Competition for water between protein (from *Haloferax mediterranei*) and cations Na$^+$ and K$^+$: A quantum approach to problem.

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Abstract The competition between Na$^+$ and K$^+$ with a protein for water was investigated by using Density Functional Theory (DFT) calculations. The optimized potential energy curves have been made in the DFT, together with balanced basis sets of split valence Def2-SV(P). Initially, calculations were performed in order to know the organization of the hydration shell of the sodium and potassium ions, when up to sixteen molecules of water are added. The results indicate the structure and stability of these cations with water clusters. Then, this knowledge was used for the analysis of the hydrated protein when potassium or sodium cations approach to them, showing that cation has a dehydration process more favourable energetically, and indicating for which cation, potassium or sodium, is the competition with the protein for water more favorable.

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1 Introduction

In the study of proteins, one of most important parameters is the stability of the protein in the medium where it has to function. An understanding of the phenomena that play a role in protein stability is essential whether for proteins in vitro or in vivo. In industry, it is very important to obtain a much longer life time for the proteins that are being used. But in vivo, it is essential for understanding the roles that proteins play in different phenomena like cell signaling, catalytic processes, etc., or in many physiological disorders, such as Alzheimer's disease or cataracts for example[1,2].

Interactions between ions, water and proteins in aqueous solutions continue to garner attention due to its importance in understanding numerous chemical and biological processes.

A large number of theoretical calculations and experiments have been performed to investigate hydrated alkali-metal cluster ions from a variety of perspectives (see ref [3] and their references). How these ions move from the aqueous phase into biological systems, such as at the entrance of an ion channel, depends on the interplay between competing intermolecular forces, which first must involve ion–water and water–water interactions, and the cation–protein binding energy[4,5].

The protein–solvent interactions have demonstrated that the stability of halophilic enzymes depends on the formation of a hydration shell that is a consequence both of the number of negative charges in the surface and how important is the reduction of the hydrophobic surface as a result mainly from a loss of surface lysine[6]. The extent of the hydration shell is strongly dependent on the salt type. For example, in malate dehydrogenase from *Haloarcula*

marismortui [7], it has been demonstrated that anions and cations have a different effect on the stability of the protein, whereas anions do not determine the composition of the hydration shell cations or affect its formation[8].

The formation of the hydration shell and the influence of the cations of the media are not limited to halophilic protein. The effects of salting-in or -out of different ions on protein is a macroscopic example of the competing interactions between the dissolved ions, the water, and the protein. It supposes the extent of the influence of the ions on the microscopic structure of water. The hydrated cations can alter the water structure around the particles, giving rise to the formation of a hydration barrier that prevents particle aggregation[9]. The ion hydration shell is highly specific to individual ions. Cations such as sodium and potassium have completely different hydration shells [10]. This fact implies a different influence on the protein hydration shell.

There have been different theoretical studies of the interaction of the cations Na^+ and K^+ with water. Some are theoretical and were made by molecular dynamics and *ab initio* calculations, and others are experimental [3,10–19]. These papers have reported that the hydration shell for sodium has fewer water molecules than that for potassium, (the hydration number for sodium is 5 or 6 water molecules, with that for potassium having a probability distribution ranging from 5 to 10), but that the energy of the ion–water interactions are stronger with sodium. A more recient *ab initio* molecular dynamic calculation (QM/MM) [20] estimate a range of 5.7–5.8 water molecules for Na^+ and 6.9–7.0 for K^+.

In this paper we have tried to shed some light on the protein–solvent–cation interaction and also contribute to a deeper understanding of what really matters in the solvation of positive ions, something which is crucial for understanding more complex systems of biological interest. To this end, we apply directly the DFT method to find the most stable geometric conformation and the comparison of the relative stability of a fragment of a protein of

Haloferax mediterranei, with their water hydration and one monovalente cation (Na^+ or K^+) near the protein surface.

From the study of this interaction, it is possible to infer some of the reasons that could be used to explain certain aspects that clarify why the intracellular cation is potassium, when potassium has an ionic radii greater than that of the sodium cation and therefore, needs more space in the cell.

