Electric field effects on alanine tripeptide in sodium halide solutions

Loukas G. Astrakas, Christos Gousias, and Margaret Tzaphlidou

Laboratories of Medical Physics, Medical School, University of Ioannina, Ioannina, Greece

Abstract

The electric field effects on conformational properties of trialanine in different halide solutions were explored with long-scale molecular dynamics simulations. NaF, NaCl, NaBr and NaI solutions of low (0.2 M) and high (2 M) concentrations were exposed to a constant electric field of 1000 V/m. Generally, the electric field does not disturb trialanine’s structure. Large structural changes appear only in the case of the supersaturated 2.0 M NaF solution containing NaF crystals. Although the electric field affects in a complex way, all the ions–water–peptide interactions, it predominantly affects the electroselectivity effect, which describes specific interactions such as the ion-pair formation.

Keywords

Electric fields, electroselectivity effect, molecular dynamics, trialanine

Introduction

One of the many proposed mechanisms explaining the potentially hazardous effects of electromagnetic (e/m) radiation on the living matter is based on the induced conformational changes of the exposed biomolecules, which result to aberrations of their biological activity (Sheppard et al., 2008). These changes are often attributed to excitations of the biomolecules’ vibrational, rotational or electronic modes. The opponents argue that similar resonance phenomena are unlikely due to dissipating noise factors, which originate from fluctuations in temperature, concentration, mechanical stress and background electric fields (Adair, 2003). Other studies have focused on the direct e/m effect either on chemical reactions, such as the chemical binding on active sites of proteins or on structural changes, such as the disruption of hydrogen bonds (Chiabrera et al., 2000). Methods of molecular dynamics (MD) have been used to model these changes but they have shown that, within their limited timescales, only unrealistic electric fields of $10^8$ or $10^9$ V/m can cause structural changes (Astrakas et al., 2011; Budi et al., 2004; English & Mooney, 2007).

Usually, the MD studies focusing on the effects of e/m radiation assume that the biomolecules are immersed in pure water solutions (Astrakas et al., 2011; Astrakas et al., 2012; Budi et al., 2004, 2005, 2007, 2008; Damm et al., 2012; English & Mooney, 2007; English et al., 2009; Solomentsev et al., 2010, 2012). In living tissues though, the biomolecules exist in mixed salt solutions interacting with the surrounding ions. It has been shown, both theoretically and experimentally, that these interactions affect significantly their structural and thermodynamic properties (Baldwin, 1996; Curtis et al., 1998; Ghosh et al., 2005; Goto et al., 1990; Hassan, 2005; Kinoshita & Harano, 2005; Perkyns et al., 1996). Characteristic example of the profound effects that salts can exert on biomolecules is the classification of ions in order of their ability to salt out or salt in proteins, known as the Hofmeister series.

In principle, ions, due to their small size and increased mobility, are very sensitive to external electric fields, at least more sensitive than the bonds holding a biomolecule together. Thus, the presence of an external electric field in an ionic solution containing biomolecules might disturb the subtle balance of ionic interactions with the biomolecular backbones or with the individual side chains. The electric field might also affect the competition between the biomolecules and the ions for water resulting to hydration changes. This study tests the above hypotheses using a MD approach, which allows on a molecular level the study of the electric effects on biomolecular stability and solubility in ionic solutions.

In our MD simulations, we have chosen the alanine tripeptide, which has been extensively studied because it is one of the simplest examples of a biomolecule, which contains the essential features of proteins. In addition, many studies have already analyzed alanine peptides in salt solutions in the absence of external electric field, shedding light into the complicated mechanisms of ion–peptide–water interactions (Dzubiella, 2008, 2009; Fedorov et al., 2006, 2007, 2009; Ioannou et al., 2011). These baseline studies have been proven very useful for the present work, which aims to understand ion–protein interactions on a molecular structural level, in the presence of external electric field.
For our MD simulation, we have chosen sodium halide solutions (NaCl, NaBr, NaF and NaI) with high (2 M) and low (0.2 M) concentrations. Our choice was based on chemical rather than biological criteria. Specifically, it allowed us to investigate how an external electric field affects the Hofmeister effect and the electroselectivity (direct binding) effect. Both these effects influence protein stability gradually from NaF to NaI, but in an opposite way, i.e. the Hofmeister effect decreases it, whereas the electroselectivity effect increases it (Goto et al., 1990; Westhof, 1993). We used salt concentrations (2 M) higher than at typical physiological conditions (0.1–0.3 M) not only for their applicability in protein crystallization, food industry and biotechnology (Dumetz et al., 2007; Dyer, 1951; Lanyi, 1974) but also for their ability to amplify salt-specific effects that can be sampled more efficiently in MD simulations.

