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Review

Ions in water: Characterizing the forces that control chemical processes and biological structure

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Abstract

The continuum electrostatics model of Debye and Hückel [P. Debye and E. Hückel, On the theory of electrolytes. I. Freezing point depression and related phenomena., Phys. Z. 24 (1923) 185–206.] and its successors utilize a macroscopic dielectric constant and assume that all interactions involving ions are strictly electrostatic, implying that simple ions in water generate electric fields strong enough to orient water dipoles over long distances. However, solution neutron and X-ray diffraction indicate that even di- and tri-valent ions do not significantly alter the density or orientation of water more than two water molecules (5 Å) away. Therefore the long range electric fields (generated by simple ions) which can be detected by various resonance techniques such as fluorescence resonance energy transfer over distances of 30 Å (about 11 water diameters) or more must be weak relative to the strength of water–water interactions. Two different techniques indicate that the interaction of water with anions is by an approximately linear hydrogen bond, suggesting that the dominant forces on ions in water are short range forces of a chemical nature.

Keywords: Hofmeister series; Water (hydration); Specific ion effects; Solution neutron diffraction; Ions; Dielectric constant; Chaotrope; Kosmotrope

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Contents

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1. Introduction

A quantitative understanding of the forces in aqueous solution that control chemical processes and give rise to biological structure is essential for progress in such disparate fields as process design in the chemical industry; drug design in the pharmaceutical industry; and the modeling of biological processes such as signal transduction *via* ionic fluxes and protein folding, localization, and function in health and disease. We have studied the interaction of ions with water as a way to understand these forces, and used the Hofmeister series [2,3] to systemize our results. The Hofmeister series orders ions as a monotonic function of their surface charge density and thus water affinity, with the strength of water–water interactions separating strongly hydrated from weakly hydrated species; it is most convenient to generate a separate series for anions and for cations.

Continuum electrostatics models such as that of Debye and Hückel [1] utilize a macroscopic dielectric constant and assume that all interactions involving ions are strictly electrostatic, implying the existence of long range electric fields strong relative to the strength of water–water interactions. In these models, ions are often thought of as point charges and water as a dipole which orients in the long range electric field; such models are unable to accurately describe such simple ion-specific behaviors as their tendency to form contact ion pairs, which is a major determinate of the solubility of specific salts and of the role of specific ions in biological systems. For example, models employing a macroscopic dielectric constant predict that all ions are strongly hydrated and will be repelled from nonpolar surfaces by image forces. In fact, weakly hydrated ions do exist (e.g., ammonium, chloride, potassium, and the positively charged amino acid side chains) and actually adsorb to nonpolar surfaces [3–5] and interfaces [6,7]. The driving force for this adsorption of weakly hydrated ions to an air/water interfaces has been shown by electrospray ionization mass spectrometry to be the release of weakly bound water to become strongly interacting bulk [8], a process not included in the calculations utilizing the macroscopic dielectric constant. Sophisticated microscopic calculations have indicated a role for the polarizability [9,10] of weakly hydrated ions (as opposed to their dehydration energy) and dispersion forces [11] in driving them to neutral interfaces. Microscopic calculations also find a role for the polarizability of water in driving the weakly hydrated Cl^- ion to an air/water interface [9,10,12], but since “polarizability appears to be important primarity for its role in facilitating a larger average dipole moment on the water model” [12] and the interaction of water with Cl^- is *via* an approximately linear hydrogen bond rather than a dipolar interaction [13–15], it is difficult to evaluate the significance of these calculations. The continuum electrostatic models also assume that anions and cations have exactly analogous interactions with water, which is not consistent with the data in Fig. 5.

Over the last 25 years, new approaches have been developed to study the hydration of ions in the Hofmeister series, and a very different picture of how ions interact with water and other ions is emerging. This new perspective provides simple explanations for the tendency of specific ions to form contact ion pairs and to manifest other behaviors.

In this review we survey the information available on how ions interact with water, and find that the known properties of ion–water interactions are inconsistent with the assumptions underlying continuum electrostatics. We arrive at the important conclusion that the dominant forces on ions in water are short range forces of a chemical nature (mediated by electrons in atomic orbitals) and that the long range electric fields generated by simple ions are weak relative to the strength of water–water interactions. We demonstrate that many processes involving ions in water are actually dominated by hydration–dehydration, a non-electrostatic component which is not included in the calculations utilizing a macroscopic dielectric constant.

2. Over what distance do ions in water have an influence on water?

Simple ions in water generate long range electric fields which can be detected by various resonance techniques, such as fluorescence resonance energy transfer, over distances of 30 Å (about 11 water diameters) or more [16]. It has usually been assumed that the long range electric fields generated by simple ions in water are strong enough to orient water dipoles over long distances. But solution neutron and X-ray diffraction techniques developed in the 1970s and applied to various ions in water in the years since have produced a very different picture.

When more than one stable isotope of an ion is available, two identical aqueous salt solutions that vary only in that isotope can be used to study the spherically averaged structure around that ion by neutron diffraction [17,18]. In particular, radial distribution functions that measure the density of the solution as a function of the distance from the isotopic ion can be calculated. When neutron diffraction experiments are carried out on aqueous solutions of a salt in heavy water, correlations between the isotopically substituted ion and both the oxygen and the deuteron of the solvent can be detected; this allows one to determine quantitatively, nearest neighbor distances, coordination numbers, and from the viewpoint of this review the most probable orientation of nearby water molecules and a qualitative understanding of the strength of the ion–water correlations.

