Ion pairing as a possible clue for discriminating between sodium and potassium in biological and other complex environments

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Sodium and potassium possess the same charge and differ only slightly in size. Nevertheless, many biological processes depend on discrimination between these two ions. K^+ to Na^+ concentration ratio of roughly 10 in the cytosol, maintained by energetically costly ion pumps, is vital for proper functioning of cells.[1] Sodium is generally more efficient than potassium in initiating processes involving proteins, such as salting-out or polymerization.[2-4] While traditionally ion-specific effects are rationalized in terms of water structuring around ions (kosmotropes vs. chaotropes)[5] modern experiments and computer simulations indicate that direct ion-protein interactions may be more important.[6] In our recent study, we have shown that sodium exhibits a stronger affinity than potassium to surfaces of proteins, the discriminating action coming primarily from the negatively charged carboxylate groups in the side chains of aspartate and glutamate. Note that this sodium preference is in accord with the empirically derived "Law of matching water affinities" stating that small cations preferentially pair with small anions or anionic groups (such as $-COO^-$) and *vice versa*.[7]

If the selective association of sodium vs potassium with large (bio)molecules is indeed primarily due to local interactions with charged and polar groups[6] then a lot can be learned by investigating ion pairing with simple anionic solutes in water. Such a study can thus provide a clue to ionic specificity between Na^+ and K^+ and to the ordering of different counter-anions into the Hofmeister series.[2] To this end, we performed an extended computational study of pairing of sodium and potassium with a broad set of biologically relevant anions. Similarly as in the related case of ion hydration, accurate calculations of ion pairing in water are difficult and results are very sensitive to the interaction potential and other parameters of the simulations.[8,9] In order to minimize the effect of such possible inaccuracies we focused at evaluating the difference between the strength of ion pairing involving Na^+ vs K^+. This computed difference is much more reliable than the absolute values for the two ions thanks to a favorable cancellation of errors. Within the calculations we combined two different approaches: classical molecular dynamics (MD) simulations of aqueous salt solutions and *ab initio* calculations employing a polarizable continuum model for water. The former approach includes explicit solvent molecules interacting, however, with each other and with ions via a simple empirical potential, while the latter mathod very accurately describes the cation-anion interactions at the price of a continuum treatment of the solvent.

MD calculations were performed using a non-polarizable forcefield.[10-13] Each of the systems contained roughly 800 water molecules with a single cation and anion (in few cases up to three ion pairs). After sufficient equilibration, several ns of production runs were performed at 300 K and 1 atm employing a 2 fs time step. All ab initio calculations[14] were performed at the MP2/aug-cc-pVTZ level of theory. Pseudopotentials were used for Br and I, and additional core-valence basis functions were employed for F, C, O, S, Cl, P, and N (cc-pCVTZ basis).[15] For each of the ion pairs the geometry was obtained from a gas phase *ab initio* optimization except for the anion-cation distance a realistic aqueous value of which was obtained as the most probable one from MD simulations (*vide infra*). Water was described as a polarizable continuum solvent using the COSMO model.[16]

From MD simulations we extracted sodium-anion and potassium-anion radial distribution functions, all of which exhibited a pronounced short distance peak corresponding to a contact ion pair, as well as a secondary peak at a longer distance representing a solvent-separated ion pair. By integrating these distribution functions second virial coefficients were obtained for cation-anion interactions in water.[17] These coefficients allow for ordering of the investigated anions in decreasing relative preference for Na^+ (and, consequently, increasing relative preference for K^+) as: $HCOO^- > OH^- > HSO_4^- > F^- > Cl^- > Br^- > H_2PO_4^- > NO_3^- > SCN^- > CH_3SO_3^- > ClO_4^- > I^-$. It is worth noting that practically the same ordering is also obtained using the relative heights of the peaks at the radial distribution functions corresponding to contact ion pairs. The anions can be divided in three groups: i) those binding preferentially with sodium (formate to fluoride) ii) anions which have roughly the same affinity to the two alkali cations (chloride to nitrate), and iii) anions which bind more strongly to K^+ than Na^+ (thiocyanate to iodide). The ordering of the anions follows well the Hofmeister series[2,18] with smaller anions preferring sodium and larger ones potassium, in accord with several empirical observations for these ions.[7]

