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Sodium versus potassium effects on the glutamic acid side-chains interaction on a heptapeptide

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Equilibrium peptide conformations in solution, especially in the presence of salts, has been of interest for several decades. The fundamental interactions that determine the dominant peptide conformations in solution have been experimentally and computationally probed; however, a unified understanding has not yet emerged. In a previous study, we performed metadynamics simulations on the heptapeptide AEAAAEA in Sodium Chloride (NaCl) and Potassium Chloride (KCl) solutions at concentrations ranging from 0.5–2.0 M. Using a three-dimensional collective variable coordinate system, we computed the free energy landscapes in each saline environment as well as in pure water. We found that the presence of Na^+ and K^+ ions induces some changes in the stability of the conformers that define the state space, but does not alter the overall energetics between conformers and does not favor helical conformations. We investigate here, how the presence of salts (NaCl and KCl) affects the glutamic–glutamic interaction and its consequences on the stability of each equilibrium conformation. We perform this study through fixed backbone simulations for the most populated conformations identified in our previous work: the α-helix, 3_{10}-helix, π-helix, the extended polyproline II (PPII) and 2.5_1-helix conformations. It was found that for each conformation, there exists stable substates determined by the glutamic acid side-chains distance and orientation, and that Na^+ and K^+ cations (de) stabilize preferentially each conformation. It was also found that intramolecular single water mediated hydrogen bonds play a crucial role in the observed (de) stabilization of each equilibrium conformation.

Keywords: Sodium; potassium; salt effects; small peptides.

1. Introduction

In our previous metadynamics simulation of the heptapeptide AEAAAEA, in Sodium chloride (NaCl) and Potassium Chloride (KCl) solutions, at concentrations ranging from 0.5–2.0 M, we computed the free energy landscapes for the peptide in each saline environment as well as in the absence of ions.[1] Salt induced stabilization effects of some conformers were found in the computed free energy maps, but the

mechanisms involved in such observed effects are not clear. Some conformations, like the extended conformations have been stabilized in environments with high ionic strength, but other more compact conformations do not show changes in presence of salt. The identity of the salts also plays an important role in the stabilization mechanisms. Interestingly, we found that some conformations were stabilized in one salt, but not in the other, and other conformations were stabilized in both salts. Specifically, the extended random coil was stabilized in KCl, but was unchanged in NaCl, π-helix conformer was stabilized in both salts, and a semi-formed helix was stabilized in NaCl, but not in KCl. A simple electrostatic explanation for a salt induced stabilization effect would be that salts screen the glutamic acid side-chain interaction. The results obtained in our previous work suggest that the studied salts do not screen the glutamic acid side chains interaction in the same way and that the change of the side-chains interaction could affect the stability of a given peptide conformation.[1] Since, for each conformation the distance and relative orientation of the side-chains are different, the effect of each salt on the side-chains interaction may also be different for a given conformation. Stabilization of helical peptides as a result of a change in the side-chain interaction has been previously investigated in the literature. Using Circular dichroism (CD) spectroscopy, Smith and Scholtz have studied the effects of NaCl on the stability of a host peptide Ala–Gln with different oppositely charged side-chains (Glu–Lys, Asp–Lys, Glu–His), at concentrations ranging from 0.01 M–2.5 M NaCl.[2] The interaction free energy between the side-chains was calculated and ΔG values between 0 and 2.7 kJ/mol (0.64 kcal/mol) were found. Although these values are very small, they are of the same order of helix propensity. Hence, a change in the side-chain interaction induced by the salt solution can make a big difference in the helical content of the peptide and could lead to a change in the peptide preferred conformations. It was also found in this study that ΔG values decrease in NaCl. The conclusions of this study are: the side-chains interaction depends strongly on their relative spacing and orientation and that the major effect involved in the helical peptide stabilization is hydrogen bonding and not a simple electrostatic interaction. The relative orientation between side-chain as an important factor in the resulting side-chain interaction has been observed in previous studies. Masunor and Lazaridis used the Spherical Solvent Boundary Potential (SSBP) method to calculate Potential Mean Forces (PMFs) between ionizable amino acid side-chains (Arg, Lys, His, Glu, Asp) for two different orientations: C_δ and Cγ atoms in the same line (head to head) and Cδ and Oε1 (O1?) in the same line (side to side).[3] For the residue which we are interested here, glutamic, it was found that when glutamic acid residues are oriented head to head, the PMF has a stable minimum at 3.8 Å. For an orthogonal orientation (side to side), a flat PMF is obtained. Additionally, if one or both glutamic residues are forming H-bonds with water, the energy decreases 8.2 kJ/mol with respect to glutamic not forming H-bonds with water. The dependence of the side-chains interactions with their relative orientations has been quantified by Thomas and Elcock through molecular dynamics (MD) simulations of acetate and methylamonium ions.[4] They simulated the effects of NaCl

