J|A|C|S COMMUNICATIONS

Published on Web 07/20/2009

To Switch or Not To Switch: The Effects of Potassium and Sodium Ions on α-Poly-L-glutamate Conformations in Aqueous Solutions

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Protein−ion interactions play a very important role in various biological processes,[1] and this subject has attracted much attention since the pioneering work of Hofmeister in the 19th century.[2] The effects of sodium and potassium cations are of great importance because these ions are the most abundant in the human body. The interactions of these cations with proteins are critical in allowing ion channels to be specific for particular metals.[3] Salts of small monovalent cations are commonly used to control ionic strength of solutions, and it is often assumed that nonspecific charge screening is mainly responsible for conformational changes in proteins, thus neglecting the often observed ion-specific differences.[1,4−6] However, this fails to account for the large specific effects often observed for different monovalent cations. On the basis of charge densities and electrostatic arguments, the empirical "law of matching water affinities" proposed by Collins[4−6] states that ions with similar free energies of hydration tend to form contact ion pairs, which could explain ion-specific differences in the interaction of salts with proteins. Accordingly, small cations such as sodium are expected to form contact ion pairs with carboxylate groups, while larger cations such as potassium are expected to be less strongly associated with the carboxylate side chains of proteins. This has recently been supported by several theoretical[7−10] and experimental studies[11,12] demonstrating that sodium and potassium have different affinities for carboxylate groups. In these studies, the results on the strength of carboxylate−ion interactions were focused on isolated carboxylate groups and monovalent cations. Savelyev and Papoian[13] recently demonstrated that the high charge density of polyanions can be an additional factor in driving the selective association of sodium over potassium.

The aim of this work is to relate the ion-specific conformational change of α-poly-L-glutamic acid (α-PGA) to differences in the specific interactions of sodium and potassium with the carboxylate side chains in α-PGA. Carboxylate groups are the most abundant anionic groups in proteins, so an understanding of the molecular mechanism of ion interactions with α-PGA side chains should have an impact on many areas of biological science, including rationalization of the discrimination between sodium and potassium ions in biological environments such as ion channels and the operation of sodium and potassium pumps.[3]

We recently reported the details of a study of the effects of sodium ions on α-PGA in solution.[14] In the present work, we concentrated on the comparative effects of sodium and potassium ions on conformational properties of the polypeptide, and here we present results of molecular dynamics simulations of α-PGA (containing 21 glutamates) in 0.30 M aqueous solutions of two different salts, sodium chloride and potassium chloride, with explicit water molecules. α-PGA was chosen as the model system because of the conformational plasticity of the polypeptide, which can take a number of different stable conformations, including α-helical, *β*-sheet, and extended, depending on the solvent conditions. The conformational landscape of the polypeptide has been extensively studied using various methods,[15−17] and the optical properties of this macromolecule in different solutions are considered to be the primary reference for the α-helical, *β*-sheet, polyproline II (PPII), and extended 2.5(1)-helix conformations.[17,18]

There is experimental evidence for the different effects of sodium and potassium salts on α-PGA conformations. At high pH (>5.0), the α-PGA side chains are ionized, and the polypeptide unfolds from a compact α-helical conformation to an extended conformation as a result of the electrostatic repulsion between the side chains.[15,17,33,34] However, the addition of a salt to the solution suppresses the unfolding by screening the electrostatic interactions as ions interact with the charged glutamates.[19−23,31,33] The degree of counterion interaction depends on the specific binding properties of the counterions, which results in differential stabilization of the compact α-PGA conformation by different ions. CD measurements have shown that potassium chloride has a smaller stabilizing effect on the α-helical conformation of α-PGA in aqueous solutions and alcohol−water mixtures than sodium chloride over various pH and salt concentration ranges.[21,23,31] In these studies, the different effects of potassium and sodium ions were attributed to the smaller carboxylate ion binding affinity of potassium ions relative to sodium ions resulting from the larger size of the potassium ions and the correspondingly lower charge density on the ion surface. The results of recent theoretical and experimental studies of different ions binding to a single carboxylate[8,9,11,12] have also shown much weaker interactions of potassium ions than sodium ions with carboxylates. However, the experimental data do not give a consistent picture of the behavior of α-PGA under different solution conditions. It is difficult to estimate the right absolute value of α-helical content and also to compare different experiments, as different experimental techniques show different helical content for similar systems;[30,32] thus, computational analyses are needed to help interpret the results.