For direct interactions of alkali cations with charged groups at the surfaces of proteins and other macromolecules local binding, i.e., the strength of the contact ion pair plays a prominent role.[6] To this end, we used *ab initio* calculations with a polarizable continuum solvent model to quantify for each anion the relative cation-anion association free energy, i.e., the value of $\Delta\Delta G$ connected with replacing sodium by potassium in the contact ion pair. In order to employ realistic aqueous phase geometries the cation-anion distance of the contact ion pair corresponded to the first peak of the radial distribution function from the above MD simulations. The difference $\Delta\Delta G$ between association free energies for contact ion pairs with K^+ vs Na^+ is presented for each of the studied anion in Figure 1. Since the two investigated cations do not differ much in size, this $\Delta\Delta G$ value semi-
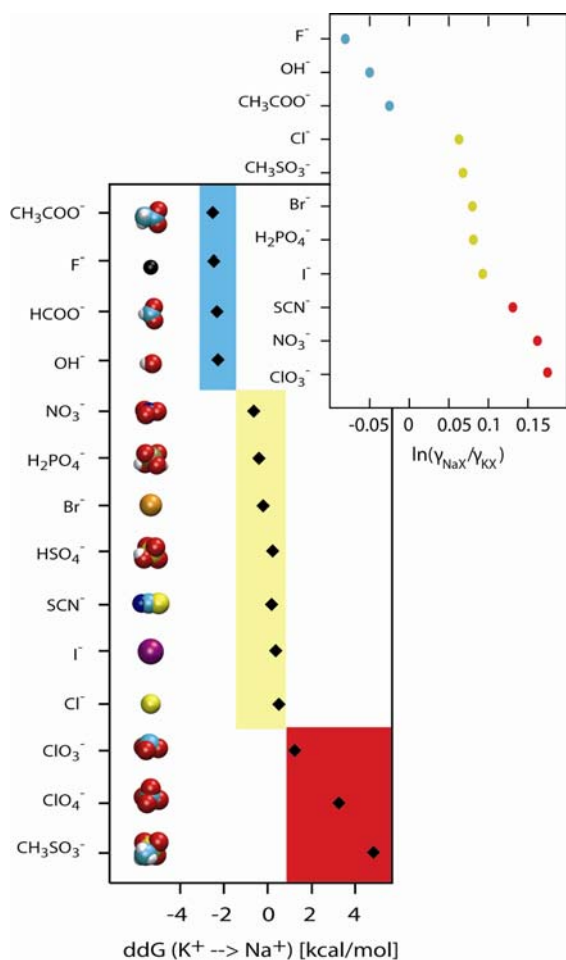
Figure 1: Free energy change upon replacing potassium with sodium in a contact ion pair with a series of anions. Inset: The corresponding differences in excess chemical potentials obtained experimentally for 0.7 M solutions.[17-19] Blue, red, or yellow color indicates anions preferring sodium, potassium, or having little preference between the two cations.

quantitatively represents the relative binding strength to a given anion, despite the implicit disregard of the slightly different positions and widths of the first peaks of the radial distribution functions for sodium and potassium. As within the analysis of second virial coefficients, the anions again fall into three groups (anions preferring sodium, ambivalent, and preferring potassium). The ordering correlates well with that obtained from MD simulations, except maybe for chloride which should probably be above bromide; nevertheless, the energy differences involved within the group of ambivalent anions are very small. Focusing in Figure 1 on contact ion pairs underscores the difference between the two cations, which exemplifies the fact that ion specificity is strongest at short interionic separations.

Figure 1 also presents the ion-pairing preferences for sodium vs potassium extracted from excess chemical potential differences, as obtained from experimental activity coefficients.[19-21] Note that these coefficients are concentration dependent and, therefore, can be only qualitatively compared with the calculations. Nevertheless, the correlation between experiments and simulations in terms of anionic preference for Na$^+$ vs K$^+$ (or *vice versa*) is very good.