The Method section shows the model, the procedure, and the programs used. The next sections contains the results and discussion, Some conclusions are drawn in the last sections.

2 Method

This paper treats a fragment of a protein that interacts on its surface with a potassium cation. The protein used for the study was the glucose dehydrogenase from of *Haloferax mediterranei*[6] (ID protein in RSC Protein Data Bank: 2B5W), because in their crystal structure there are several potassium ions interacting with the surface of the protein. More specifically, the K^+ referred to in the pdb file as 904 (HETATM 2808) and the surface of the protein neighboring to it has been used. We consider only this protein fragment because the focus of our study is on the hydration shell of a cation when it is near a protein, and their reciprocal influence.

In a preliminary study was performed a theoretical calculation in order to know the organization of the hydration shell of the sodium and potassium ions when several molecules of water are added, from four to sixteen molecules. The geometry of these systems has been full optimized using the B3LYP[21,22] and CAM-B3LYP[23] hybrid density functionals and several basis sets (Def2-SV(P)[24] and 6-31+G*[25,26]).

From this preliminary study on the interaction of Na$^+$ and K$^+$ with water molecules, there has been determined the distance around the cation including water molecules (up to 10 Å) and that a similar distance was used to choose the protein fragment to study, so, the residues that have some atom at a distance less than 10 Å from cation K(904) have been considered. As a result, the protein fragment includes the following amino acid residues: Tyr 203, Thr 231, Glu 234, Asp 235, Val 236, Pro 237, Asp 238, Val 239, Tyr 240 , Glu 241, Gln 242, Met 243, Ala 262. This radius of 10 Å involves the number of water molecules considered (69). The system to study is formed by this set of amino acid residues, the 69 water molecules and the cation (sodium or potassium). It was considered as a closed shell, so a negative charge has been set, justified by the Aspartic residue presence, which is distributed among the atoms belonging to the protein fragment.

In our model, the potassium cation is approximately 2.8 Å from the mass center, which lies virtually on the surface of the protein fragment used. A scan of the optimized potential energy curve versus the cation position (distance to mass center), between 2.3 Å to 5.8 Å, has been carried out.

In all calculations, the coordinates of the atoms belonging to the protein fragment were kept fixed except for hydrogen, since the protein structure corresponds to a crystal structure which is one of the possible stable conformations for a protein. So, for each coordinates of the protein fragment and the cation, there has been optimized the geometry of the water molecules and the hydrogen belonging to the protein fragment. This optimization has been made using the B3LYP/Def2-SV(P) procedure, which at the DFT level gives accurate results, and its size allows making the calculations to be performed. The criterion for considering the geometry to be optimized was when the maximum force is less than 0.001 hartrees/bohr.

All calculations were made for both the potassium and the sodium cations.

Finally, with the aim to analyze the competition of the cations and of the protein for the water, it has been considered a simplified system, which include only the water molecules within a distance of 4 Å from the cation (See Table 1), as the solvation shell, together the protein fragment and the cation. With this system single point energy calculations have been carried out to obtain the interaction energies between their different components. These calculations have been made using the optimized geometries obtained previously, and with the B3LYP/6-31+G* method (the reduction of number of atoms in these single point energy calculations, allow the use of a more large basis set). We take all systems as closed shells, conserving the total charge, which implies that the protein fragment is considered with two negative charges and the cation with one positive.

In these calculations, it always the energy calculations of the diverse components has been carried out with the basis set of all atoms of the full system (protein fragment plus cation plus solvation shell), to avoid the basis set superposition error.

The software packages used are Amber14[27] and Avogadro[28] to perform the selection of amino acid residues and the inclusion of the water molecules in a semi-sphere of radius 10 Å, and Gaussian09-D.01[29] for the DFT calculations. The results and the data were visualized with the Molden and Xmgrace programs.

3 Results

A preliminary calculation was performed in order to know the organization of the hydration shell of the sodium and potassium ions when several molecules of water are added, from four to sixteen molecules. The geometry of these systems has been full optimized using the B3LYP and CAM-B3LYP hybrid density functionals and several basis sets.