2.1. Simple monovalent cations affect only the first water layer

Fig. 1 shows the neutron and X-ray diffraction of the IA cations Li^+, Na^+, K^+, and of water. As the surface charge density of the ion decreases from Li^+ to Na^+ to K^+, the density peak of the nearest water oxygen is lower and further away, indicating weaker binding. The Na^+–oxygen distance is smaller than the oxygen–oxygen distance of pure water, indicative of strong hydration for Na^+, while the K^+–oxygen distance is larger than the oxygen–oxygen distance of pure water, indicative of weak hydration for K^+. A surface charge density between that of Na^+ and K^+ is exactly where the Jones–Dole viscosity *B* coefficient (a measure of water affinity) changes sign, marking the strength of water–water interactions. Additionally, Figs. 2–4 use neutron diffraction of deuterium

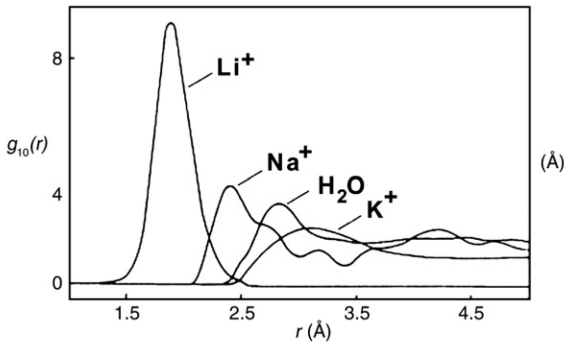

Fig. 1. The radial distribution functions g_{10}(r) for Li^+, Na^+, water, and K^+ in liquid water. These curves measure the density of the solution as a function of the distance from the isotopically substituted ion, and effectively measure the distance from the monovalent cation to the nearest solvent oxygen. The curve labeled "H_2O" measures the oxygen–oxygen distance in liquid water. Both neutron and X-ray diffractions were used to generate these data. Reprinted from [19].

oxide solutions to determine the orientation of the deuterium oxide molecules adjacent to Li^+, Ag^+ (an analog of Na^+), and K^+; strong hydration (Li^+) is associated with strong orientation of solvent, intermediate hydration (Ag^+) is associated with intermediate orientation of solvent, and weak hydration (K^+) is associated with no orientation of solvent. Thus a chaotrope (such as K^+) is weakly hydrated: the immediately adjacent water is far away and not oriented. In fact, nuclear magnetic resonance can be used to show that the water immediately adjacent to a chaotrope is actually tumbling more rapidly than is water in bulk solution [23], as might be expected for a water molecule that is "not being held" by one of its neighbors. Neutron diffraction has recently been used to characterize the strong denaturants guanidinium and thiocyanate in solution, verifying the weakly hydrated character of chaotropes [24]. Gel sieving chromatography on Sephadex G-10 has been used to indicate that Li^+ has 0.6 tightly bound water molecules, Na^+ has 0.25 tightly bound water molecules, and K^+ has no tightly bound water [25]. Thus solution neutron and X-ray diffraction combined with gel sieving chromatography indicate that only tightly bound water is oriented and that the major effect of ions on water is short range.

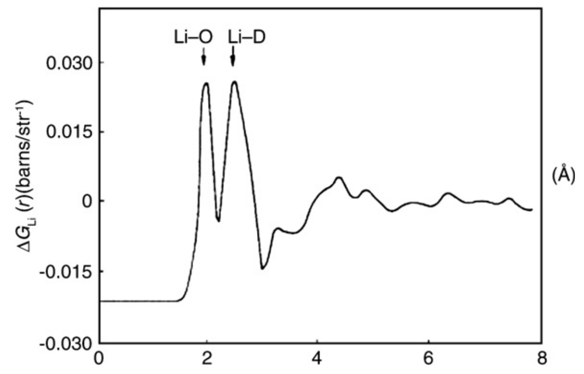

Fig. 2. The first-order difference function G_{Li}(r) for Li^+ in D_2O. This curve measures the distance from the isotopically labeled Li^+ to the nearest solvent oxygen or deuteron. These are the neutron diffraction data of Newsome, Neilson and Enderby [20].

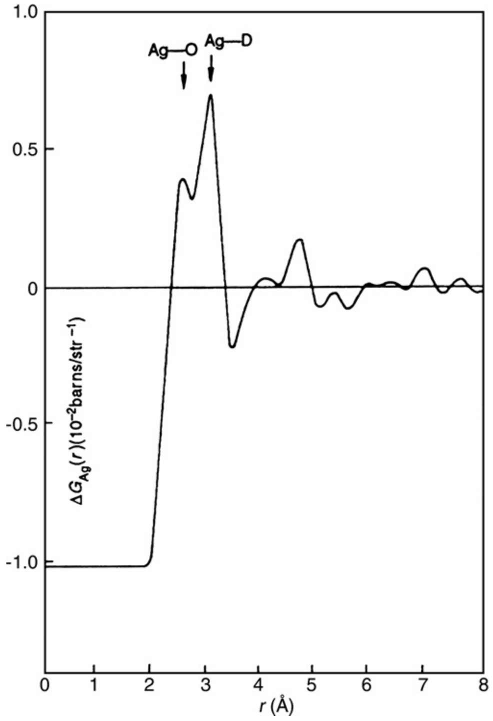

Fig. 3. The first-order difference function G_{Ag}(r) for Ag^+ (an analog of Na^+) in D_2O. This curve measures the distance from the isotopically labeled Ag^+ to the nearest solvent oxygen or deuteron. These are the neutron diffraction data of Sandstrom et al. [21].

Gel sieving chromatography can also measure the number of tightly bound waters for H^+ and OH^-: at 0.1 M, H^+ has 1.9 tightly bound waters and thus exists as the dihydrate (the Zundel proton), whereas OH^- has 2.8 tightly bound waters and thus exists as the trihydrate [25].

2.2. Ions of high charge density affect both the first and second water layers

Gel sieving chromatography [25] has been used to establish that the chaotrope Cl^- has no tightly bound water (and is thus weakly hydrated), whereas F^- has 5 tightly bound waters. Gas

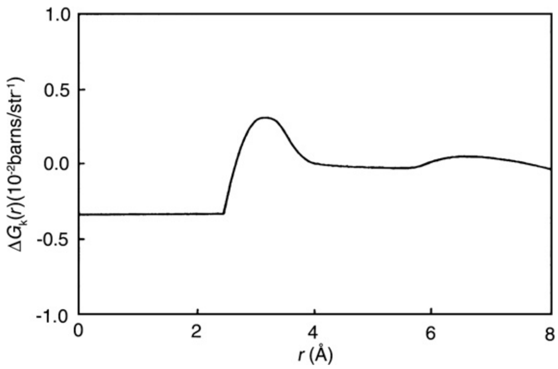

Fig. 4. The first-order difference function G_K(r) for K^+ in D_2O. This curve measures the distance from the isotopically labeled K^+ to the nearest solvent oxygen or deuteron. These are the neutron diffraction data of Neilson and Skipper [22].