Methods

Following the simulation protocol by Fedorov et al. (2006, 2007, 2009), MD simulations were performed with the GROMACS 4.5.5 software package (Groningen University, Groningen, The Netherlands) (Hess et al., 2008) and the OPLSSA fully atomic force field (Kaminski et al., 2001). A cutoff of 1 nm was applied to short-range non bonded interactions. For long range electrostatic interactions, the Particle Mesh Ewald method (Petersen, 1995) was used with grid spacing of 0.12 nm and fourth-order interpolation. The alanine tripeptide was designed with the PyMOL open source molecular visualization software (Schrödinger, New York, NY) with an alpha helix initial conformation and central (Φ and Ψ) dihedral angles equal to (−57° and −47°). Then it was placed in a periodic dodecahedron box together with 1206 TIP5P EW (Rick, 2004) water molecules. Ionic solutions of NaCl, NaBr, NF and NaI with high (2 M) and low (0.2 M) concentrations were created with random substitution of water molecules with the corresponding ions. Energy minimization was performed with the protein frozen using steepest descent for 20,000 steps. Then two equilibrations, one in a constant volume (NVT) ensemble of 1 ns duration and another in a constant pressure (NPT) ensemble of 27 ns duration were performed successively at a temperature of 300 K. Finally, the production MD simulations were run for 27 ns at 300 K temperature using the Berendsen thermostat (Berendsen et al., 1984) (time constant, 0.1 ps) and with 1 atm pressure using the Berendsen barostat (Berendsen et al., 1984) (time constant, 0.5 ps). The LINear Constraint Solver (LINCS) algorithm was used to apply holonomic constraints to bonds, allowing a 2 fs time step in all MD simulations. Atomic coordinates were saved every 250 steps, resulting in 54,000 trajectory frames. Trying to mimic realistic conditions, we have chosen a constant electric field of 1000 V/m, a value close to the low frequency Federal Communications Commission (FCC) limit of 614 V/m (FCC-13-39). All MD simulations were run with and without a constant electric field in the same arbitrary direction, i.e. along the x axis of the starting equilibrated conformation. Baseline simulations with the peptide in pure water solution with and without an electric field were also conducted.

Structural changes of the alanine tripeptide were assessed by clustering the conformational space of the tripeptide. This space was populated by the various conformations of the tripeptide during the production MD simulations. Clusters were created using the GROMOS method and the g_cluster program of the GROMACS software package. Every cluster contained structures with root mean square difference less than 0.1 nm. The central member of the three most populated clusters was calculated along with its dihedral angles phi, psi, its total solvent accessible area (SAS) and the hydrophobic percentage of SAS. In addition, Ramachandran plots were created for every production simulation. The Pearson correlation coefficient between Ramachandran plots, which were obtained with and without the electric field, was used to estimate possible structural changes.

In order to investigate the peptide–ion–water interactions, we examined the radial distribution functions (rdf’s) between the ions, water and peptide termini (NH\(^+\), COO\(^-\)). Changes of rdf’s (Δg(r)) have been calculated according to the formula Δg(r) = gE(r) − g(r), where gE(r), g(r) are the rdf’s between the same group of atoms with and without electric field, respectively.

Results

The baseline simulation without salts showed that the electric field cannot inflict any structural changes to the peptide. Table 1 lists the phi, psi angles of the central member of the three most populated clusters for the various solutions. It clearly shows that in the absence of ions, the peptide preserves its conformation after the application of the external electric field. This is also confirmed by the perfect correlation coefficient (Table 2) between the Ramachandran plots before and after the application of the external field.

The electric field barely disturbs the peptide’s structure in the various salt solutions with the noticeable exception of the 2 M NaF. In most cases, the peptide is close to the Polyproline II conformation \(\approx (−80° \text{ and } 140°)\). The electrical field induces large structural changes only in the 2 M NaF solution. This is also confirmed from the two Ramachandran plots in Figure 1 and their low correlation coefficient 0.06 in Table 2. Figure 2 depicts these structural changes revealing a transition from an extended to a compact conformation.

Furthermore, looking at phi, psi value changes in Table 1, the peptide in the 0.2 M NaBr solution appears to be severely affected from the external electric field. However, in this case, the Ramachandran plots in Figure 3 look similar containing two almost equally populated clusters, and their correlation coefficient is high (0.93) in Table 2. Therefore the changes in Table 1 in the NaBr 0.2 M case reflect small changes in the population number of the first and second cluster, which reversed their order after the application of the external field.

Plots of rdf’s g(r) and their changes Δg(r) between Na\(^+\), halide anions and peptide’s terminal groups are presented in Figures 4–8. Rdf’s between water and ions or between water and peptide terminal groups are not presented because the external electric field does not affect them. Note the large
values of rdf’s in the supersaturated 2.0 M NaF solution (Figure 4), which contains NaF crystals.