To calculate the stabilization energy between the cation and n molecules of water ($E_S^{(n)}$), we used the following equation:

$$E_S^{(n)} = E_{(C^+ - (H_2O)_n)} - \left[n \cdot E_{(H_2O)} + E_{(C^+)} \right] \tag{1}$$

Here, $E_{(C^+ - (H_2O)_n)}$ is the energy of the system cation with n water molecules, $E_{(H_2O)}$ is the isolated water molecule energy, and $E_{(C^+)}$ is the cation energy.

The Fig 1 shows that the relative stability of the sodium and potassium clusters is not dependent on the basis quality and that the interactions of the water molecules with the cations are stronger with Na^+ than with K^+. The distances between the oxygen atoms and the cation, the averaged distance ($\langle d_{c-w} \rangle$) and the stabilization energy ($E_S^{(n)}$) for clusters of from 4 to 16 molecules of water, using the B3LYP/Def2-SV(P) method, are shown in Table 1. It can be seen that the solvation shell of Na^+ and K^+ are very different (See Table 1 and Fig. 2). In the case of Na^+, the first solvation shell appear with up to 6 water molecules(WM) (5 for 13 to 16 WM) at distances less than 2.5 Å, however, the first solvation shell of K^+ is composed of 7 WM at distances close to 3 Å. Then, we have a second layer of molecules of water at similar distances to both cations. These values are coincident with the results obtained in the dynamic QM/MM calculations by Rowley and Roux[20]. In light of these numbers, a water column over the protein fragment of height 10 Å was selected. This is consistent, as long as the cation is not displaced at distances from the surface greater than 6 Å.

With these considerations, the geometry optimization was performed with the procedure previously indicated, for the system composed of the protein fragment, 69 water molecules, and the cation (sodium or potassium), at several distances from the surface (d_{c-p}). The results are plotted in Fig. 3. In this figure, with the aim of showing the two potential energy curves in the same graph, different amounts of energy have been taken in both the Na^+ and

K^+ representations, but have remained at the same relative order of energy. These potential energy curves show the optimum distance for the interaction of the hydrated cation with the surface of the protein fragment. Both cations show the calculated optimum distance close to the distance where the potassium is located in the crystal structure ($\approx 2.8\ Å$). In addition it can seen that the stabilization energy when the cation is potassium is considerably greater than in the case of Na^+.

As we have already mentioned, to analyze the effect of the solvation shell, there have been made specific single point energy calculations considering only the molecules of water up to a distance of 4 Å from the cation. The expressions for the interaction energies are:

The full system interaction energy ($E^{int}_{(P^=-S-C^+)}$)

$$E^{int}_{(P^=-S-C^+)} = E_{(P^=-S-C^+)} - \left[E_{(P^=)} + E_S + E_{(C^+)}\right] \tag{2}$$

where $P^=$, S and C^+ are the protein fragment with two negative charges, the solvation shell and the cation (K^+ or Na^+) respectively.

The interaction energy between the solvation shell and the cation ($E^{int}_{(S-C^+)}$) and the protein fragment and the solvation shell ($E^{int}_{(P^=-S)}$) one.

$$E^{int}_{(P^=-S)} = E_{(P^=-S)} - \left[E_{(P^=)} + E_S\right] \tag{3}$$

$$E^{int}_{(S-C^+)} = E_{(S-C^+)} - \left[E_S + E_{(C^+)}\right] \tag{4}$$

The results have been plotted in Fig. 4. In these plots, the solvation layer is considered as a whole, which depends on the distance of the cation to the protein fragment.