on the interaction between these two ions which serve as a model of the interaction between glutamate and lysine side-chains. They found a set of minima in a 2D free energy surface that fall in two categories: minima associated with direct charged groups and minima associated with hydrophobic interactions. The latter category lies 10.5 kJ/mol lower than the one associated with direct charge–charge interactions. Comparing with pure water, the depths of the free energy wells associated with the charge–charge interaction are less favorable in NaCl, but the electrostatic interactions are not completely screened, not even at 2 M NaCl. The minima associated with hydrophobic interactions are only slightly unfavorable. Following the idea that the side-chains interaction is orientation dependent, Scheraga *et al.* calculated the PMF for a glutamic acid model as a function of the relative distance between side-chains.[5] The PMFs were calculated for four different orientations. The conclusions were the same. The position of the minima and the values of the potential depths depend strongly on the glutamic acid distance, and their relative orientation. The 'side to side' orientation is the one with lower energy. In addition to the distance and relative orientation between side-chains, another factor that influences the effect of salts on the stability of peptides with charged side-chains is the location of the side-chains in the peptide. Friedman showed that charged amino acids and ions with opposite charge attract, but these electrostatic effects are quite residue specific, with some residues being much more attractive to the ions than others.[6] Based on the evidence, that different arrangements of the glutamic acids will lead to different interactions, that could stabilize the peptide in different ways, the aim of this study is to investigate the most probable glutamic acid arrangements in the AEAAAEA peptide (distances between glutamic acid side-chains and relative orientations) and how each given geometry will promote stabilization of the corresponding peptide conformation. The second objective is to compare the effects of NaCl and KCl on the glutamic side-chain interaction and hence, the resulting stabilization of each conformation. Finally, motivated by the evidence that the position of the side-chains is an important factor in the side-chains ions interaction, we will investigate if the two glutamic acid residues interact in the same way with Na^+ and K^+ ions. In order to address the mentioned objectives, we performed fixed backbone MD simulations for five conformations identified previously in Asher's work as the most stable conformations: α-helix, π-helix, 2.5_1-helix, 3_{10}-helix, and Poly proline II (PPII).[7] The simulations were performed for a capped form of the peptide (Ace-AEAAAEA-Nme) in KCl and NaCl solutions, and also in pure water.

2. Methods

The peptide sequence examined in this work is AEAAAEA, where A represents alanine and E represents glutamic acid. The peptide was simulated with blocking groups on each termini, consisting of an acetylated N-terminus and an N-methyl group on the C-terminus. Glutamic acid residues were deprotonated in all

simulations. The peptide was immersed in a box of TIP3P pure water, and 1.0 M concentrations of either KCl and NaCl. Electric neutrality of each condensed phase system was maintained with the addition of two additional K^+ and two additional Na^+ cations for the KCl and NaCl simulations respectively. Specific conformations of the peptides in the various saline environments were sampled by building the peptide in question in a given conformation (*i.e.* α-helix) and geometrically constraining its backbone atoms throughout the simulation. Since the conformation of a peptide is determined by the Φ and Ψ dihedral angles of the backbone, holding the atoms of the latter fixed, allows selected conformations of the peptide to be sampled for long periods of time, whereas, these same conformations may rarely be sampled in classical MD simulations. In turn, it is possible to probe how water molecules and ions interact with side-chains and the peptide backbone atoms of high and low energy conformers, in order to elucidate the driving force behind ionic influence on peptide conformational equilibrium. Peptides were built using MOE software in five different conformations based on the published values of Φ and Ψ dihedral angles for each conformation of interest.[8] These include extended conformations such as the 2.5_1-helix and PPII conformer, as well as folded conformations such as the α-helix, π-helix and 3_{10}-helix. Values of the dihedral angles used for each conformation are shown in Table S1 in the Supplementary Information. The aforementioned conformations were selected because they are the common motifs observed naturally in proteins, they have also been observed in experimental studies on similar small peptides by our collaborator,[7] and these conformations were local minima, in the free energy maps constructed by our previous metadynamics simulations.[1] Initially, fixed backbone MD simulations consisted of 10,000 energy minimization steps to eliminate steric clashes between atoms within each system, 40 ns of equilibration in the isothermal–isobaric ensemble (NPT) and 100 ns production at a constant pressure and temperature of 1 atm and 298 K, respectively. Coordinates of all atoms were written every 2 ps. NAMD was used to perform all calculations and the CHARMM36 forcefield was used for the peptide and ions.[9,10]

3. Results

Fixed backbone simulations were performed for the five conformations previously described. These five conformations were identified by Asher's group as being the most populated conformations in the capped peptide at room temperature. For the five conformations, the glutamic acid side-chains relative distance and orientation is different. Also, as it can be seen in Fig. 1, the angle between the glutamic acid side-chains and the backbone is different for each conformation, e.g. for π-helix and α-helix, the glutamic acid side-chains lie at the same side of the backbone, but for PPII they are found at opposite sides. For this reason, and as it was discussed in the Introduction, we expect that salt effects on the side-chains electrostatic repulsion and on the ion adsorption to the peptide will be different for each conformation, affecting the side-chains interactions and hence, the stability of each conformation.

