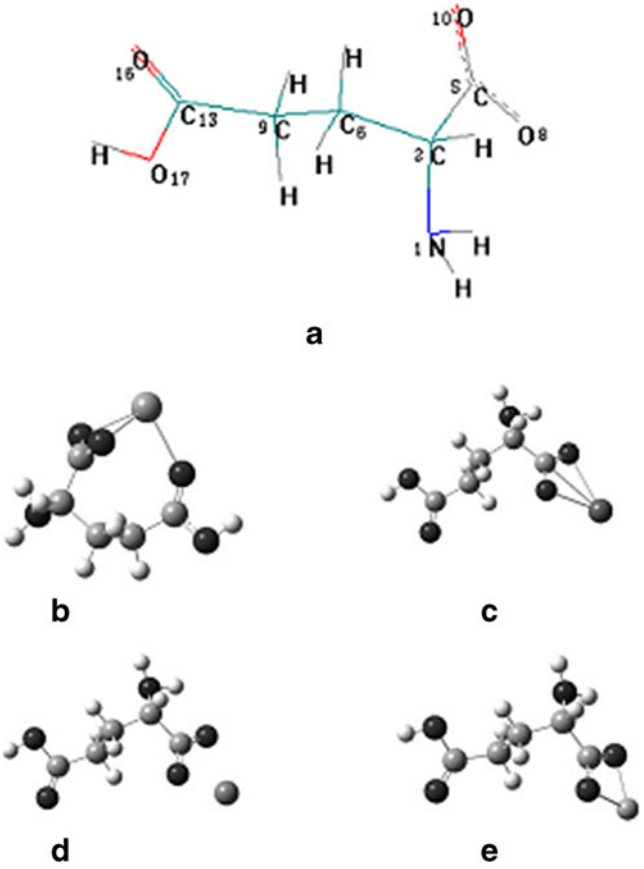

Fig. 2 Glutamate and its metal ion compound. (**a**) glutamate, (**b**) glutamate-Ca^{2+}, (**c**) glutamate-K^{+}, (**d**) glutamate-Na^{+}, (**e**) glutamate-Li^{+}

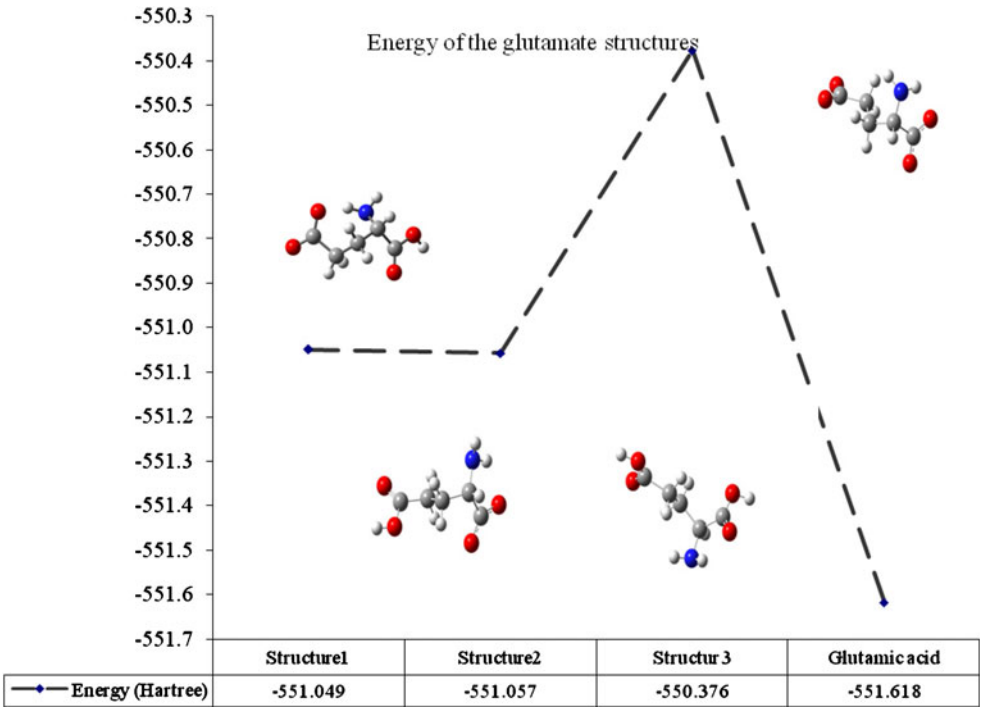

Fig. 1 The energy profile for glutamic acid and different glutamate structures. The glutamic acid data are taken from [43]