4 Discussion

The distances showed in Table 1 are coincident with the experimental results obtained using neutron diffraction[11]. These authors found that the average distance of the first solvation shell is slightly different for these cations, being 2.50 ± 0.10 Å and 2.70 ± 0.10 Å for sodium and potassium, respectively. However, unlike the cited studies, our results show significant differences between the behaviour of sodium and potassium. Although, a priory, one would think that distance differences between sodium and potassium was put down to atomic radius of the cation, the results show that the difference is related to the number of water molecules and its distribution around the cation. This fact supports the work of Mancinelli et al.[14] who proposed that the water molecules in the K^+ hydration shell are orientationally more disorganized and tend to bring their dipole moments more tangential to the Na^+ hydration shell. These features are shown in Fig. 2, showing the geometrical conformation of the cations hydrated with 16 water molecules. Another difference is the interaction energy between the water molecules (solvation shell) and the cation, as can be seen in Table 1 and Fig. 1. These behavior differences of the two cations remain in the presence of protein and even some of them are amplified, which leads to the different potential energy curves shown in Fig. 3.

The Fig. 4 shows the interaction energy between the solvation shell, the protein fragment, and the cation, as has been indicated in Eqs. 2–4. Taking into account the interaction between the solvation shell and the protein fragment, ($E^{int}_{(P=-S)}$ in Fig. 4 a)), distances lower than 3.3 Å increases the stability when there are K^+, instead of Na^+. At these distances, the solvated protein will more stable when the K^+ is present in place of Na^+, however, greater distances than 3.3 Å result in similar energy for the two cations analyzed.

Regarding the interaction energy of the solvation shell with the Na$^+$ cation ($E^{int}_{(S-Na^+)}$), this is more stable that the interaction energy of solvation shell with K$^+$ ($E^{int}_{(S-K^+)}$). But this interaction energy with sodium reaches an equilibrium with 8–10 molecules of water in the solvation shell and to 2.8 to 3.8 Å distance from the protein. Related to potassium, this equilibrium is reached with a content of 11–14 water molecules in the solvation shell and at distances from 3.8 to 4.8 Å from the protein surface. As a consequence, when the potassium cation is at 3.8 Å from the protein, it is more energetically favorable to lose water (dehydration) than for the sodium cation, which is located in the area of equilibrium around at a distance of 2.8 Å. These data indicate that the competition for water between the protein and the cation is more favorable in the case of potassium than with sodium. In other words, it could be suggested that it is to easier to compete for the water of hydration with the potassium cation than with the sodium cation for the protein. This fact should be taken into account, since the soluble proteins must maintain their solubility, in order to be functional. That is, they must compete efficiently with ions and other substances for the water present in the medium.

Fig. 4 b), shows the contribution to the total energy of the interactions between the components of the system (protein fragment, solvation shell and cation). Over long distances the dominant interaction is the cation-solvation shell interaction. When the cation is close to the protein fragment, 4.8 Å and 4.3 Å for K$^+$ and Na$^+$ respectively, the effect of interaction with the protein have two different behaviors. In the case of the K$^+$ there is a decrease of energy up to the value obtained at long distances, but in Na$^+$ case, the decrease in energy is 3-fold less than in K$^+$, without reaching to the values that is had at long distances. So, when the cation is close to the fragment occurs a reorganization of the water molecules in the solvation shell, increasing the energy. However, in the case of Na$^+$, the effect of stabilization very low since the variation of energy, from 4.3 Å to 2.8 Å, is very small. This

means that between 4.3–2.8 Å, the effect of proximity to the protein fragment is not reflected in the reorganization of the interactions, while in the presence of K^+, a significant change is observed. Then, this figure shows that the approach to the fragment is more favorable for the K^+ that for Na^+, resulting more easy dehydration of K^+ cation.

At last, the variation of the distances between the water molecules and the cation, versus the cation distance to protein fragment, for potassium and sodium, are represented in Figs. 5 and 6, respectively. They show the relative positions of the water molecules with respect to cation when this cation get away from protein fragment.

It can be observed that the protein interferes in the number of water molecules, when the distance between the cation and the surface is less than 3.3 Å, the water molecules are moved away from the cation positions. In the analysis of potassium, it is observed that as the cation is farther from the surface, there appear more water molecules next to the cation, since at 4.8 Åits two solvation layers are practically complete. The sodium cation does not completely restore its first solvation shell until 5.8 Å, where the last molecule of water is introduced.