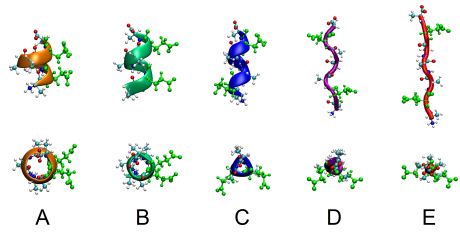

Fig. 1. Side and top views of the five conformations studied: π-helix (A), α-helix (B), 3_{10}-helix (C), PPII (D), and 2.5_1-helix (E). Glutamic acid side-chains are colored green.

The glutamic acid side-chains distances and their relative orientation was tracked and the respective 2D PMF for the different conformations in each environment (pure water, 1 M KCl and 1 M NaCl) was constructed as a function of these two variables. The glutamic acid side-chains distance was calculated between C_δ atoms and their relative orientation is monitored by the torsional angle C_δGLU2–C_αGLU2–C_αGLU6–C_δGLU6. Each PMF is measured with respect to a different reference state (each one has a different zero). The 2D PMF for α-helix in pure water spans glutamic acid side-chains distances between 6 Å –10 Å and wide values for torsions, in the range of 40 deg–100 deg (Fig. 2, left). The global minimum corresponds to a glutamic acid side-chain distance of 7 Å and a torsion value of 40 deg. There is a second minimum that only differs in the glutamic acid side-chain distance, at about 9 Å . 1 M NaCl has the effect of inverting the depth of the main two minima, stabilizing the second minimum (at 9 Å). Also, in this environment, the torsion values sampled are reduced, compared with pure water (Fig. 2, center). From here, the net effect of Na^+ ions seems to be to increase the glutamic acid side-chain repulsion. In 1 M KCl on the other hand, the first minimum is stabilized (shorter glutamic acid side-chain distances), also in this salt environment, glutamic acid side-chains have more mobility given by the wide range of torsion values observed (Fig. 2, right). The positions of each glutamic acid side-chain corresponding to the two deep minima were identified by the construction of clusters by root mean square deviation (rmsd) over the entire

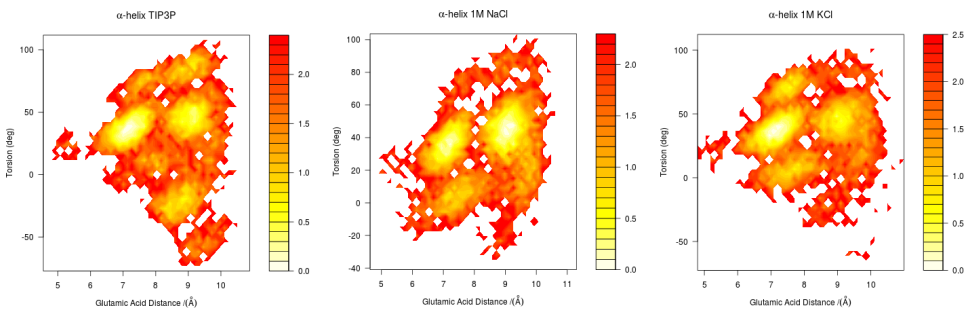

Fig. 2. 2D PMF as a function of the glutamic acid side-chains distance (Å) and the torsional angle C_δGLU2–C_αGLU2–C_αGLU6–C_δGLU6 (deg) for the α-helix conformer in pure water (left), 1 M NaCl (center) and 1 M KCl (right).

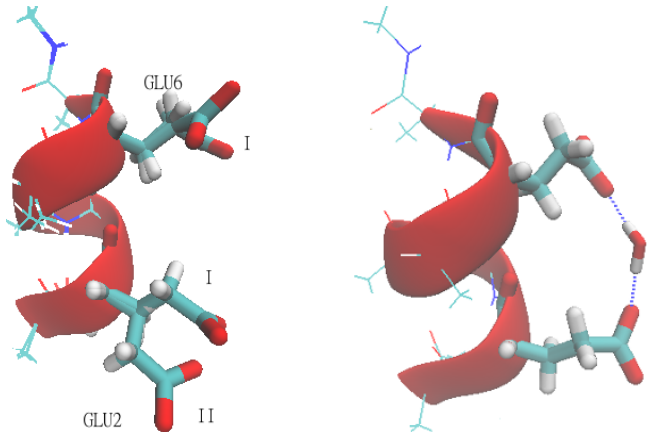

Fig. 3. Positions I and II adopted by the glutamic acid side-chains in the two deep minima for the α-helix conformer (left). Intramolecular water mediated hydrogen bond (GLU6–WAT–GLU2) observed in the α-helix conformer (right).

trajectory. It was found that GLU6 remains at the same orientation relative to the backbone for both minima. GLU2 moves from an upward position to a downward position. Rotations around the glutamic acid axis are also observed (Fig. 3, left). The global minimum found in pure water and 1 M KCl corresponds to both glutamic acid side chains in position I, the second minimum has GLU6 in position I and GLU2 in position II (This is the global minimum for 1 M NaCl).