As the theoretical and experimental data do not show the details of how the conformation of the whole macromolecule is influenced by the local ion−carboxylate pairing effects, we performed long (200−300 ns) molecular dynamics simulations of α-PGA in 0.30 M KCl and NaCl solutions to reveal the

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10.1021/ja9030374 CCC: $40.75

molecular mechanism of the ion effects on the polypeptide conformation. Reference 30 shows that folding of a short α-PGA sequence requires milliseconds, which is far longer than is feasible for any explicit-solvent MD simulations at present. Therefore, we simulated α-PGA unfolding rather than folding, because this is a much faster process.[14,27] The 21 amino acid α-PGA oligopeptide as well as the ions were modeled using the OPLS-AA force field[24] (ion parameters are listed in the Supporting Information). The simulations used ambient conditions with fully ionized carboxylate groups. The simulation box contained 10 200 SPC/E water molecules.[25] We randomly substituted 112 water molecules by 56 alkali chloride pairs to create the 0.3 M alkali chloride solutions. The total charge of the simulation cell was neutralized by addition of 21 extra alkali cations into the simulation cell to balance the charges on the α-PGA side chains. We used the GROMACS 3.3 simulation package[26] and employed the same simulation methodology as in our previous study on sodium chloride effects on α-PGA,[14] in which we demonstrated that these molecular mechanics methods are capable of reproducing the subtle effects of salts on the oligopeptide conformations. A recent paper[35] has shown that the GROMOS force field is not effective at high concentrations, and Joung and Cheatham[36] have shown that the best choice of ion parameters is not straightforward. However, for the concentrations and conditions in our study and with the OPLS-AA force field, our earlier results[14] demonstrate that the approach is appropriate, and additional data on the hydration of sodium and potassium ions in water at this level of theory are available in the Supporting Information along with full computational details. The calculations show that sodium is hydrated more strongly than potassium; the experimentally measured[37] difference in the hydration energies of the K−Cl and Na−Cl ion pairs (20.5 kcal/mol) is ∼60% of the calculated value (35.0 kcal/mol).

We calculated the difference in binding energies of sodium and potassium ions to a *single* carboxylate as 2.3 kcal/mol.[29] This is in the range of experimental and ab initio values, which vary from ∼1.8 kcal/mol[12] to ∼2.5 kcal/mol.[11] We calculated[29] the average equilibrium contact distances for cation−carboxylate oxygen pairs as 2.3 Å (sodium) and 2.6 Å (potassium), which are the same as reported in ref 11.

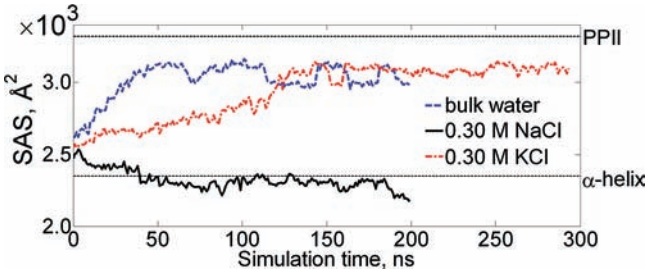

Figure 1. Solvent-accessible surface area (SAS) values for α-PGA in 0.30 M NaCl (black line), 0.30 KCl (red line), and bulk water (blue line) plotted against simulation time. The SAS values for an ideal α-helix (2.35×10^3 Å^2) and an ideal polyproline II (PPII) conformation (3.32×10^3 Å^2) of α-PGA are shown as horizontal dashed lines.