energy difference over a series of more closely related states. Using this parameter, the free energy difference between states A and B becomes the following:

$$\Delta G=\sum_{i=1}^{N}\Delta G_i=\sum_{i=1}^{N}-RT\ln\left\langle\exp-\left[E_{\lambda(i+1)}-E_{\lambda(i)}\right]/RT\right\rangle_{\lambda i} \quad (5)$$

In Eq. 5, each ΔG_i represents a "window" in the nonphysical pathway linking the two states. This procedure reduces some of the sampling problems which arise from the different potential energy surfaces of states A and B.

In fact the relationship between the initial, final and intermediate states is usefully described in terms of a coupling parameter (λ). As λ is changed from 0 to 1, the internal energy varies from E_A to E_B. Each of the terms in the force field for an intermediate state λ can be written as a linear combination of the values for A and B:

$$\text{Electrostatic}: \; q_i(\lambda)=\lambda\, q_i(B)+(1-\lambda)q_i(A) \quad (6)$$

$$\text{Van der waals}: \; \varepsilon(\lambda)=\lambda\,\varepsilon(B)+(1-\lambda)\varepsilon(A) \quad (7)$$

$$\sigma(\lambda)=\lambda\,\sigma(B)+(1-\lambda)\sigma(A) \quad (8)$$

The solvation free energy of the species A (glutamate and its metal complexes) can be written in terms of perturbations where the species disappear to nothing in the gas phase and in solution:

$$\Delta G_{sol}(A)=\Delta G_{gas}(A\rightarrow 0)-\Delta G_{sol}(A\rightarrow 0) \quad (9)$$

Absolute free energies of the reaction of glutamate with metal ions have been calculated using thermodynamic cycle [37]. At last the free energy of complex formation in solution is given by:

$$\Delta G_r=\Delta G_{sol}(L\rightarrow 0)+\Delta G_{sol}(M\rightarrow 0)-\Delta G_{sol}(ML\rightarrow 0) \quad (10)$$

$\Delta G_{sol}(L\rightarrow 0), \Delta G_{sol}(M\rightarrow 0), \Delta G_{sol}(ML\rightarrow 0)$, are solvation free energies of ligand(glutamate), metal ion and metal-ion complex respectively.

Computational details

Geometries

The isolated solute molecules (glutamate and its metal compounds (monovalent cations, Li^+, K^+, Na^+ and divalent cation, Ca^{2+})) were first modeled in gas phase by quantum mechanical calculations.

Table 4 Quantum mechanical calculated partial charges for solutes

Atom	Atomic charges				
	Glutamate	Glu-K	Glu-Na	Glu-Li	Glu-Ca
N1	−0,93671	−0,91649	−0,91281	−0,90969	−0,91119
C2	−0,17098	−0,15617	−0,15377	−0,15459	−0,15659
H3	0,34519	0,36904	0,372	0,37595	0,39679
H4	0,39759	0,39817	0,3987	0,39982	0,40601
C5	0,77907	0,79241	0,79792	0,7972	0,79503
C6	−0,45044	−0,45725	−0,45809	−0,45932	−0,45045
H7	0,20398	0,22748	0,23191	0,23785	0,26264
O8	−0,7766	−0,81526	−0,8119	−0,83027	−0,83126
C9	−0,5767	−0,57092	−0,57207	−0,57313	−0,58899
O10	−0,76962	−0,80967	−0,80607	−0,82562	−0,83399
H11	0,24449	0,24905	0,25037	0,25231	0,25807
H12	0,21239	0,23735	0,2401	0,24371	0,26931
C13	0,83943	0,8369	0,83599	0,83537	0,91113
H14	0,27106	0,27579	0,27634	0,27759	0,28969
H15	0,27193	0,25415	0,2568	0,25807	0,29421
O16	−0,63235	−0,61292	−0,60847	−0,60418	−0,83247
O17	−0,72641	−0,72118	−0,72198	−0,72245	−0,64332
H18	0,47468	0,49047	0,49173	0,49342	0,51582
K		0,92903			
Na			0,89331		
Li				0,90796	
Ca					1,84955