phase infrared vibrational predissociation spectroscopy indicates that 4 of these tightly bound waters are in the first hydration layer of F^- [26], leaving one for the second layer. Similarly, Mg^{2+} has 5.8 tightly bound water molecules, all in the first hydration layer, while Be^{2+} (which is smaller and has a higher charge density than Mg^{2+}), has only 5.3 tightly bound water molecules, 4 in the first hydration layer and 1.3 in the second layer [25]. As ions get smaller and their surface charge density increases, they typically have more tightly bound water; but whereas Mg^{2+} has room for six water molecules in its first hydration layer, the smaller Be^{2+} has room for only four. Gel sieving chromatography finds 9.6 tightly bound waters associated with the Cr^{3+} ion, in excellent agreement with X-ray and neutron diffraction studies, which identify 6 of those tightly bound waters as first layer [27], leaving 3.6 for the second layer. The accuracy of determining tightly bound water using gel sieving chromatography is supported by its excellent agreement with (1) the demarcation between strong and weak hydration as defined by Jones–Dole viscosity *B* coefficients [28,29] as well as by solution neutron and X-ray diffraction [18]; (2) calculations indicating a drop in first hydration layer water molecules from 6 for Mg^{2+} [30] to 4 for Be^{2+} [31]; and (3) X-ray diffraction of Mg^{2+} in water which shows six water molecules in the first hydration layer, but no large alteration in the density of the second hydration layer [32].

In summary, simple small ions perturb water over only a short distance, e.g., <5 Å.

3. Defining an abbreviated Hofmeister series for anions and for cations

The IA cations and VIIA halides of the periodic table of the elements form abbreviated Hofmeister series and each can be separated into strongly hydrated (small size, high surface charge density) and weakly hydrated (large size, low surface charge density) species relative to the strength of water–water interactions:

$$\text{kosmotropes} \quad H^+, Li^+, Na^+//K^+, Rb^+, Cs^+ \quad \text{chaotropes}$$

$$(\text{strongly hydrated}) \quad F^-//Cl^-, Br^-, I^- \quad (\text{weakly hydrated})$$

"//" indicates the strength of water–water interactions; it is the position at which the Jones–Dole viscosity *B* coefficient changes sign [29]. NH_4^+ has hydration properties similar to K^+ [29,33]. All multivalent small ions are strongly hydrated [18,25,28,29,34]. The positively charged amino acid side chains (derivatives of ammonium) are weakly hydrated whereas the negatively charged amino acid side chains (the carboxylate) are strongly hydrated [29].

4. What is the evidence for the chemical nature of ion–water interactions?

Cl^- is known to be weakly hydrated because coordinated water molecules exchange on a timescale of less than 10^{-11} s and because it has a negative Jones–Dole viscosity *B* coefficient. Even so, solution neutron diffraction by isotopic substitution has been used to show apparently well defined geometry of the six water molecules coordinated to the ion; each water molecule has a linear hydrogen bond (within experimental error) with Cl^-, indicative of a primarily chemical interaction (a dipolar interaction would have an angle about one-half that of the water H–O–H angle of 104.5°, which is 52.25°) [13].

Additionally, gas phase infrared vibrational predissociation spectroscopy of halide–water binary or cluster complexes has been carried out with weakly bound argon atoms [14,15] and combined with *ab initio* calculations [35]. These studies find that the anions interact with one water hydrogen in a near-linear hydrogen bond. As the charge density on and proton affinity of the anions increases, the covalent character of the hydrogen bond increases. Charge transfer to solvent is also found to be important; there is vibrationally mediated charge redistribution within the anion–water complex. In the sequence $I^- < Br^- - Cl^- < O^- < OH^-$, there is an increasing tendency for the anion in a binary complex with a single water molecule to "pry apart" the water molecule. These findings support the view of ion–water interactions as primarily chemical, and are consistent with the emerging view of hydrogen bonds. "The strength of H-bonding interaction ranges from about 1 to 40 kcal/mol, indicating the existence of a continuum of strength. It is important to note that weak H-bonds are hardly distinguishable from van der Waals interaction. The strength of the classical H-bonding varies from 4 to15 kcal/mol. For strong H-bonds, the strength falls in the range 15–40 kcal/mol. Although [the] electrostatic interaction is taken to be the primary factor responsible for the classical type of H-bonding, pronounced covalent character is found in strong H-bonding and a dominance of dispersive interaction is observed in weak H-bonding" ([36]; see also [37]).

Measurements of the oxygen K-edge X-ray absorption spectrum (XAS) of aqueous sodium chloride, bromide and iodide solutions, combined with density functional theory calculations showed that "the observed *red shift* of the water transitions as a function of salt concentration arises from a strong, direct perturbation of the unoccupied molecular orbitals on water by anions", indicative of a chemical interaction [38]. Similar measurements on aqueous Cr^{3+} and Fe^{3+} solutions were interpreted as evidence for

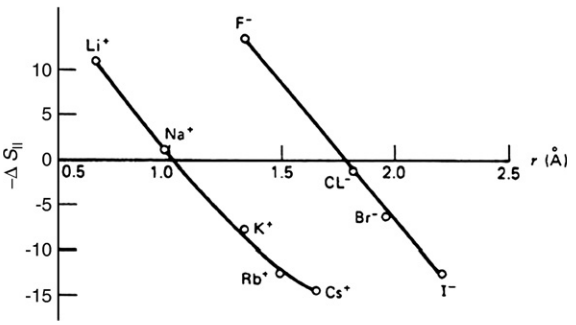

Fig. 5. The entropy of water near an ion minus the entropy of pure water in cal °K^{-1} mol^{-1}. The crystal radii of the ions in angstroms are plotted along the abscissa. Positive values of ΔS_{II} (*lower portion of figure*) indicate water that is more mobile than bulk water. Negative values of ΔS_{II} (*upper portion of figure*) indicate water that is less mobile than bulk water. Kosmotropes are in the upper portion of the figure; chaotropes are in the lower portion of the figure. Adaption of data of Krestov as presented by Samoilov [40]. Reprinted with permission of John Wiley and Sons ©1972.

"mixing between the molecular orbitals of water and the open d shell of the transition-metal ion" [39].