The main message from the present calculations is that the relative ion pairing preference of sodium vs potassium is strongly anion-specific. Small hard anions prefer sodium over potassium,

while the situation reverses for large soft anions. There is also a set of intermediate anions which are ambivalent, exhibiting virtually no preference between Na$^+$ and K$^+$.

Among sodium-preferring anions probably the most biologically relevant are small carboxylate ions, which mimic the negatively charged amino acid side chains of glutamate and aspartate. Indeed, such a local ion pairing with the –COO$^-$ groups, as well as with oxygens in the backbone amide groups, has been recently demonstrated to be the dominant effect responsible for sodium vs potassium ion specificity at protein surfaces.[22] In contrast, the opposite anionic preference (i.e., for K$^+$ over Na$^+$) has been observed in simulations of inverse micelles with sulfonate head groups,[23] which is in perfect agreement with the present computational results for methylsulfonate. Finally, our calculations show that monovalent phosphate, which is a building block of the DNA backbone, belongs to anions which exhibit virtually no Na$^+$ vs K$^+$ selectivity (possibly with a slight preference for sodium). This rationalizes weak ion specific effect of sodium and potassium at the surface of hydrated DNA.[24] Consequently, due to the lack of appreciable specificity in ion pairing, other effects such as the overall structure of the DNA and pairing with chloride counterions[25,26] can contribute more significantly to discrimination between sodium and potassium.

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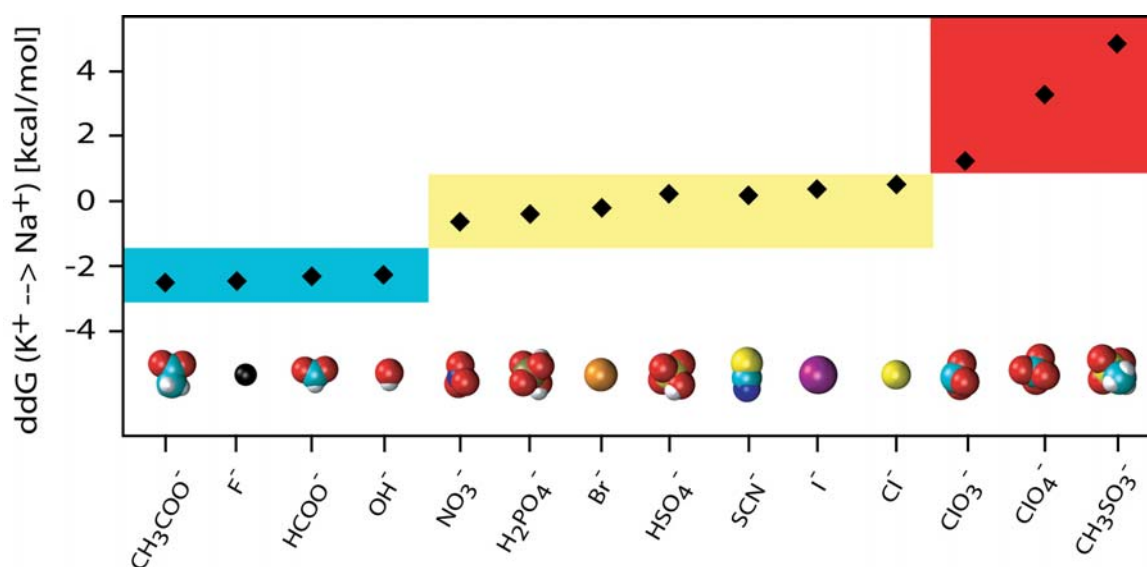
ABSTRACT FOR WEB PUBLICATION (Word Style "BD_Abstract"). Authors are required to submit a concise, self-contained, one-paragraph abstract for Web publication.

For a series of biologically relevant anions we present free energy change upon replacing potassium with sodium in a contact ion pair. Calculations performed using a combination of molecular dynamics simulations and ab initio methods demonstrate the ordering of anions in a Hofmeister series. Small anionic groups such as carboxylates preferentially pair with sodium, while intermediate cases like chloride or monovalent phosphate exhibit almost no specificity and large anions (e.g., methylsulfonate) prefer potassium. These results can rationalize different behavior of Na^+ vs K^+ at the surface of hydrated proteins, DNA, and reversed micelles.