Table 5 RDFs between different sites of glutamate and water

Site	r_1,Å	ρ_1	r_2,Å	ρ_2
N1	3	0,0364	3,9	0,0205
O8	3	0,059	3,6	0,0205
O10	2,7	0,0461	3,6	0,0487
O16	2,4	0,0461	3,6	0,0294
O17	2,7	0,0364	3,6	0,0205

First of all, different structures of glutamate (protonated and non protonated) were considered to obtain the most stable glutamate structure to react with metal ion. Figure 1 depicts the energy profile of different glutamate structures. As can be seen, the structure of glutamate numbered as 2 is more stable than the two other structures.

The calculations have been performed by using the GAMESS-US quantum chemistry package [38]. Each solute was optimized by DFT method using the standard 6-31G* basis set [39, 40] (gas phase). The structures are shown in Fig. 2. In the subsequent simulation step, these geometries were kept constant. Then a Monte Carlo (MC) simulation was applied for dilute solutions of glutamate and its metal compound in water. A brief description of the applied method is given in the next section.

Monte Carlo (MC) simulation

Monte Carlo statistical mechanical simulations were carried out in standard manner using Metropolis sampling technique [41] in canonical (T, V, N) ensemble at 300 K.

All calculations were performed in a cubic box at the experimental density of water, 1 g/cm^3. The edges of the box were 22×22×22 Å, which corresponds to 352 H_2O molecules of pure solvent. A spherical cut off for the potential at an OO separation of half the length of an edge of the cube were used. One molecule was picked and