We take Fig. 5, which shows the entropy of water near an ion minus the entropy of pure water *vs*. the crystal radius of the ion in angstroms, as probable evidence for charge transfer to solvent, indicative of a chemical interaction. Small ions of high charge density (kosmotropes, shown above the line) bind nearby water molecules tightly, thus immobilizing them, whereas large monovalent ions of low charge density (chaotropes, shown below the line) actually "free up" nearby water molecules, allowing more rapid motion than in bulk solution. F^- and K^+ are approximately the same size, but F^- is strongly hydrated whereas K^+ is weakly hydrated. The oxygen of water is very electronegative and readily accepts negative charge; therefore (negative) charge transfer from F^- to water is facile, whereas (positive) charge transfer from K^+ to water is difficult. Another factor also contributes to the stronger hydration of anions: because they interact with the hydrogen atom of water, they allow intra-shell hydrogen bonding of the solvating waters, whereas cations, which interact with the oxygen atom of water, do not [41]. *Ab initio* molecular orbital studies of $Mg^{2+}\cdot 6H_2O$ indicate the net positive charge on the central magnesium to be only +1.18, the residual being on the six waters [30]; this lowers the effective charge density of the ion. [The hydration of some of the alkaline earth divalent metal cations and first row transition metal cations has been treated within a quasi-chemical theory of solutions; this uses quantum chemical calculations to provide information on the chemically important interactions between the ion and its first-shell water molecules, and a dielectric continuum model to supply the outer-shell contribution [42]. They find that local chemical effects dominate the hydration phenomena.] A quantum mechanical treatment of the protein–water interface for a 69 amino acid protein found transfer of two full charges to solvent, largely from protein carboxylates [43]. We conclude that charge transfer to solvent is important, and consider it to be *de facto* evidence for a chemical interaction.

Models of water which are to give accurate results in computer simulations must accurately capture the chemical interactions of water with ions, including charge transfer to solvent.

5. "Volcano plots" illustrate the dominant forces on ions in water

We shall interpret Fig. 6 to indicate that oppositely charged ions with equal water affinity tend to come together in solution to form contact ion pairs whereas oppositely charged ions with differing water affinities tend to stay apart. We shall attribute the release of heat to the formation of strong bonds and the uptake of heat to the breakage of strong bonds, and shall assume that the strongest interactions in the system will tend to dominate the behavior of the system. In aqueous salt solutions of kosmotropes (small ions of high surface charge density which are strongly hydrated) and chaotropes (large ions of low surface charge density which are weakly hydrated), the interactions in order of decreasing strength are as follows: kosmotrope–kosmotrope > kosmotrope–water > water–water > chaotrope–water > chaotrope–chaotrope [28,29]. Fig. 6 shows the relationship between the standard heat of solution of a crystalline alkali halide at infinite dilution (on the vertical axis; this is a measured quantity) and the difference between the absolute heats of hydration of the constituent gaseous anion and cation (on the horizontal axis; this is a calculated quantity). [In this context, "absolute" refers to the conceptual experiment of transferring an isolated ion from the gas phase to the solution.] Fig. 6 illustrates that a necessary but not sufficient condition for the standard heat of solution of a crystalline alkali halide to be negative (exothermic) is that one ion be a chaotrope and the other ion be a kosmotrope, suggesting that kosmotrope plus chaotrope neutral salts dissociate extensively

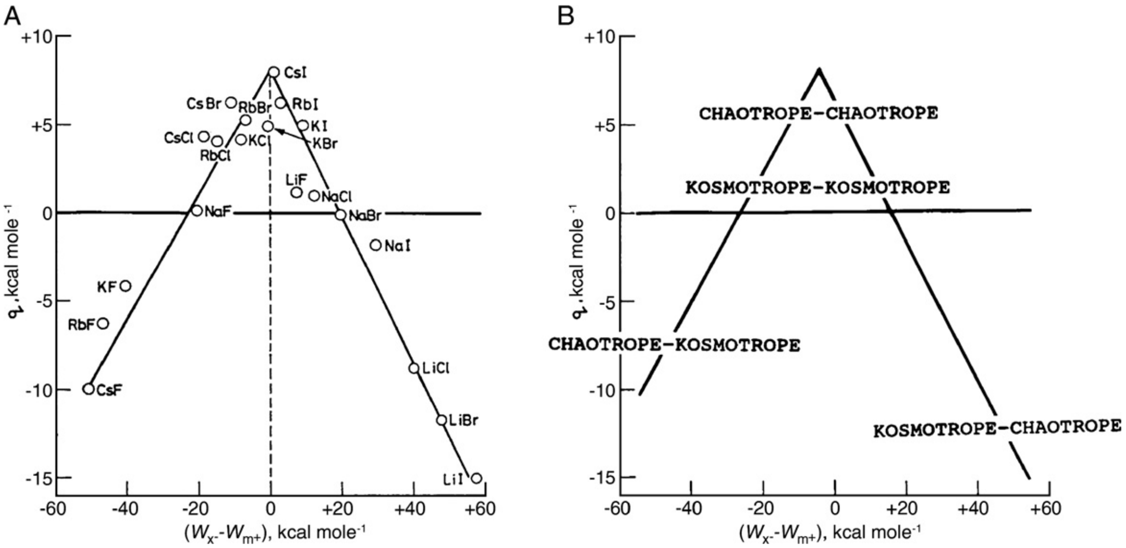

Fig. 6. (A) Relationship between the standard heat of solution of a crystalline alkali halide (at infinite dilution) in kcal mol^{-1} and the difference between the absolute heats of hydration of the corresponding gaseous anion and cation, also in kcal mol^{-1} (Source: [44] © Springer-Verlag, reprinted with permission). (B) Identification of ions as chaotropes (weakly hydrated) or kosmotropes (strongly hydrated). The enthalpy of solution of chaotrope–chaotrope and kosmotrope–kosmotrope salts tends to be positive (takes up heat), whereas for the enthalpy of solution to be negative (gives off heat), the salt must have a kosmotropic and a chaotropic ion.