Calculation of the number of Na^+ and K^+ ions in the vicinity of the peptide shows that the density of Na^+ around glutamic acid side-chains is three to four times greater than K^+ density (see Supporting Information). This result is consistent with previous works in which it is shown that Na^+ cations have much stronger attraction to carboxylate groups compared to K^+, [11–14] and it is also consistent with the Law of Matching Water Affinities,[15] which states that ions with similar hydration energy form more stable contact ionic pairs. Since Na^+ ions and carboxylate have close hydration energies, they form stable ion pairs in water. We are interested here in the consequences of the differences in ion densities in the vicinity of glutamic acid side-chains. Is the greater amount of Na^+ ions surrounding the peptide responsible for the stabilization of the substate, associated with the larger glutamic acid distance? If yes, what is the physical mechanism involved? Through a precise analysis of the first hydration shell of the peptide in the three environments, it has been found the existence of two types of intramolecular single water mediated hydrogen bonds. A single water mediated hydrogen bond connecting the two glutamic acid side-chains (GLU6–WAT–GLU2) was found in the three environments (Fig. 3, right). This type of hydrogen bond is more abundant in 1 M KCl (Table 1). In 1 M NaCl, the number of this type of hydrogen bond found is less than in pure water. A greater amount of this type of water mediated hydrogen bond in KCl (bond that connects glutamic acid side-chains), might be one of the mechanisms responsible for the stabilization in 1 M

Table 1. Number of intramolecular water mediated hydrogen bonds for each conformation, in the three studied environments.

	α-helix			3_{10}-helix			π-helix			25_1-helix			PPII		
Hbond	TIP3P	NaCl	KCl	TIP3P	NaCl	KCl	TIP3P	NaCl	KCl	TIP3P	NaCl	KCl	TIP3P	NaCl	KCl
G2–WAT–A1									1510	630					
G2–WAT–A3									5680	9990	13,310	10,430	9850	9840	
G6–WAT–A3	1280	1600	2010	1670	1900	2280									
G6–WAT–A4												3390	04840	1060	
G6–WAT–A7												3910	1590	4000	
G2–WAT–G6	1250	810	870			16,990	6600	11,640							

KCl of the minimum associated with shorter glutamic acid side-chains distances, since it has the effect of 'stapling' the side-chains. The reason for a less amount of this hydrogen bond found in 1 M NaCl might be due to a greater number of Na^+ ions in the peptide first hydration shell, disrupting in a greater amount than K^+, the water hydrogen bond network. The second type of intramolecular water mediated hydrogen bond found for the α-helix conformer is GLU6–WAT–ALA3. Although the amount of this type of hydrogen bond increases for both salts, it is still more abundant in 1 M KCl. This type of hydrogen bond will also favor short or intermediate glutamic acid distances. Since the energy to break an intramolecular hydrogen bond in pure water is about 1 kcal/mol, the energy to break the observed bonds will be about 2 kcal/mol. If the presence of these water mediated hydrogen bonds were the only mechanism stabilizing short glutamic acid distances in 1 M KCl, it is expected to have energy differences between 0–2 kcal/mol. A calculation of PMF between the peptide in each salt and pure water, and between the two salts show that the energy of each minimum differs in a range of 0–2 kcal/mol between different environments (Fig. 4), suggesting that intramolecular water mediated hydrogen bonds might be the main mechanism responsible for the (de)stabilization of each substate due to the saline environment.

The 2D PMF for 3_{10}-helix in pure water covers several glutamic acid side-chains distances (10 Å–14 Å) and wide values for the torsions, between 60 deg–150 deg. (Fig. 5). The global minimum corresponds to a distance between glutamic acid side-chains of 12 Å and a torsion value of 70 deg. At the same glutamic–glutamic distance, there is a second populated region with larger torsion values. For this conformer, 1 M NaCl has the effect of increasing torsion values for the glutamic acid distance associated with the global minimum (12 Å), also this salt destabilizes substates with larger glutamic acid side-chains distances. 1 M KCl does not change the position of the global minimum, but increases the sampling of substates with lower torsion values as well. This two deep minima are shown in Fig. 6, where the global minimum in the three environments corresponds to GLU6 in position I and GLU2 in position II.

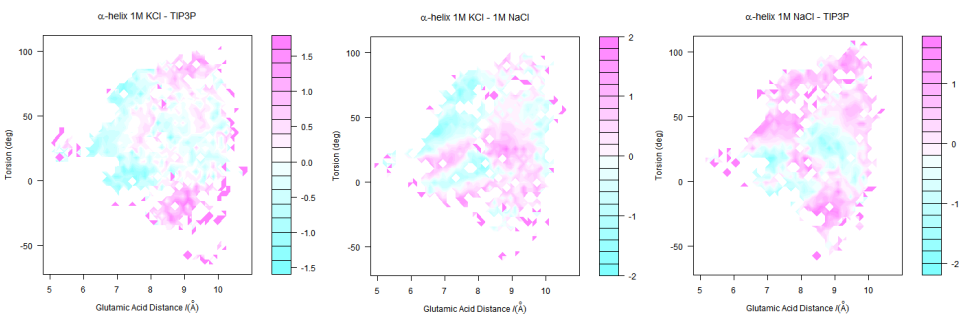

Fig. 4. 2D PMF difference between 1 M KCl and TIP3P (left), 1 M NaCl and TIP3P (center) and 1 M KCl and 1 M NaCl (right).