This study of the displacement of the water in its approach to the cation, versus their distance from the protein surface, also could give the key to the difference in the solvation shells of the two cations, potassium and sodium. For the potassium the distances between the waters of the first layer and a second layer of solvation shell are less than 0.5 Å while in the sodium this distance is still higher than that value ($\approx 0.9\ \text{Å}$). So, in the first case, the solvation shell can be see as a single layer, or at least as a continuum, while in the second one, the two layers more defined can be observed (Fig. 2).

On the other hand, the data of this analysis (Figs. 5, 6) clearly confirm that the interaction between the protein and the solvation shell of the cation occurs relatively near the protein fragment, (3.3 Å and 4 Å for potassium and sodium, respectively). This allows us to think

that the protein found water to establish its solvation shell at a distance of 3.8 Å, where the competition for water is more efficient with the potassium ion, which might be of great importance in the prevalence of this cation over sodium in a medium such as intracellular media.

These facts can not only explain the prevalence of potassium as an intracellular cation, but also give another reason for the selectivity of the potassium ion channels[5, 13], since the energy to compensate for the dehydration of the potassium cation is less than for sodium, independently of the involvement of other processes to compensate for the dehydration, such as polarization[30].

5 Conclusions

The interaction energy calculated between the protein fragment and the solvated cations potassium and sodium show a larger stabilization for the potassium process than for the sodium. This is related to the fact that their solvation shells are very different when they approach the protein, because the Na^+ solvation shell is distorted earlier than in case of K^+.

With respect to the protein's competition for water, our results suggest that it is easier to compete with the potassium cation than with the sodium, which might be of great importance in the prevalence of this cation over sodium in a medium such as intracellular media.

These results have been obtained using a fragment from a halophilic protein as model, but it shows the typical features of any protein fragment which interacts with a cation. Consequently, these results could be extrapolated to other proteins.

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Table 1 Distances between the oxygen atoms and the cation and the averaged distance ($\langle d_{c-w} \rangle$), in Å, and the stabilization energy (E_S) in kcal/mol, for the clusters of 4 to 16 molecules of water. B3LYP/def2-SV(P) calculations.