upon dissolution, and that the kosmotropic ion of this salt acquires stronger interactions with water in solution than it has with chaotropes in the crystal, thus tending to release heat. In contrast, when crystalline kosmotrope–kosmotrope alkali halides dissolve in water, the kosmotrope–kosmotrope ion pairs will tend to stay together, and insofar as the constituent ions do separate, strong kosmotrope–kosmotrope interactions are broken, thus tending to take up heat. When crystalline chaotrope–chaotrope alkali halides dissolve in water, relatively strong water–water interactions will keep the chaotrope ion pairs together, and insofar as the constituent ions do separate, relatively strong water–water interactions will be broken, thus tending to take up heat. These patterns suggest that oppositely charged ions with equal water affinities will tend to form contact ion pairs in solution, whereas those with differing water affinities will tend to separate [28,29,33]. In simplest possible terms, kosmotropes pair with kosmotropes and chaotropes pair with chaotropes; that is, like likes like. Since all of the salts in the volcano plot are monovalent, the long range electric fields generated by each salt must be very similar, and the dramatic differences in their behavior must be due to the differences in the strength of their short range chemical interactions with water. Forming a contact ion pair requires a partial dehydration of both the positive and negative ion, which occurs most readily when both ions have the same water affinity. A simple model can be used to show that the relative affinity of a monovalent ion for water closely correlates with its relative affinity for monovalent ions of opposite charge [29]. Therefore, when one ion is more strongly hydrated than its oppositely charged partner, dehydrating the more strongly hydrated ion costs more in energy than is gained by forming a contact ion pair with the more weakly hydrated ion, and thus these ions tend to stay apart. The issue of surface charge density-dependent microscopic hydration-dehydration is not included in the electrostatic calculation using the macroscopic dielectric constant, but energetically it actually dominates and controls the process of contact ion pair formation.

This “Law of Matching Water Affinities” has important consequences in chemistry and biology. [Using Jones–Dole viscosity *B* coefficients as scaling factors for the strength of water–water interactions (these being about 44.0 kJ/mol at 20 °C as measured by the heat of evaporation {references in [5]}), we were able to show a linear correlation between the tendency of various monovalent cations to form a contact ion pair with the protein carboxylate and the similarity of the cation and carboxylate water affinities [34].] For example, both Li^+ and F^- are strongly hydrated, and thus tend to form contact ion pairs (the first step in the process of coming out of solution), whereas Cs^+ is weakly hydrated [45], and will tend to stay away from F^-. The solubility of LiF in water is only 0.1 M at 18 °C. In contrast, the solubility of CsF at 18 °C is 24.2 M, or 48.4 M in ions, and since pure water is about 55.5 M, a saturated solution of CsF contains only about one water molecule per ion. Another example is the fact that the strongly hydrated Ca^{2+} and Na^+ ions are pumped out of living cells, whereas the weakly hydrated K^+ is pumped in. This is because Ca^{2+} and Na^+ are well matched to the strongly hydrated major intracellular anions (phosphate, the carboxylate, and carbonate), and tend to form contact ions pairs with them (and then come out of solution). [Ca^{2+}, $H_2PO_4^-$, and HCO_2^- each bind about 2 water molecules tightly [25]]. In fact, insoluble Ca^{2+} complexes play important biological roles. Calcium carbonate (e.g., eggshells, oyster shells) has a solubility product of $10^{-8.5}$; calcium oxalate (e.g., kidney stones) has a solubility product of $10^{-10.5}$; and calcium hydroxyphosphate (hydroxyapatite) (e.g., bones and teeth) has a solubility product of 10^{-58} [46]. K^+, in contrast, stays away from the major intracellular anions (as shown by molecular dynamics simulations of Na^+ and K^+ interactions with the carboxylate [47]), increasing the net charge and thus solubility of molecules containing these anionic groups and also leaving these anionic groups available for acting as binding determinants. As summarized above, ions interact strongly with water over only short distances through predominantly chemical mechanisms which determine water affinity, and thus ion pairing preferences are determined largely by hydration-dehydration.

The guanidinium side chain of arginine is weakly hydrated whereas phosphate is strongly hydrated; the above considerations suggest that these two ions should not readily form contact ion pairs. The fact that they spontaneously form tight complexes [48,49] indicates that specific chemical interactions (probably unusually strong hydrogen bonds) mediate their interaction, with obviously important biological implications.

Intracellular monovalent cation-dependent phosphoryl transfer enzymes utilize K^+ (the common intracellular monovalent cation) as a phosphoryl oxyanion ligand [50,51] rather than Na^+ (the common extracellular monovalent cation), even though Na^+ is more closely matched to the phosphoryl oxyanion in water affinity [34]. K^+ may be used by these enzymes simply because it is locally more abundant. Presumably the enzyme is able to “tune” the charge density on the bound K^+ to acceptable levels by its selection of protein ligands; however, a mismatch in water affinity between the bound K^+ and its phosphoryl oxyanion ligand may be useful in that it will encourage rapid exchange of the substrate.

6. Biological evidence for the short range nature of the dominant forces generated by ions in water

We shall now examine three biological systems whose behavior has been thought to be controlled by long range electric fields, and present the evidence for actual control by short range forces. (A) First, we shall examine the binding of negatively charged ligands to negatively charged regions of proteins; (B) second, we shall examine the role of charged amino acid side chains in globular protein stability; (C) and third, we shall examine the origins of “ionic strength” effects.

6.1. The binding of negatively charged ligands to negatively charged regions of proteins

The 34.4 kDa *E. coli* periplasmic phosphate-binding protein (PBP) serves as the initial high affinity receptor for active transport of phosphate. It is a member of a family of about 50 proteins which initiate transport of a wide variety of ligands in bacterial cells. PBP has a two domain structure which closes around inorganic phosphate, completely burying and dehydrating it; the phosphate interacts with the protein *via* 12 hydrogen bonds (11 with donor groups and one with a carboxylate acceptor group), as well as one salt link to an Arg that is in turn salt linked to an Asp. The calculated surface charge potential of the cleft between the two domains that contains the anion binding site is intensely electronegative. In spite of this, inorganic phosphate diffuses to the open cleft at a near diffusion-controlled rate, whereupon the two domains close, binding the phosphate with a K_d of about 13 μM at pH 7.0 [52]. Since this is a transport protein whose job is to release the phosphate to the interior of the cell, optimal function occurs at moderate affinity for phosphate; there is no reason to believe that a much higher affinity for phosphate could not be selected for with the same protein charged residues at the phosphate binding site. Enzymes are believed to use short strong hydrogen bonds to bind very tightly the small molecule transition state of the reaction which they catalyze [53–56]; and such strong interactions could also be used to bind inorganic phosphate. The 38 kDa *M. tuberculosis* phosphate-specific transport substrate binding protein-1 (PstS-1), which has 30% sequence identity with the *E. coli* PBP, has a calculated surface charge in and around the ligand binding site cleft that is almost entirely electronegative (Fig. 7). The completely dehydrated, buried phosphate is bound by 13 hydrogen bonds formed with nine residues distributed between the two domains. The two shortest hydrogen bonds are with the carboxylate side chains of Asp83 and Asp168. There is one salt link to an Arg that is in turn salt linked to an Asp. The inorganic phosphate binds with a K_d of about 33 μM at pH 7.0 [52,59,60].