Figure 1 shows a plot of the changes in solvent-accessible surface area (SAS) of α-PGA with time for pure water, potassium chloride solution, and sodium chloride solution. The figure shows that changing the dissolved salt has a striking effect on the polypeptide conformation. Substitution of sodium ions by potassium ions leads to a complete unfolding of α-PGA, as also illustrated in Figure 2. The first 150 ns of the simulation show the system changing shape, but then it seems to settle down to a steady state. We note that 150 ns lies in the range of the experimental unfolding rate of 200 ns for a 21 amino acid peptide.[27] In contrast, the SAS values for α-PGA in NaCl solution are very close to that for an ideal α-helix during the whole simulation, and examination of the trajectories confirms that the α-PGA adopts this conformation (see Figure 2).

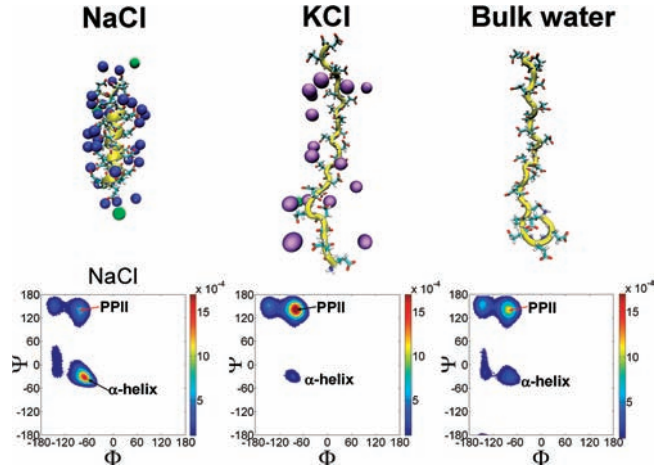

Figure 2. (bottom) Frequency distributions of Ramachandran angles between neighboring glutamates and (top) corresponding conformations of the α-PGA polypeptide dissolved in (left) 0.3 M NaCl and (middle) 0.3 M KCl. The data for pure water are shown at the right. Ions from an 8 Å shell around the macromolecule are shown as spheres. Sodium ions are shown as blue spheres, potassium ions as violet spheres, and chloride anions as green spheres. The conformations were taken from the end of the MD trajectories.

The distribution maps of the Ramachandran angles between each pair of adjacent amino acids are shown in Figure 2. The figure shows that in the potassium chloride solution the dominant Ramachandran angles correspond to extended β-sheet and PPII conformations, similar to those found in previous simulations[14] and experiments[17] for α-PGA in bulk water solutions. In the sodium chloride solution, the density distributions are significantly different: the most populated conformation of the α-PGA is the compact α-helix. The results are in line with the results of experimental studies of the effects of sodium chloride on the CD spectra of α-PGA, which show that an addition of 0.1−1.0 M sodium chloride significantly increases the helical content of ionized α-PGA.[21−23,31,33]

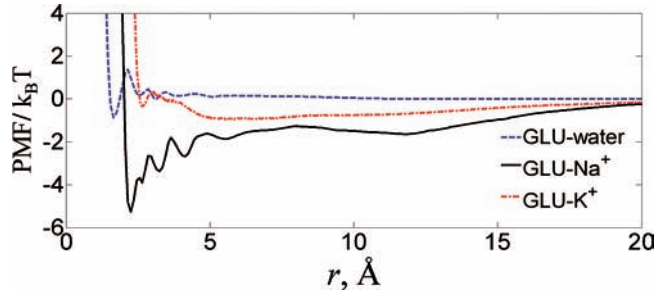

Figure 3. Glutamate−sodium ion (black) and glutamate−potassium ion (red) PMFs in comparison with the glutamate−water PMF (blue).