Rdf’s reveal that F⁻ has a significantly stronger propensity than all the other halides to approach either Na⁺ (Figure 4) or both the peptide’s terminal groups (Figures 5 and 6) despite of their charge. In the absence of an external electric field, the affinity of the halides to the other charged groups decreases nonlinearly as their ionic radius increases. Interestingly, Figures 7 and 8 show that the halides anions differently influence the affinity of Na⁺ to the peptide’s charged terminal groups. Especially in the 0.2 NaF case, the Na⁺ appears to be strongly attracted by the NH₃⁺ charged groups of the same charge (Figure 7)!

The application of the external electric field varies the rdf’s between charged groups in a complex manner. Negative values of Δg(r) in Figures 4–6 indicate that F⁻ tends to move away from the other charged groups with the exception of the 2 M NaF solution, where the NaF crystals are attached with the peptide. Conversely, the electric field increases Cl⁻ affinity with the opposite charged NH₃⁺ group (Figure 5) and decreases its affinity with the same charged COO⁻ group (Figure 6). Finally, Br⁻ slightly moves away from both termini, whereas I⁻-termini rdf’s are negligibly affected by the electric field. The electric field also disturbs Na⁺ rdf’s both with the halides and the peptide’s terminal groups. For the Na⁺-COO⁻ pair, Δg(r) depends strongly on the ionic concentration and the halide ion (Figure 8). Again with the exception of the 2 M NaF case, Δg(r) is significantly negative for the Na⁺-NH₃⁺ pair in the NaF and NaCl solutions, indicating Na⁺ removal from the vicinity of the NH₃⁺ group due to the external electric field.

**Discussion**

The influence of salts on the stability of macromolecular solutes is a well-studied phenomenon; and although various theories have been developed to explain it, many questions remain open, keeping it an active field of research. Debye–Hückel screening effect, ion pair formation (electroselectivity effect) and disruption of water structure (Hofmeister effect) have been suggested as major factors
affecting macromolecular hydration (Goto et al., 1990; Westhof, 1993). The contribution of each of these effects depends on the peptide’s structure, the type of the ion and its concentration. Inevitably, the presence of an external electric field influences all these effects directly or indirectly. In the case of the Debye–Huckel screening effect, the charge cloud around the ions gets distorted by the electric field and as the ion moves it becomes asymmetric in the motion direction. Models developed by Fuoss and Onsager have successfully quantified molar conductivity in this case (Wright, 2007). The electric field weakens the electroselectivity effect by enhancing the dissociation of ion pairs into free carriers. It will also affect ions bound on biomolecules, especially in surface shallow traps. The electric field also alters the polarization of the peptide atoms, the water molecules and the ions, which affects the Hofmeister effect. Finally, the electric field changes the concentration and the mobility of the ions around the peptide as well as its SAS. Generally, the ions influence macromolecular structure either implicitly by altering water activity and hydration or explicitly affecting direct binding and salt bridges. In principle, both these mechanisms are influenced by the electric field.

In the case of the alanine tripeptide Fedorov et al. (2006, 2007, 2009) have analyzed in detail its conformational properties in aqueous sodium halide solutions. They found that trialanine’s structure is more affected in the case of NaF solutions. The reason lies in strong electronegativity of the fluoride anion combined with its small size, which enhance the interactions with counterions, water and charged groups of the trialanine. The small size fluoride anion binds with the highly charged groups and the backbone atoms through strong, specific charge–charge interactions. Our results show that especially for the highly concentrated, supersaturated 2 M NaF solution, the electric field enhances this behavior and increases significantly the affinity of the fluoride anion to the peptide (Figures 5 and 6). In this case, NaF is expected to crystallize because its concentration is above its solubility limit, i.e. 1.0 M at 40°C. Indeed, our simulation showed fast crystallization of NaF at this concentration (Figures 2 and 4).

The electric field enhances the interaction between the peptide and the NaF crystals by directing the surface fluoride ions of the NaF crystal to bind with the trialanine causing a small structural bending (Figure 2). Conversely in the low concentration 0.2 M NaF solution, both Na and F ions are free charge carriers and the electric field moves them away from the peptide termini.

The tri-alanine structure, in the other cases of the high concentrated sodium halide solutions, gets also affected but not as much as in the NaF case. For example, Table 1 shows that the most prominent changes appear in the second and third most-populated clusters and they depend on the electronegativity and/or the size of the halide. These results combined with the absence of a systematic effect on the hydration of the peptide (Table 2) support the idea that the
electric field affects the electroselectivity effect rather than the Hofmeister effect in the highly concentrated halide solutions. The negligible changes $D_g(r)$ of the rdf’s between water and ions or peptide’s terminal groups further support the idea that the 1000 V/m electric field cannot affect hydration effects of the charged groups.