There are other proteins which bind negatively charged ligands in regions of calculated intense negative surface charge potential, including sulfate binding protein, DNase I, and redox proteins such as flavodoxin (which has a net negative charge of −17). None of these examples is consistent with the existence of long range electric fields strong relative to the strength of water–water interactions; that is, these examples are not consistent with the assumptions or predictions of the Poisson-Boltzmann equation [57]. [A reviewer has pointed out that Poisson–Boltzmann calculations also exaggerate long-range electric field effects owing to the treatment of the protein as a material with a low dielectric constant.]

The Poisson–Boltzmann equation is equally unsatisfying as a meaningful description of the mechanism by which a positively charged protein binding site combines with a negatively charged binding partner. All positive charges on proteins are derivatives of the weakly hydrated ammonium ion and are thus themselves weakly hydrated [29]. The electric field emanating from these positively charged amino acid side chains is too weak to orient the immediately adjacent water molecules, and thus cannot plausibly be expected to be “reaching out and pulling in” the negatively charged binding partner from many water diameters away.

6.2. The role of charged amino acid side chains in globular protein stability

The existence of stable proteins such as PstS-1 (above) with calculated intensely negative surface charge potentials (implying unfavorable interactions between amino acid side chains of like charge) suggests that long range electrostatic interactions between

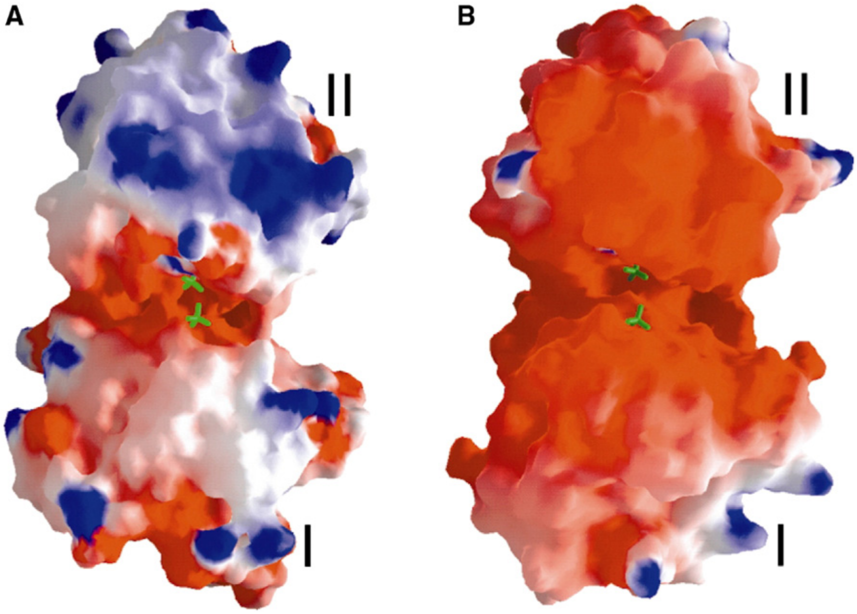

Fig. 7. Surface charge distribution of the “open cleft” ligand-free form of *E. coli* phosphate binding protein (PBP) (A) and *M. tuberculosis* phosphate-specific transport substrate binding protein-1 (PstS-1) (B). “The crystal structure of the ligand-free open PBP [57] was used to model the PstS-1 open form (B) starting from its ligand-bound “closed” structure.... Red and blue represent negative and positive electrostatic potential, respectively, contoured at 5k Te^{-1}. I and II identify the two domains. The inclusion of the two P_i molecules (green stick models) is for the sole purpose of marking the location in each domain that participates in P_i binding in the closed structures.... It does not represent two P_i molecules bound in the open form. Several receptors, including PBP ... have been shown by crystal structure analysis to undergo a hinge-bending motion between the two domains” [58].

surface charges on proteins are weak, and other studies support this conclusion. “To quantitate the contribution of the ionizable amino acids to the stability of the native state of staphylococcal nuclease, each of the 23 lysines, 5 arginines, 4 histidines, 12 glutamic acids, and 8 aspartic acids was substituted with both alanine and glycine. This collection of 104 mutant proteins was analyzed by guanidine hydrochloride denaturation, using intrinsic tryptophan fluorescence to quantitate the equilibrium between native and denatured states…. For the residue pairs that form ten salt bridges and ten charged hydrogen bonds between side chains…. Little or no significant correlation was found between the magnitude of the loss in stability and the local electrostatic potential calculated from the three-dimensional structure by numerical and model dependent solutions of the linearized Poisson–Boltzmann equation…. These results suggest that the ionizable amino acids contribute to stability predominantly through packing and bonding interactions that do not depend on their electrostatic charge” [61].

The CspB-Bs protein from *Bacillus subtilis* was substantially stabilized by redesigning the surface charge distribution to maximize favorable long range electrostatic interactions [62]. Even if long range electrostatic interactions are weak, the sum of many weak interactions could be significant, and protein “breathing” motions could bring widely separated charged groups close together (providing a short range explanation for apparent long range effects).

The Poisson–Boltzmann assumption that behavior near ions can be accurately described utilizing a macroscopic dielectric constant is inconsistent with the over-shifted calculated pK_a’s for contact ion pairs in proteins [63,64]. That macroscopic dielectric constants mask more complex processes has been pointed out [65,66]. That hydrogen bonding markedly reduces the pK of buried carboxyl groups in proteins [67] suggests that chemical interactions with ions which delocalize charge may be important for ions in proteins as well as for ions in water.