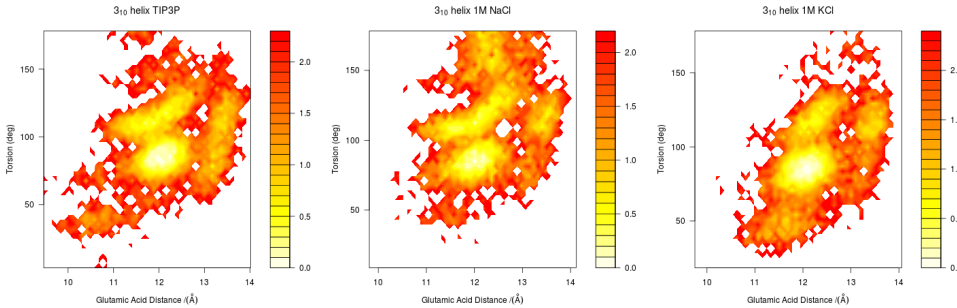

Fig. 5. 2D PMF as a function of the glutamic acid side chain distance (Å) and the torsional angle C_δGLU2–C_αGLU2–C_αGLU6–C_δGLU6 (deg) for the 3_{10}-helix conformer in pure water (left), 1 M NaCl (center) and 1 M KCl (right).

For this conformer, only one type of water mediated intramolecular hydrogen bond has been found: GLU6–WAT–ALA3 (Fig. 6, right). Similar to the hydrogen bond found in the α-helix conformer, this hydrogen bond is more abundant in 1 M KCl, and there is a less amount of this hydrogen bond in 1 M NaCl. Less hydrogen bonds in NaCl might be due to a more density of Na^+ ions in the first hydration shell of the peptide, only that, in this case it does not influence the stability of the global minimum, since it is not connecting glutamic acid side-chains. The energetics is similar to the α-helix conformer, energy differences between 0–2 kcal/mol between environments have been observed (Supplemental Information). These values are the same for all conformers studied.

In pure water, π-helix conformer has its global minimum at a glutamic distance of 5.7 Å and a 40 deg. torsion value (Fig. 7, left). 1 M NaCl increases the population of substates with 7 Å–8 Å glutamic distances and 80 deg. torsion values. In 1 M KCl, the sampled substates are reduced and the global minimum is stabilized. Torsions

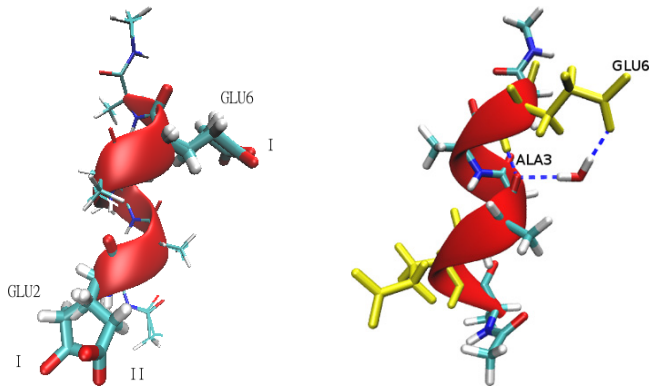

Fig. 6. Positions I and II adopted by the glutamic acid side-chains in the two deep minima for the 3_{10}-helix conformer (left). Intramolecular water mediated hydrogen bond (GLU6–WAT–ALA3), glutamic acid side chains are colored in yellow for a better visualization.

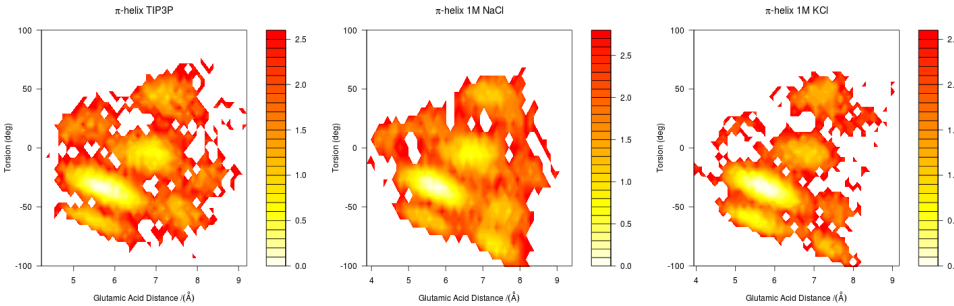

Fig. 7. 2D PMF as a function of the glutamic acid side-chains distance (Å) and the torsional angle C_δGLU2–C_αGLU2–C_αGLU6–C_δGLU6 (deg) for the π-helix conformer in pure water (left), 1 M NaCl (center) and 1 M KCl (right).

with values greater than zero are considerably reduced. For π-helix, the most stable substate has GLU2 in position I (Fig. 8, left) in the three environments. Here, the only intramolecular water mediated hydrogen bond observed is connecting the two glutamic acid side-chains (GLU6–WAT–GLU2). It is surprising the amount of this type of hydrogen bond observed is 20 times larger than in the α-helix conformer.