| Na^+:	4	5	6	7	8	9	10	11	12	13	14	15	16
	2.252	2.237	2.405	2.229	2.240	2.242	2.243	2.296	2.342	2.343	2.320	2.333	2.312
	2.252	2.278	2.405	2.423	2.371	2.401	2.422	2.406	2.347	2.355	2.324	2.336	2.327
	2.252	2.381	2.406	2.432	2.437	2.402	2.437	2.418	2.350	2.360	2.360	2.379	2.400
	2.252	2.415	2.407	2.470	2.447	2.442	2.464	2.419	2.355	2.381	2.424	2.420	2.432
		2.463	2.408	2.501	2.498	2.513	2.497	2.423	2.470	2.534	2.431	2.463	2.506
			2.408	2.575	2.733	2.542	2.729	2.516	2.625	2.995	3.017	2.863	2.890
				3.374	3.368	3.156	3.337	3.163	3.040	3.319	3.265	3.287	3.327
					3.448	3.208	3.398	3.234	3.059	3.623	3.511	3.555	3.381
						3.266	3.676	3.420	3.511	3.632	3.557	3.559	3.489
							3.841	3.496	3.596	3.656	3.585	3.705	3.583
								3.648	3.750	3.691	3.621	3.713	3.675
									4.083	3.784	3.696	3.938	3.892
										3.819	3.786	3.963	4.017
											4.055	3.997	4.204
												4.134	4.282
													4.283
$\langle d_{c-w} \rangle$	2.25	2.36	2.41	2.57	2.69	2.69	2.91	2.88	2.94	3.11	3.14	3.24	3.31
E_S	-110.9	-129.2	-149.4	-173.7	-197.3	-214.0	-240.4	-256.8	-274.8	-300.9	-318.9	-339.9	-361.2
K^+:	4	5	6	7	8	9	10	11	12	13	14	15	16
	2.633	2.574	2.599	2.568	2.731	2.761	2.760	2.783	2.724	2.846	2.799	2.678	2.660
	2.786	2.649	2.648	2.760	2.800	2.774	2.770	2.790	2.780	2.849	2.803	2.820	2.756
	2.787	2.823	2.894	2.832	2.833	2.776	2.788	2.855	2.853	2.851	2.837	2.844	2.811
	2.788	2.862	2.914	2.836	2.871	2.823	2.826	2.862	2.861	2.857	2.853	2.887	2.956
		3.709	2.936	3.607	2.876	2.824	2.837	2.863	2.886	2.888	2.882	2.896	2.990
			3.640	3.663	3.601	3.552	3.492	2.886	2.887	2.940	2.937	2.976	3.023
				3.810	3.665	3.567	3.697	3.477	3.378	2.975	2.942	3.035	3.097
					3.964	3.933	4.065	3.945	3.926	3.286	3.292	3.321	3.532
						4.658	4.067	3.946	3.977	3.356	3.463	3.410	3.562
							4.545	4.131	4.086	3.708	3.538	3.661	3.657
								4.184	4.267	3.802	3.560	3.677	3.791
									4.623	3.895	3.635	3.697	3.842
										4.053	3.777	3.920	3.972
											3.802	4.036	4.264
												4.341	4.341
													4.573
$\langle d_{c-w} \rangle$	2.75	2.92	2.94	3.15	3.17	3.30	3.39	3.34	3.44	3.26	3.22	3.30	3.49
E_S	-88.2	-108.1	-131.7	-155.3	-173.9	-186.1	-215.4	-230.4	-255.5	-271.8	-300.9	-316.3	-331.9
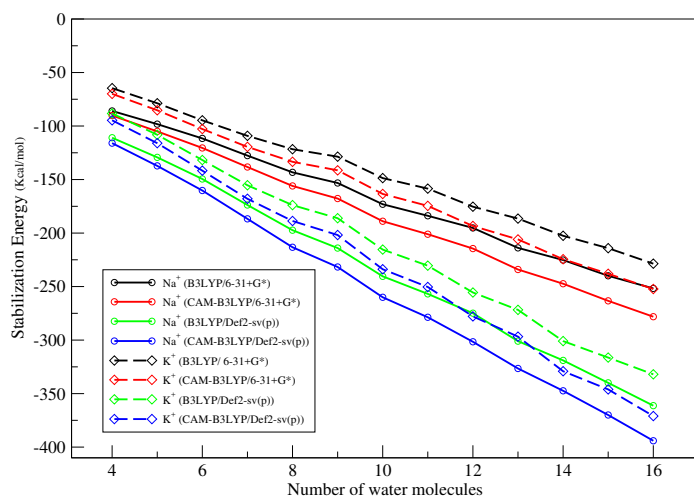
Fig. 1 Stabilization energy of K$^+$ and Na$^+$ solvated with *n* molecules of water, using several methods and basis sets. In kcal/mol.
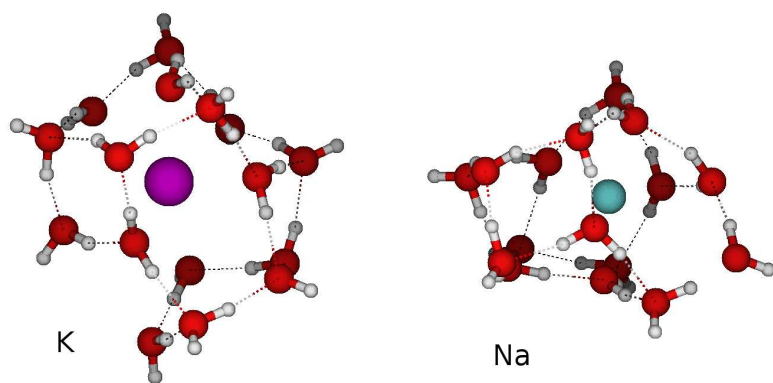