6.3. The origins of “ionic strength” effects: direct and indirect effects

The ionic strength of a salt solution is defined as $I = [\Sigma_i M_i \cdot z_i^2]/2$, where M_i is the molarity of each ion, z_i is the charge on each ion and I is the ionic strength [68]. (ionic strength was originally defined in terms of molality) The ionic strength of a salt solution has usually been attributed to strong long range electric fields emanating from the ions [1], but the hydrated size of an ion is roughly proportional to the square of the charge on the ion [25], suggesting short range explanations for the origins of ionic strength effects. NaCl or KCl have most commonly been used to control ionic strength, and the classical explanation for ionic strength has never been able to explain the often large specific ion effects manifested by other monovalent salts in the same system. A direct and an indirect effect involving short range forces has been identified that together may be responsible for most or all ionic strength effects. (1) *The direct effect is ion binding*. One example is the difference between coarse and fine fibrin clots that had been interpreted for nearly 50 years as a nonspecific ionic strength effect, suggesting that fibrin polymerization was largely controlled by long range electrostatic forces. But while Cl^- is active in this system, F^- is not. Cl^- and F^- should have similar long range electric field effects, and thus the origin of their different behaviors cannot be long range electric fields. However, F^- has a high surface charge density, generating 5 tightly bound water molecules interacting *via* a chemical process (see above); in contrast, Cl^- has a much lower surface charge density, and is thus weakly hydrated with no tightly bound water molecules [25]. These surface charge density-dependent short range differences in water affinity must be the basis for their different behavior in the fibrin system. It was subsequently shown that Cl^- binding to fibrin fibers was responsible for fine fibrin clots, not generic ionic strength or long range electrostatic effects. Binding of Cl^- opposed the lateral aggregation of protofibrils and produced thinner fibers that were also more curved than those grown in the presence of inert anions such as F^-. From the pH dependence of the Cl^- effect it was suggested that Cl^- exerted its effect by increasing the pK_a of a basic group ionizing around pH 9.2 [69]. F^- is inert in this system because it is strongly hydrated whereas the positive charges on proteins are weakly hydrated, and ions of differing water affinities stay apart (see above).

This same effect is seen in protein crystallization. Only neutral complexes crystallize, so positively charged proteins such as lysozyme must first bind anions. Weakly hydrated anions such as Cl^-, NO_3^-, and SCN^- bind readily to the weakly hydrated positive charges on lysozyme by the Law of Matching Water Affinities, causing crystallization, whereas the strongly hydrated anions acetate, phosphate, and citrate do not bind readily to the protein and are much less effective crystallizing agents [70].

(2) *The indirect effect occurs when ions (particularly anions) with high water affinity bind water near other solutes and surfaces*. F^- promotes the folding of a 30 residue peptide into a coiled coil, while Cl^- does not [71]. Since the long range electric fields produced by F^- and Cl^- should be very similar, these different effects on peptide folding must be due to their short range surface charge density-dependent differences in water affinity, which arise from their chemical interaction with water. Water is unique in that the second water layer (each layer being one molecule thick) surrounding a solute such as a protein makes a large contribution to the energy of solvation of the protein [3,28,72]. Hofmeister effects on proteins arise when anions compete for this second layer of water surrounding the protein, making the water more or less available to help solvate the protein. Anions with high water affinity such as F^- compete effectively for this second water layer, making the solution a less good solvent and encouraging the protein to minimize its solvent exposed surface area by acquiring a compact form. In contrast, Cl^- is slightly weakly hydrated and does little to make this second water layer more or less available, and usually has only small effects on protein stability. Anions with high water affinity not only stabilize proteins, they also encourage their crystallization [28,34], since crystallization also minimizes the solvent exposed surface area (strongly hydrated cations tend to bind to the protein surface [73]).

Salts also alter the surface tension and osmotic pressure of solutions, but these are fundamentally “particle effects” which do not act through long range electric fields.

7. Conclusion

In conclusion, solution neutron diffraction, gas phase infrared vibrational predissociation spectroscopy, X-ray absorption spectroscopy, and *ab initio* molecular orbital studies indicate that water interacts with ions *via* a chemical bond, and that water is affected over only a short distance from simple, small ions (i.e., <5 Å). This chemical interaction involves substantial charge transfer to solvent for strongly hydrated ions of high surface charge density, resulting in delocalization of charge. Solution neutron diffraction also indicates that long range electric fields arising from simple, small ions in solution must be weak relative to the strength of water–water interactions. The rapid, tight binding of negatively charged ligands to negatively charged regions of proteins also indicates that the protein–ligand interactions are chemical in nature and that negatively charged regions of proteins do not generate long range electric fields which are strong relative to the strength of water–water interactions. Analysis of site-specific mutations of the charged amino acids in staphyloccal nuclease indicates that long range electric fields in this protein are weak. The dramatically different behavior of different monovalent ions on contact ion pair formation (and therefore solubility) and on protein structure and function (effects associated with “ionic strength”) can be explained by the differences in water affinity of ions that result from their differences in surface charge density, but not long range electric fields; contact ion pair formation is actually dominated by hydration–dehydration. We conclude that while calculations utilizing a macroscopic dielectric constant attribute the forces on ions in water entirely to electrostatics (implying the existence of long range electric fields strong relative to the strength of water–water interactions), the dominant forces on ions in water are actually short range forces of a chemical nature, and that the long range electric fields generated by simple ions in water are weak relative to the strength of water–water interactions.

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References

[1] P. Debye, E. Hückel, On the theory of electrolytes. I. Freezing point depression and related phenomena, Phys. Z. 24 (1923) 185–206.

[2] F. Hofmeister, On the understanding of the effect of salts. Second report. On regularities in the precipitating effect of salts and their relationship to their physiological behavior, Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. (Leipzig) 24 (1888) 247–260.

[3] K.D. Collins, M.W. Washabaugh, The Hofmeister effect and the behaviour of water at interfaces, Q. Rev. Biophys. 18 (1985) 323–422.

[4] M.W. Washabaugh, K.D. Collins, The systematic characterization by aqueous column chromatography of solutes which affect protein stability, J. Biol. Chem. 261 (1986) 12477–12485.

[5] K.D. Collins, Sticky ions in biological systems, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 5553–5557.

[6] S. Gopalakrishnan, D. Liu, H.C. Allen, M. Kuo, M.J. Shultz, Vibrational spectroscopic studies of aqueous interfaces: salts, acids, bases, and nanodrops, Chem. Rev. 106 (2006) 1155–1175.

[7] P.B. Petersen, R.J. Saykally, On the nature of ions at the liquid water surface, Annu. Rev. Phys. Chem. 57 (2006) 333–364.

[8] J. Cheng, C.D. Vecitis, M.R. Hoffmann, A.J. Colussi, Experimental anion affinities for the air/water interface, J. Phys. Chem., B Condens. Mater. Surf. Interfaces Biophys. 110 (2006) 25598–25602.