For the 2.5_1-helix conformer, the main difference between the most populated states is the glutamic acid distances with reduced torsion values for each one. Three regions are clearly populated with glutamic acid distances of: 14, 16 and 18 Å. The global minimum corresponds to larger distances in pure water (18 Å). 1 M KCl solution stabilizes short and intermediate distances, and destabilizes the minimum with larger distances. 1 M NaCl has the same effect but not as intense as 1 M KCl (Fig. 9). The three minima are shown in Fig. 10. In pure water, both glutamic acid are located in position II (center minimum), and GLU6 in position I and GLU2 in position II (right minimum). The less populated substate has GLU6 in position II and GLU2 in position I (left minimum). When the peptide is in 1 M NaCl solution, the population of the minimum associated with shorter glutamic acid distances increases, favoring GLU6 in position II and the population of the minimum associated with larger

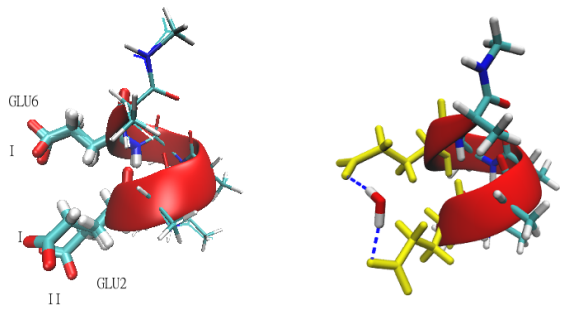

Fig. 8. Positions I and II adopted by the glutamic acid side-chains in the two deep minima for the π-helix conformer (left). Intramolecular water mediated hydrogen bond (GLU6–WAT–ALA3). Glutamic acid side-chains are colored in yellow for a better visualization.

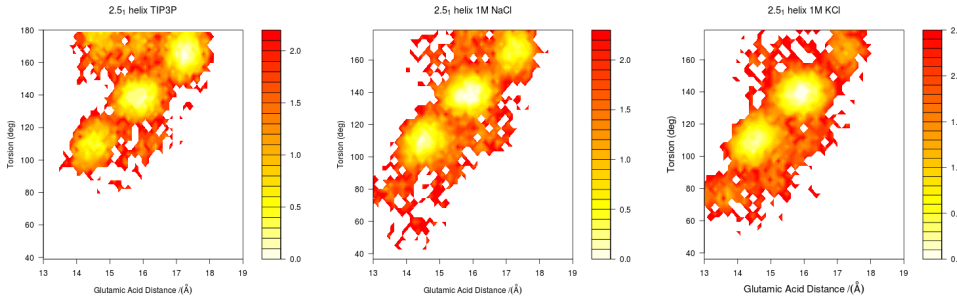

Fig. 9. 2D PMF as a function of the glutamic acid side-chains distance (Å) and the torsional angle C_δGLU2–C_αGLU2–C_αGLU6–C_δGLU6 (deg) for the 2.5$_1$-helix conformer in pure water (left), 1 M NaCl (center) and 1 M KCl (right).

glutamic distances decreases, favoring GLU2 in position I. When the peptide is immersed in 1 M KCl solution, the third peak decreases. In this case, only GLU6 moves to either position I or II. For this conformer, two types of water mediated hydrogen bonds involving GLU2 were found: GLU2–WAT–ALA1 and GLU2–WAT–ALA3. Such a hydrogen bond will cause the movement of GLU2 between positions I and II. The number of GLU2–WAT–ALA1 hydrogen bonds strongly decreases for 1 M NaCl and disappears completely for 1 M KCl. This hydrogen bond favors large glutamic acid distances and accordingly, is observed mainly for the substate with largest C_δ–C_δ distances. The substate with largest C_δ–C_δ distances