Fig. 2 Graphical representation of K$^+$ and Na$^+$ with 16 molecules of water. Optimized geometries using B3LYP/def2-SV(P) method.
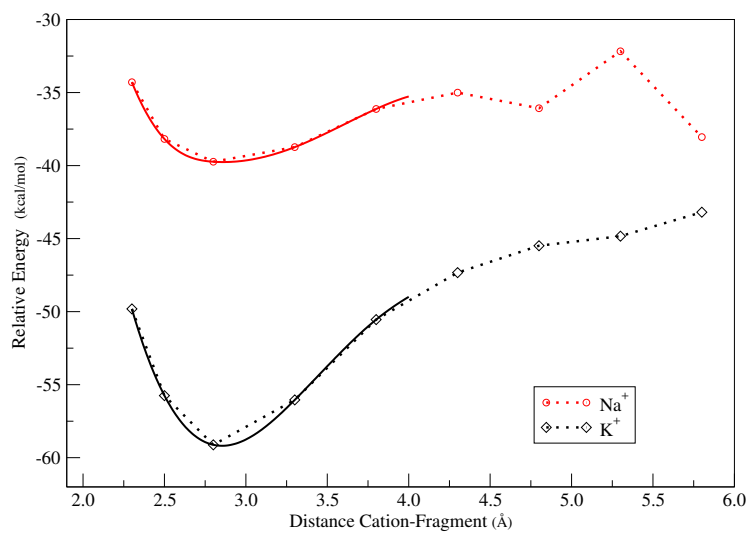
Fig. 3 Relative potential energy curves of protein fragment, cation (K^+ or Na^+), and 69 water molecules, versus the distance of the cation from the surface of the protein (d_{c-p}). B3LYP/def2-SV(P) method. Distance in Å, energy in kcal/mol. The continuous lines are the interpolated energies.
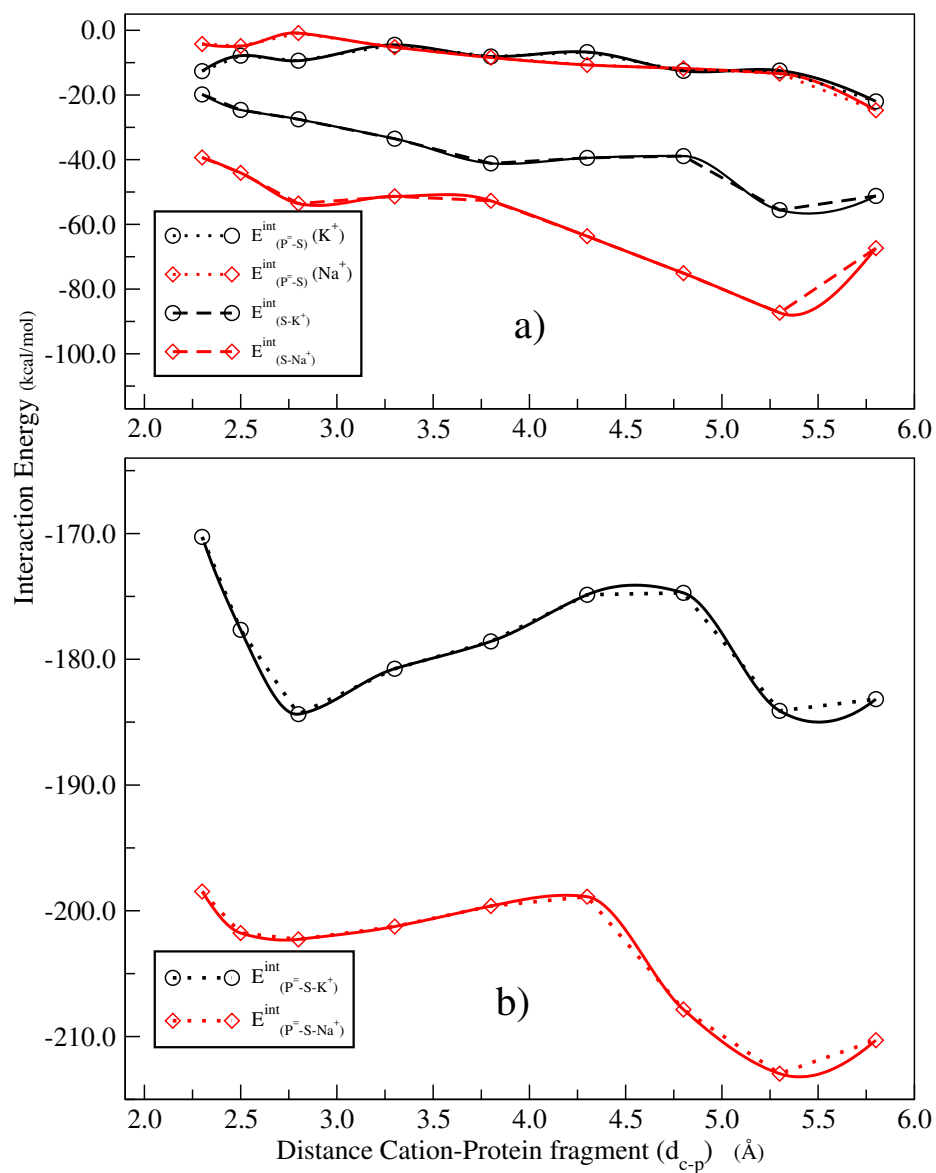