To understand the mechanism of ion binding to the side-chain glutamates, we calculated potentials of mean force (PMFs) between glutamates and ions using the same methodology as in our previous work on ion effects on the conformational properties of a trialanine peptide.[28,29] The results are presented in Figure 3, where we compare the glutamate−ion PMFs to the glutamate−water PMF. The positions of the first minima on the PMFs correspond to the equilibrium distances of direct contact of the species with carboxylic oxygens (see above). The figure shows that the potassium ions have lower binding affinity for glutamates than water, as the depth of the first minimum of the glutamate−potassium ion PMF is less than the one for the glutamate−water PMF. As a result, the potassium ions cannot effectively compete with water for the first solvation shell of the glutamates and prefer to form a cluster of ions in the second and third solvation shells at a distance of 5−10 Å from the polypeptide, averaging 2.5 times the concentration of the bulk solution; this corresponds to the second minimum on the glutamate−potassium ion PMF. In contrast, the sodium cations have much stronger affinity for glutamate groups than for water, so the depth of the first minimum on the glutamate−sodium ion PMF is $\sim 5k_BT$ greater than the one for the glutamate−water PMF. As a result, sodium ions form a compact, high-density cluster at a contact distance of 2.3−3.5 Å from the polypeptide; the sodium ion concentration in this cluster is ∼50 times greater than the sodium ion concentration in the rest of the solution.

The main conclusions of our study are as follows: (*i*) Because of their lower charge density, potassium ions have much weaker affinity for the anionic side chains of α-PGA than do sodium ions and thus cannot effectively compete with water for the first solvation shell of the glutamates. Potassium cations form a smeared-out, low-density cluster at distances corresponding to the second and third solvation shells of α-PGA. (*ii*) As a result of the weak ion-specific interactions with the polypeptide side chains, the addition of potassium ions to an α-PGA solution has no significant effect on the polypeptide conformation relative to that in bulk water, and as in bulk water, the oligopeptide unfolds into an extended rodlike conformation. The potassium ions may slow down this conformational transition. (*iii*) The net effect of ions on the oligopeptide conformation is determined by the strength of local interactions of the ions with the oligopeptide surface. Water plays a very important role in the mechanism of ion interactions with α-PGA because the strength of these interactions depends on the balance between peptide−water and peptide−ion interactions.

Acknowledgment. M.V.F. and J.M.G. thank Unilever for funding. We are grateful to R.S. Farr (Unilever R&D Vlaardingen), K. Collins (University of Maryland), and G. Chuev (MPI MIS Leipzig) for useful discussions. We thank V. Sergiievskiy (MPI MIS Leipzig) for helping us with computer graphics. We thank R. Kleinrensing and R. Kriemann (MPI MIS Leipzig) for their help in carrying out the supercomputer calculations.