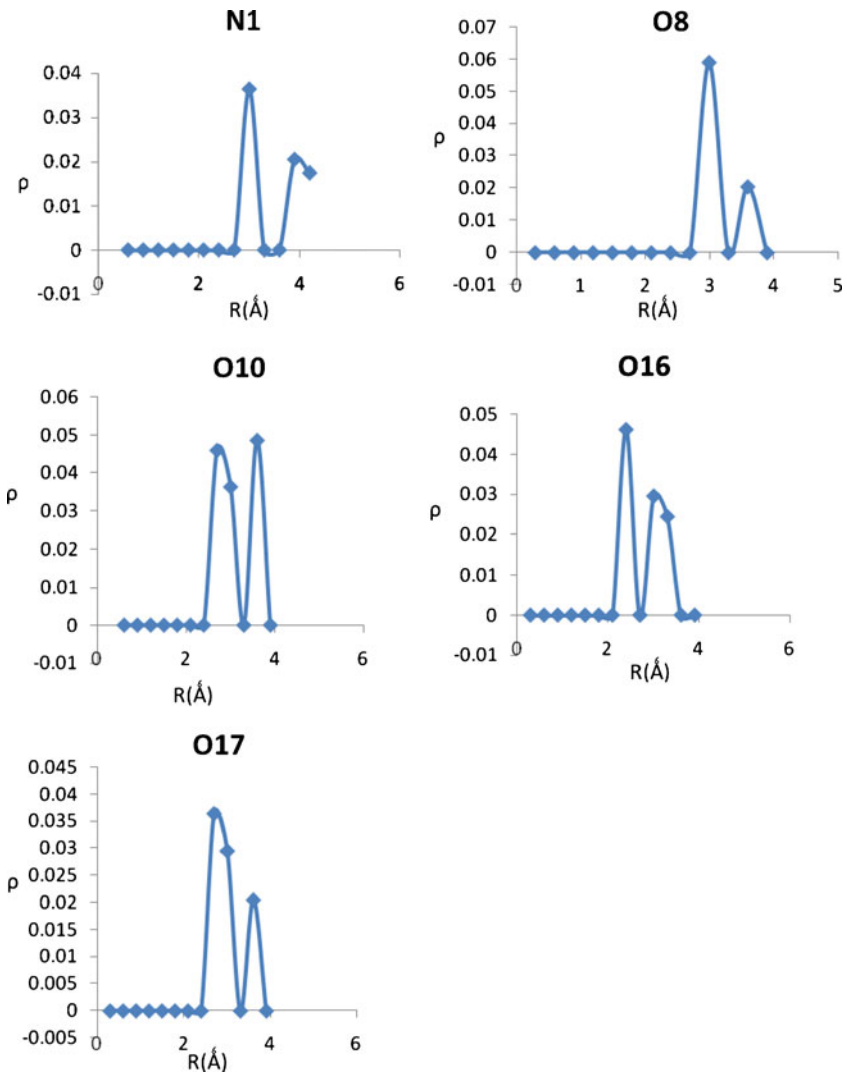

Fig. 3 The calculated radial distributions between water and (**a**) N1, (**b**) O8, (**c**) O10, (**d**) O16 and (**e**) O17 atoms of glutamate

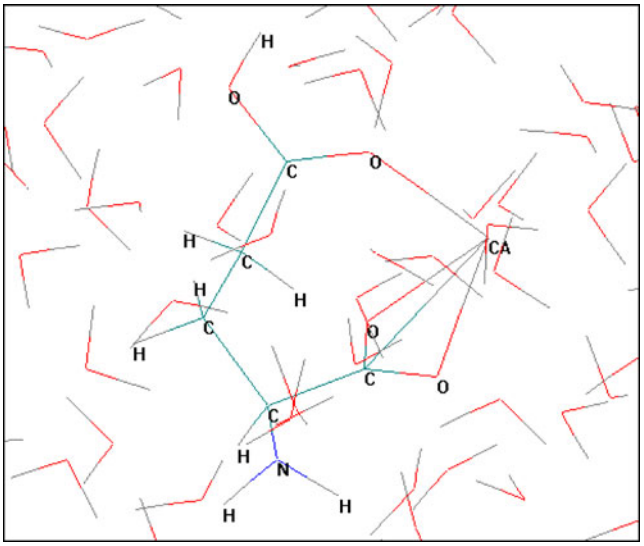

Fig. 4 The snapshot of glutamate-Ca^{2+} complex in aqueous solution

displaced randomly on each move. An acceptance rate of 50% for new configurations was achieved by using suitable ranges for translations and rotation about a randomly chosen axis.

Periodic boundary conditions were employed in computation of energy of the initial configuration, in cut off, in translations and rotations, and computation of the energy of each produced configuration. The system was thoroughly equilibrated using several hundred thousand configurations. The energy of a configuration was obtained from the pair wise sum of the dimerization energies for each monomer as usual. Each run consisted of 10^6 attempted moves. Initial steps (roughly 5×10^5) were disregarded for equilibrium. Every calculation was extended to include as many configurations as were necessary to reduce the statistical error to the level at which calculated energy differences have quantitative significance.

Results and discussion

Partial charges

As is mentioned above we used quantum mechanical calculations for isolated solute molecules. The results are tabulated in Table 4. We show only partial atomic charges since in further calculations (Monte Carlo simulation) the charges have been used.

As can be seen in Table 4, atoms O16, O17, O8, O10 and N1 have the highest negative charges. As a result, they can be considered as the possible binding sites to metal ions.

In the following section we studied the stability of glutamate and its metal ion complexes in aqueous solutions.

Radial distribution functions

Radial distribution functions (RDF) between water molecules and each site of the solute molecule are important in hydrogen bonding and interaction of that site with ions. Therefore radial distribution functions were computed between water molecules and the probable active sites in glutamate (O16, O17, O8, O10 and N1 atoms) to determine the most activated site that metal ion binds to it. Only the RDFS under 4 Å were considered, since the distances (R) greater than 4 Å correspond to hydrogen bonding of water molecules with each other. Beneath this distance (4 Å) there are two coordination layers of water molecules around each site. ρ_1 is the first coordination layer which is located at the distance r1, and ρ_2 is the second coordination layer at the r2 distance. The results are reported in Table 5.