Fig. 4 Interaction energies of protein fragment–solvation shell–cation versus the distance of the cation from the surface of the protein fragment (d_{c-p}) (see the text). In kcal/mol. The continuous lines are the interpolated energies.
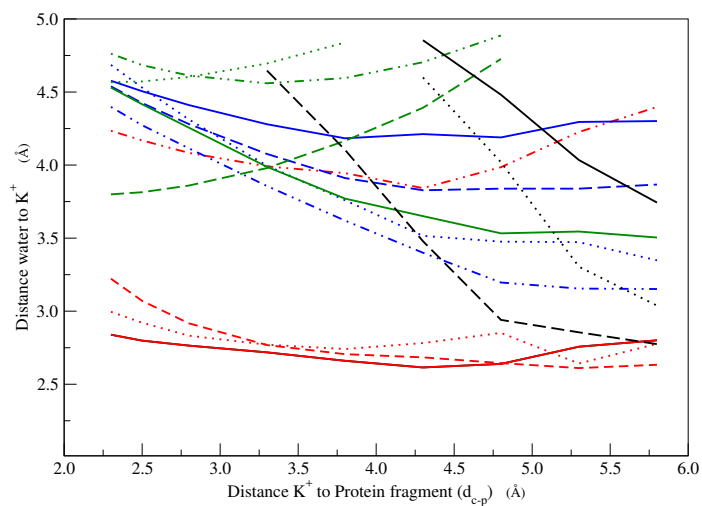
Fig. 5 Distances between the water molecules in the sphere of radius 5 Å around the cation, and the cation, versus the cation–protein fragment distances. Results for K^+ cation.
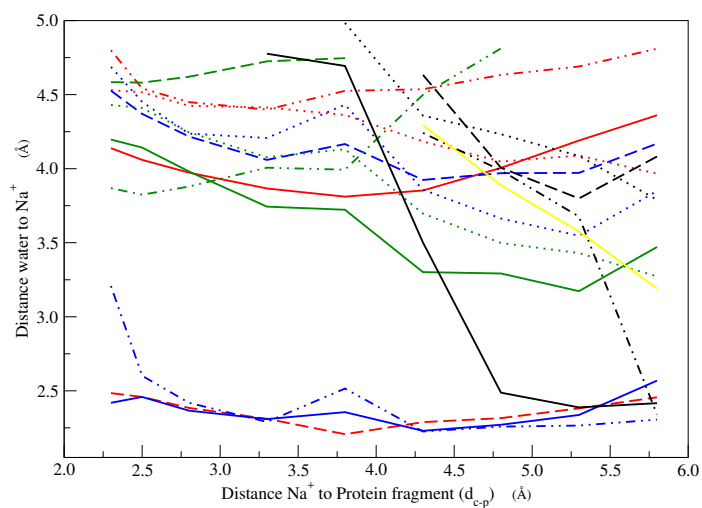

Fig. 6 Distances between the water molecules in the sphere of radius 5 Å around the cation, and the cation, versus the cation–protein fragment distances. Results for Na^+ cation.