Supporting Information Available: Simulation methodology, trajectory analysis, calculations of thermodynamic properties, and initial conformation of α-PGA. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Collins, K.; Washabaugh, M. *Q. Rev. Biophys.* **1985**, *18*, 323–422.
(2) Hofmeister, F. *Arch. Exp. Pathol. Pharmakol. (Leipzig)* **1888**, *24*, 247.
(3) Darnell, J.; Lodish, H.; Baltimore, D. *Molecular Cell Biology*; Scientific American Books: New York, 1990; p 1105.
(4) Collins, K. D. *Biophys. J.* **1997**, *72*, 65–76.
(5) Collins, K. D. *Biophys. Chem.* **2006**, *119*, 271–281.
(6) Collins, K. D.; Neilson, G. W.; Enderby, J. E. *Biophys. Chem.* **2007**, *128*, 95–104.
(7) Vrbka, L.; Jungwirth, P.; Bauduin, P.; Touraud, D.; Kunz, W. *J. Phys. Chem. B* **2006**, *110*, 7036–7043.
(8) Vrbka, L.; Vondrasek, J.; Jagoda-Cwiklik, B.; Vacha, R.; Jungwirth, P. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15440–15444.
(9) Jagoda-Cwiklik, B.; Vacha, R.; Lund, M.; Srebro, M.; Jungwirth, P. *J. Phys. Chem. B* **2007**, *111*, 14077–14079.
(10) Dzubiella, J. *J. Am. Chem. Soc.* **2008**, *130*, 14000–14007.
(11) Aziz, E. F.; Ottosson, N.; Eisebitt, S.; Eberhardt, W.; Jagoda-Cwiklik, B.; Vacha, R.; Jungwirth, P.; Winter, B. *J. Phys. Chem. B* **2008**, *112*, 12567–12570.
(12) Uejio, J. S.; Schwartz, C. P.; Duffin, A. M.; Drisdell, W. S.; Cohen, R. C.; Saykally, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6809–6812.
(13) Savelyev, A.; Papoian, G. A. *J. Phys. Chem. B* **2008**, *112*, 9135–9145.
(14) Fedorov, M. V.; Goodman, J. M.; Schumm, S. *Chem. Commun.* **2009**, 896–898.
(15) Wada, A. *Mol. Phys.* **1960**, *3*, 409–416.
(16) Song, S.; Asher, S. *J. Am. Chem. Soc.* **1989**, *111*, 4295–4305.
(17) Mikhonin, A. V.; Myshakina, N. S.; Bykov, S. V.; Asher, S. A. *J. Am. Chem. Soc.* **2005**, *127*, 7712–7720.
(18) Yamaoka, K. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3499–3500.
(19) Jacobson, A. *Biopolymers* **1964**, *2*, 237–244.
(20) Iizuka, E.; Yang, J. *Biochemistry* **1965**, *4*, 1249–1257.
(21) Satoh, M.; Fujii, Y.; Kato, F.; Komiyama, J. *Biopolymers* **1991**, *31*, 1–10.
(22) Satoh, M.; Komiyama, J.; Iijima, T. *Biophys. Chem.* **1981**, *14*, 347–355.
(23) Satoh, M.; Komiyama, J.; Iijima, T. *Biopolymers* **1982**, *21*, 1927–1931.
(24) Kaminski, G. A.; Friesner, R. A.; Tirado-Rives, J.; Jorgensen, W. L. *J. Phys. Chem. B* **2001**, *105*, 6474–6487.
(25) Nezbeda, I.; Slovak, J. *Mol. Phys.* **1997**, *90*, 353–372.
(26) Berendsen, H. J. C.; van der Spoel, D.; van Drunen, R. *Comput. Phys. Commun.* **1995**, *91*, 43–56. Lindahl, E.; Hess, B.; van der Spoel, D. *J. Mol. Model.* **2001**, *7*, 306–317.
(27) Mikhonin, A. V.; Asher, S. A.; Bykov, S. V.; Murza, A. *J. Phys. Chem. B* **2007**, *111*, 3280–3292.
(28) Fedorov, M. V.; Goodman, J. M.; Schumm, S. *Phys. Chem. Chem. Phys.* **2007**, *9*, 5423–5435. Fedorov, M. V.; Goodman, J. M.; Kolombet, V. V.; Schumm, S.; Socorro, I. M. *J. Mol. Liq.* **2009**, *147*, 117–123.
(29) See the Supporting Information.
(30) Kimura, T.; Takahashi, S.; Akiyama, S.; Uzawa, T.; Ishimori, K.; Morishima, I. *J. Am. Chem. Soc.* **2002**, *124*, 11596–11597.
(31) Tiffany, M.; Krimm, S. *Biopolymers* **1969**, *8*, 347–359.
(32) Holtzer, A.; Hawkins, R. B. *J. Am. Chem. Soc.* **1996**, *118*, 4220–4221.
(33) Daoust, H.; Daniel, S.-C. *Biopolymers* **1988**, 1267–1281.
(34) Bordi, F.; Cametti, C.; Paradossi, G. *J. Phys. Chem.* **1992**, *96*, 913–918.
(35) Hass, B.; Holm, C.; van der Vegt, N. *J. Chem. Phys.* **2006**, *124*, 164509.
(36) Joung, I. S.; Cheatham, T. E. *J. Phys. Chem. B* **2008**, *112*, 9020–9041.
(37) Pliego, J. R., Jr.; Riveros, J. M. *Phys. Chem. Chem. Phys.* **2002**, *4*, 1622–1627.

JA9030374