Figure 3 depicts the RDF diagrams for glutamate sites. As can be seen, all of the RDF diagrams have two peaks that correspond with the first and second solvent shells. As shown in Fig. 3, the first peak of all sites occurs at about 2.8Å and is a sharp peak. Atom O8 has the highest first peak. For all sites, the second coordination shell can be seen at about 3.6Å.

These results reveal those O8 atoms are the most hydrophilic atoms in glutamate. It means that this atom as a site is the most active one in glutamate and has the potential for taking part in hydrogen bonding or interaction with metal ions. On the other hand, glutamic acid has negative charge in pH above 3 and it is in the form of glutamate. The experimental results showed that the best site for binding of glutamate with metal ion was the atom

Table 6 Summary of Monte Carlo runs

Solute	N	NSTEP	NSTEPav	Esoln.	Esolv.	Etotal
Glutamate	342	1×106	5×105	−2,7328	−56,1252	−58,8580
Glu-k	342	1×106	5×105	−3,1648	−66,2918	−69,4566
Glu-Na	341	1×106	5×105	−3,2652	61,3875	−64,6527
Glu-Li	343	1×106	5×105	−2,9532	40,4407	−43,3939
Glu-Ca	344	1×106	5×105	−3,2786	−69,5255	−72,8041

O8 [42]. Clearly, the simulation outcomes are in good agreement with the experimental data.

Total energies

Obviously, the interaction between the solute and the solvent molecules plays a crucial role in understanding the various molecular processes involved in chemistry and biochemistry. In this study, the solvation of glutamate-complexes in the presence of water was studied. A very dilute solution of glutamate and its metal ion compounds was used, so one molecule of solute has merged in water and then average energies were calculated from Monte Carlo simulations.

We used only intermolecular potential function that has Columbic and Lennard-Jons terms and did not consider internal coordinate. The goal of each simulation procedure is simplicity of the system together with quality of results. We have obtained similar results by a simpler force field in other research [45]. Our results are compatible with outcomes of more complicated force fields such as CHARMM [45], AMBER [46] and QM/MM [47]. Furthermore the purpose of this work is comparison of solvation energies of solute molecules in water and we have considered all of the interaction of water molecules with solute in solvent phase. The results have shown that effect of small changes in the structure of solute molecules in total interaction of solute-solvent have been negligible. This simplicity is correct for small molecules and reduces the time required for calculations.

As an instance, the resultant configuration of the MC simulation of Glutamate-Calcium in water is shown in Fig. 4. This gives a qualitative idea of the formation of the solvation shell around the selected solute. The total energy of the compounds (including van der Waals and Coulomb interaction) in water has been calculated. The average energy (E_{tot}) calculated from Monte Carlo simulations, as well as the energies of solute–solvent (E_{soln}) and solvent–solvent (E_{solv}) components, are given in Table 6. This table also includes the number of solvent molecules (N), the total number of MC steps (NSTEP), and the actual number of configurations ($NSTEP_{av}$) used in calculating ensemble averages for every run.

Table 7 Simulation errors

Solute	<E>	STDEV	Relative error
Glutamate	−58,8580	1,1629	0,0200
Glu-Li	−43,3939	0,6568	0,0151
Glu-Na	−64,6527	3,1151	0,0482
Glu-K	−69,4566	0,9859	0,0140
Glu-Ca	−72,8041	1,2632	0,0170

Table 8 Computed solvation free energies (in kcal mol^{-1})

Species	ΔG_{sol}
Glutamate	−3,2721
Glu-Li	−3,0682
Glu-Na	−12,4994
Glu-K	−14,0558
Glu-Ca	−47,7164

The obtained energies indicate that the solvation energies of glutamate compounds in water appears in the following order:

$$E_{total}(\text{Glutamate-}Ca^{2+}) > E_{total}(\text{Glutamate-}K^{+}) > E_{total}(\text{Glutamate-}Na^{+}) > E_{total}(\text{Glutamate}) > E_{total}(\text{Glutamate-}Li^{+})$$