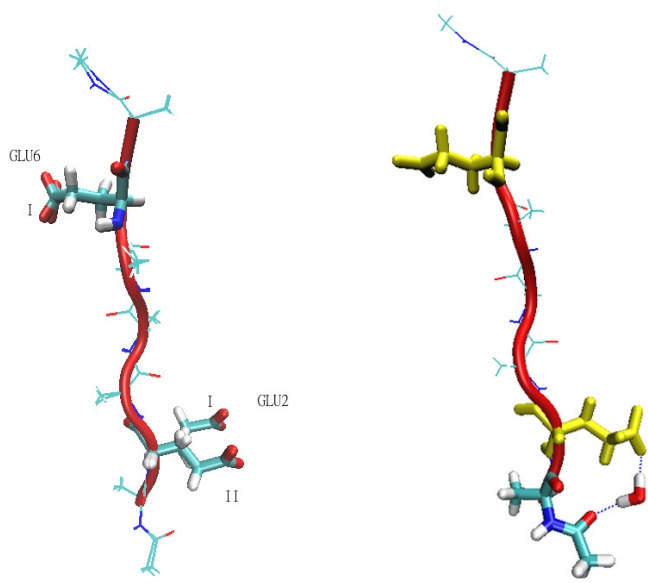

Fig. 10. Positions I and II adopted by the glutamic acid side-chains in the two deep minima for the 2.5$_1$-helix conformer (left). Intramolecular water mediated hydrogen bond (GLU2–WAT–ALA3). Glutamic acid side-chains are colored in yellow for a better visualization.

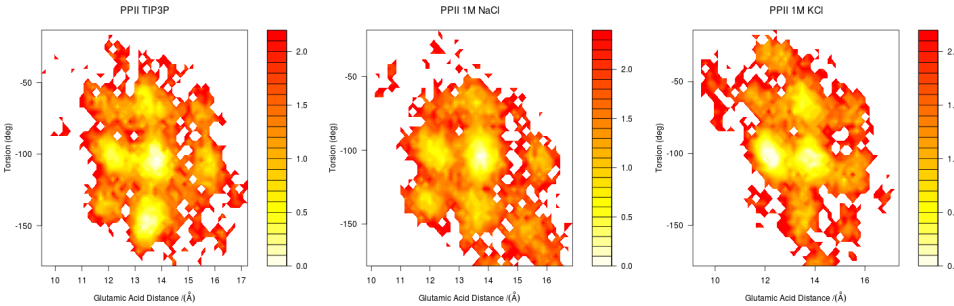

Fig. 11. 2D PMF as a function of the glutamic acid side-chains distance (Å) and the torsional angle C_δGLU2–C_αGLU2–C_αGLU6–C_δGLU6 (deg) for the 2.5_1-helix conformer in pure water (left), 1 M NaCl (center) and 1 M KCl (right).

also decreases its population in 1 M KCl. GLU2–WAT–ALA3 hydrogen bond, the bond that favors shorter glutamic distances, increases in both salts, but for 1 M KCl, the number of this hydrogen bond observed is 2.5 times larger than in pure water.

Glutamic acid side-chains in the PPII conformer have higher mobility than in 2.5_1-helix, more substates are sampled in this conformer for the three environments (Fig. 11). In pure water, the most populated substates correspond to a glutamic acid distance of 13.5 Å in two different orientations given by the torsion values. In 1 M NaCl, these torsions are reduced and substates with higher glutamic acid separations

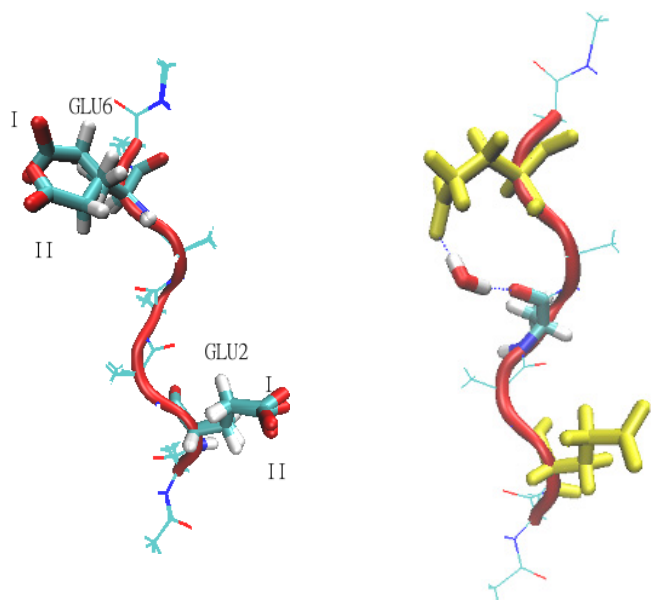

Fig. 12. Positions I and II adopted by the glutamic acid side-chains in the two deep minima for the PPII conformer (left). Intramolecular water mediated hydrogen bond (GLU2–WAT–ALA3). Glutamic acid side-chains are colored in yellow for a better visualization.

and larger torsions appear. In 1 M KCl, the substate with shorter glutamic distances (12 Å) is strongly stabilized, while larger glutamic acid distances are destabilized. The minima are shown in Fig. 12 with both glutamic acid side-chains in position I for the global minimum in pure water. The global minimum in 1 M KCl has GLU6 in position II and GLU2 in position I. In PPII conformation, three types of water mediated H-bonds involving both glutamic acid side-chains have been observed: GLU2–WAT–ALA3, GLU6–WAT–ALA4 and GLU6–WAT–ALA7. The number of GLU2–WAT–ALA3 decreases for both salts. The number of GLU6–WAT–ALA4 increases in 1 M NaCl and decreases in 1 M KCl, and the number of GLU6–WAT–ALA7 decreases in 1 M NaCl and is about the same in 1 M KCl.