The electric field does not induce severe structural changes when the tri-alanine is immersed in the low concentrated halide solutions. The ion distribution in the immediate vicinity of the peptide changes about 10% (Figures 5–8), and in most cases, the phi and psi angles vary slightly indicating subtle structural distortions (Table 1). As a result, the solvent-accessible area remains fairly constant (Table 2). Apparently, the electric field cannot massively relocate ions either to or from the peptide and minor changes in ion distribution have been detected. These changes are more intense for the small with high charge density kosmotropes $F^-$, $Na^+$, and less intense for the large with small charge

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**Figure 3.** Ramachandran plots of the distortion angle Phi, Psi of the trialanine peptide conformation during the production simulation immersed in a 0.2 M NaBr solution before (left) and after (right) the application of an electric field.

**Figure 4.** Radial distribution functions $g(r)$ (up) and changes of radial distribution function $\Delta g(r)$ (down) between Na$^+$ and halide anions in the low 0.2 M (left) and high 2 M (left) concentration solutions.
Figure 5. Radial distribution functions $g(r)$ (up) and changes of radial distribution function $\Delta g(r)$ (down) between halide anions and NH$_3^+$ terminal group in the low 0.2 M (left) and high 2 M (left) concentration solutions.

Figure 6. Radial distribution functions $g(r)$ (up) and changes of radial distribution function $\Delta g(r)$ (down) between halide anions and COO$^-$ terminal group in the low 0.2 M (left) and high 2 M (left) concentration solutions.
Figure 7. Radial distribution functions $g(r)$ (up) and changes of radial distribution function $\Delta g(r)$ (down) between Na$^+$ and NH$_3^+$ terminal group in the low 0.2 M (left) and high 2 M (left) concentration solutions.

Figure 8. Radial distribution functions $g(r)$ (up) and changes of radial distribution function $\Delta g(r)$ (down) between Na$^+$ and COO$^-$ terminal group in the low 0.2 M (left) and high 2 M (left) concentration solutions.
density chaotropes Cl\(^-\), Br\(^-\) and I\(^-\). They also differ between NH\(_2\)- and COO\(^-\) mainly due to hydration differences between these groups (Fedorov et al., 2007). Because of the strong hydration of NH\(_2\)- terminal, big anions prefer to interact with this group by water mediated interaction, whereas for the less hydrated COO\(^-\) terminal direct binding is more favorable.

Although we have demonstrated that an electric field can alter the conformation of a biomolecule indirectly by disturbing the ionic concentration around it, our results are still far from providing a realistic mechanism of e/m effects on the living matter. Inherent limitations of the MD methodology and the simplicity of our model compared with the complexity of the real biomolecular environments restrict the applicability of our results. In addition, the most prominent electric field effects appear in the case of high NaF concentration (2 M), which is not met in living tissues. NaF can be administered to the human body through the fluoridate water (~0.5 mg/L), the toothpastes (~0.3% w/w) and as a drug for osteoporosis treatment (20–34 mg/d), but it is very unlikely that it will ever occur in crystal form. Nevertheless, there are many cases where crystallization or nucleation occurs in pathological conditions in the human body (Poloni & Ward, 2014). Typical examples are the calcium pyrophosphate dehydrate in pseudo-gout and the monosodium urate in gout. In the former example, molecular dynamic simulations have shown how the electrostatic interactions between the charged surface of the crystal and the extracellular layer of a phospholipid bilayer lead to membranolysis (Wierzbicki et al., 2003). We believe that our findings promote the investigation of similar effects under the influence of an external electric field.

**Conclusion**

Relatively small electric fields cannot inflict significant structural changes in biomolecules immersed in salt solutions. The most prominent changes occur in the case of the supersaturated (2 M) NaF solution due to the strong interactions between the NaF crystals and the peptide charged groups. In the other high concentration cases, minimal structural changes appear in the second and third most populated clusters of the conformational space, and they depend on the electronegativity and/or the size of the halide. The electric field does not inflict significant structural changes to the peptide in the cases of the low concentrated (0.2 M) halide solutions. Although many different effects contribute to ion–water–peptide interactions, the electric field seems to affect mainly the electrosensitivity effect. Future studies with more advance methodologies (i.e. polarizable force-field, quantum mechanics/molecular mechanics (QM-MM)) are needed to further improve the modeling of the electric field effects on macromolecular hydration in electrolyte solutions and salt crystals and confirm our results.

**Declaration of interest**

The authors report no declarations of interest.

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