Error estimation

There is no denying the fact that a simulation can generate an enormous amount of data that should be properly analyzed to extract relevant properties and to check that the calculation has behaved properly. The three most important factors that determine the accuracy of Monte Carlo calculations are the quality of intermolecular potentials, the sample size effect, and statistical fluctuations of calculated ensemble averages. The first was briefly discussed. The second factor arises because locating a limited number of molecules in a box followed by subsequent application of periodic boundary conditions introduces an error into the molecular correlations. For a given system, this effect decreases with the sample size. In most cases of interest, we do not know how to choose the size of the system in order to minimize an effect of periodic boundary conditions. The most straightforward test is to perform a series of calculations in which the sample size is systematically increased until calculated values remain unchanged. The statistical errors are often reported as standard deviations. The errors have been reported in Table 7. STDEV is the standard deviation of the calculated average in the simulation of finite number of steps. As can be inferred from Table 7, the simulation error is 1-2%.

Table 9 Computed complexation free energies and stability constants of the complexes

Complex	ΔG_r	log K
Glu-Li	−3,3869	2,48
Glu-Na	−2,0158	1,48
Glu-K	−0,1205	0,088
Glu-Ca	−5,1431	3,77

Free energies

The solvation free energy calculations for each of the solutes were carried out. To model the solvation of glutamate and its compounds, a simulation was done on the system with the solute fully represented and then it's electrostatic and van der Waals parameters decreased to zero. The computed solvation free energies, ΔG_{sol}, are presented in Table 8.

Several biological processes involve ligand binding to amino acids and therefore hydration of amino acids and displacing the water hydration is very important. As can be seen in Table 8, glutamate has the minimum value of the solvation free energy and Ca^{2+}-glutamate complexes has the most solvation free energy and so it is the most stable compound in biological processes like ion selectivity in ion channels.

Eventually, free energies of reaction of complex formation, ΔG_r, were computed using Eq. 10. For calculating each free energy we need three solvation free energies: solvation free energy of glutamate, metal ion and glutamate-metal ion. The results are tabulated in Table 9. Free energy of complex formation for calcium-glutamate species is larger than other complexes. The increase in the ion atomic number or z/r ratio leads to a decrease in the stability of the complexes and therefore decreases of the complexation free energies and stability constants. In this systems the binding strength which is related to complexation free energies and stability constants varies with the metal ion such that Li^+ binds more strongly than Na^+, which in turn binds more strongly than K^+

As a result we can discuss how protein channels select between ions with the same sign but different magnitude of charge. The calcium channel conducts monovalent ions when no divalent ions are present in the surrounding solution, but the channel allows only divalent ions to pass as soon as they are present [17]. The opening and closing of ion channels may depend on the binding of ligand. In fact this selectivity can be explained by free energies of complex formation of channels with the metal ions. Free energy has a relationship with the stability constant of the complex. As can be seen in Table 9, complex of calcium ion with glutamate has the greatest absolute free energy of complex formation, therefore it is a stabilized compound. Thus Ca^{2+} is a stronger ion for reacting with channels than other metals. Between one charge metal ions, Li^+ has the most absolute free energy of complex formation and therefore the channel select Li^+ between other monovalent ions. So the selectivity for either divalent or monovalent ions arises directly from the interaction with the partial charges in the channel walls. Increasing the atomic radius between the four interested metal ions makes a more stable complex. This fact is proven by the results of the frequency calculations.

Conclusions

In this research study, we calculated solvation free energies for the glutamate, glutamate-Ca^{2+}, glutamate-Na^+, glutamate-K^+, and glutamate Li^+. These energies are unattainable experimentally because of the lack of volatility of these compounds. Our computations show that glutamate-Ca^{2+} is the most stable solute in the water. Glutamate has the minimum value of the solvation free energy and glutamate-calcium complex has the maximum value. So we conclude in protein channels, calcium ion is preferred to other ions.

The radial distribution functions between the active sites in glutamate and water molecules were computed in order to determine the most active site that metal ions bind to. The outcomes showed that oxygen of the backbone carboxilate group has the most radial distribution function and therefore in further calculations, metal ions were binding to this site. On the other hand, it was found that the more motion in ions; less stability of the structure would result, revealed by the frequency calculations in gas phase. Interestingly, the same trend can be seen in total energies of the structures in water and the free energies of solvation.

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