In summary, for each studied conformation glutamic acid side-chains are found in two or three different arrangements (substates). Substates can be described by the relative distance between glutamic acid side-chains and torsional angles. The presence of Na^+ and K^+ ions in the vicinity of the peptide has the consequence of (de) stabilizing specific substates, depending on the nature of each ion.

4. Conclusion

We have compared the effects of sodium and potassium salts on the glutamic acid side-chains interaction, through fixed backbone simulations of five different conformations of the AEAAAEA peptide. It was found that for each studied conformation, a small number of stable substates exists, given by different combinations of glutamic acid side chains orientations. It was also found that the net effect of each salt on the peptide is to preferentially stabilize the substates. The main physical mechanism involved in the observed stabilization is related with the number of intramolecular single water mediated hydrogen bonds formed in the first peptide solvation shell. In some cases, these hydrogen bonds act as staplers, holding the glutamic acid side-chains together and thus, decreasing the electrostatic repulsion. In other cases, the hydrogen bonds staple one glutamic acid side-chain with the backbone of the peptide, with the final effect of decreasing the side-chains repulsion. The number of each kind of single water mediated hydrogen bond depends strongly on the identy of the salt. Hence, the main difference between the observed sodium and potassium stabilizing effects lies in their ability to disrupt intramolecular single water mediated hydrogen bond in the peptide first layer. This result is important because a tangible cause has been introduced as a consequence of the different behaviour of Na^+ versus K^+ on the peptide vicinity.

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References

1. Gaborek T, Chipot C, Madura JD, Conformational free-energy landscapes for a peptide in saline environments, *Biophys J (USA)* **103**(12):2513–2520, 2012.
2. Smith JS, Scholtz JM, Energetics of polar side-chain interactions in helical peptides: Salt effects on Ion pairs and hydrogen bonds, *Biochem* **37**:33–40, 1998.
3. Masunov A, Lazaridis T, Potentials of Mean force between Ionizable Amino acid side-chains in water, *J Am Chem Soc* **125**:1722–1730, 2003.
4. Thomas AS, Elcock AH, Direct observation of salt effects on molecular interactions through explicit-solvent molecular dynamics simulations: Differential effects on electrostatic and hydrophobic interactions and comparisons to Poisson-Boltzmann theory, *J Am Chem Soc* **128**:7796–7806, 2006.
5. Makowski M, Liwo A, Sobolewski E, Scheraga HA, Simple physics-based analytical formulas for the potentials of mean force of the interaction of amino-acid side chains in water. V. Like-charged side chains, *J Phys Chem B* **115**(19):6119–6129, 2011.
6. Friedman R, Ions and the protein surface revisited: Extensive molecular dynamics simulations and analysis of protein structures in alkali-chloride solutions, *J Phys Chem B* **115**:9213–9223, 2010.
7. Xiong K, Lu M, Asher SA, Conformation of poly-L-glutamate is independent of ionic strength, *Biophys Chem* **162**:1–5, 2012.
8. Molecular Operating Environment (MOE), 2012.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite 910, Montreal, QC, Canada, 2012.
9. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K, Scalable molecular dynamics with NAMD, *J Comput Chem*, **26**:1781–1802, 2005.
10. Vanommeslaeghe K, Hatcher E, Acharya C, Kundu S, Zhong S, Shim J, Darian E, Guvench O, Lopes P, Vorobyov I, MacKerell Jr AD, CHARMM general force field (CGenFF): A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields, *J Comput Chem* **31**:671–690, 2010.
11. Aziz EF, Ottosson N, Eisebitt S, Jagoda-Cwiklik B, Vacha R, Jungwirth P, Winter B, Cation-specific interactions with carboxylate in amino acid and acetate aqueous solutions: X-ray absorption and ab initio calculations, *J Phys Chem B* **112**:12567–12570, 2008.
12. Uejio JS, Schwartz CP, Dufn AM, Drisdell WS, Cohen RC, Saykally RJ, Characterization of selective binding of alkali cations with carboxylate by x-ray absorption spectroscopy of liquid microjets, *Proc Natl Acad Sci (USA)* **105**:6809–6812, 2008.
13. Vlachy N, Jagoda-Cwiklik B, Vacha R, Touraud D, Jungwirth P, Kunz W, Hofmeister series and specific interactions of charged headgroups with aqueous ions, *Adv Colloid Interface Sci* **146**:42–47, 2009.
14. Fedorov MV, Goodman JM, Schumm S, To switch or not to switch: The effects of potassium and sodium ions on alpha-poly-L-glutamate conformations in aqueous solutions, *J Am Chem Soc* **131**:10854–10856, 2009.
15. Collins KD, Charge density-dependent strength of hydration and biological structure, *Biophys J* **72**:65–76, 1997.