[9] L. Vrbka, M. Mucha, B. Minofar, P. Jungwirth, E.C. Brown, D.J. Tobias, Propensity of soft ions for the air/water interface, Curr. Opin. Colloid Interface Sci. 9 (2004) 67–73.

[10] P. Jungwirth, D.J. Tobias, Specific ion effects at the air/water interface, Chem. Rev. 106 (2006) 1259–1281.

[11] M. Bostrom, F.W. Tavares, B.W. Ninham, J.M. Prausnitz, Effect of salt identity on the phase diagram for a globular protein in aqueous electrolyte solution, J. Phys. Chem., B Condens. Mater. Surf. Interfaces Biophys. 110 (2006) 24757–24760.

[12] S.J. Stuart, B.J. Berne, Effects of polarizability on the hydration of the chloride ion, J. Phys. Chem. 100 (1996) 11934–11943.

[13] D.H. Powell, A.C. Barnes, J.E. Enderby, G.W. Neilson, P.S. Salmon, The hydration structure around chloride-ions in aqueous-solution, Faraday Discuss. (1988) 137–146.

[14] W.H. Robertson, M.A. Johnson, Molecular aspects of halide ion hydration: the cluster approach, Annu. Rev. Phys. Chem. 54 (2003) 173–213.

[15] J.R. Roscioli, E.G. Diken, M.A. Johnson, S. Horvath, A.B. McCoy, Prying apart a water molecule with anionic H-bonding: a comparative spectroscopic study of the X-.H2O (X=OH, O, F, Cl, and Br) binary complexes in the 600–3800 cm (−1) region, J. Phys. Chem., A Mol. Spectrosc. Kinet. Environ. Gen. Theory 110 (2006) 4943–4952.

[16] L. Stryer, R.P. Haugland, Energy transfer: a spectroscopic ruler, Proc. Natl. Acad. Sci. U. S. A. 58 (1967) 719–726.

[17] G.W. Neilson, J.E. Enderby, Aqueous solutions and neutron scatttering, J. Phys. Chem. 100 (1996) 1317–1322.

[18] J.E. Enderby, Ion solvation via neutron-scattering, Chem. Soc. Rev. 24 (1995) 159–168.

[19] N.T. Skipper, G.W. Neilson, X-ray and neutron-diffraction studies on concentrated aqueous-solutions of sodium-nitrate and silver-nitrate, J. Phys., Condens. Matter 1 (1989) 4141–4154.

[20] J.R. Newsome, G.W. Neilson, J.E. Enderby, Lithium ions in aqueous-solution, J. Phys. C: Solid State Phys. 13 (1980) L923–L926.

[21] M. Sandstrom, G.W. Neilson, G. Johansson, T. Yamaguchi, Ag^+ hydration in perchlorate solution, J. Phys. C: Solid State Phys. 18 (1985) 1115–1121.

[22] G.W. Neilson, N. Skipper, K^+ coordination in aqueous-solution, Chem. Phys. Lett. 114 (1985) 35–38.

[23] L. Endom, H.G. Hertz, B. Thul, M.D. Zeidler, A microdynamic model of electrolyte solutions as derived from nuclear relaxation and self-diffusion data, Dtsch. Bunsenges. Phys. Chem. 71 (1967) 1008–1031.

[24] P.E. Mason, G.W. Neilson, C.E. Dempsey, A.C. Barnes, J.M. Cruickshank, The hydration structure of guanidinium and thiocyanate ions: implications for protein stability in aqueous solution, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 4557–4561.

[25] M.Y. Kiriukhin, K.D. Collins, Dynamic hydration numbers for biologically important ions, Biophys. Chem. 99 (2002) 155–168.

[26] W.H. Robertson, E.G. Diken, E.A. Price, J.W. Shin, M.A. Johnson, Spectroscopic determination of the OH− solvation shell in the OH−.(H_2O) n clusters, Science 299 (2003) 1367–1372.

[27] R.D. Broadbent, G.W. Neilson, M. Sandstrom, The hydration structure of $Cr3^+$ in a concentrated aqueous-solution, J. Phys., Condens. Matter 4 (1992) 639–648.

[28] K.D. Collins, Ions from the Hofmeister series and osmolytes: effects on proteins in solution and in the crystallization process, Methods 34 (2004) 300–311.

[29] K.D. Collins, Charge density-dependent strength of hydration and biological structure, Biophys. J. 72 (1997) 65–76.

[30] C.W. Bock, A. Kaufman, J.P. Glusker, Coordination of water to magnesium cations, Inorg. Chem. 33 (1994) 419–427.

[31] C.W. Bock, J.P. Glusker, Organization of water around a beryllium cation, Inorg. Chem. 32 (1993) 1242–1250.

[32] N.T. Skipper, G.W. Neilson, S.C. Cummings, An X-ray-diffraction study of Ni-2+(Aq) and Mg-2+(Aq) by difference-methods, J. Phys., Condens. Matter 1 (1989) 3489–3506.

[33] N.A. Hewish, G.W. Neilson, Ammonium ion coordination in concentrated aqueous ammonium-chloride, Chem. Phys. Lett. 84 (1981) 425–427.

[34] K.D. Collins, Ion hydration: implications for cellular function, polyelectrolytes, and protein crystallization, Biophys. Chem. 119 (2006) 271–281.

[35] W.H. Thompson, J.T. Hynes, Frequency shifts in the hydrogen-bonded OH stretch in halide-water clusters. The importance of charge transfer, J. Am. Chem. Soc. 122 (2000) 6278–6286.

[36] R. Parthasarathi, V. Subramanian, N. Sathyamurthy, Hydrogen bonding without borders: an atoms-in-molecules perspective, J. Phys. Chem., A Mol. Spectrosc. Kinet. Environ. Gen. Theory 110 (2006) 3349–3351.

[37] G. Gilli, P. Gilli, Towards an unified hydrogen-bond theory, J. Mol. Struct. 552 (2000) 1–15.

[38] C.D. Cappa, J.D. Smith, K.R. Wilson, B.M. Messer, M.K. Gilles, R.C. Cohen, R.J. Saykally, Effects of alkali metal halide salts on the hydrogen bond network of liquid water, J. Phys. Chem., B 109 (2005) 7046–7052.

[39] L.A. Naslund, M. Cavalleri, H. Ogasawara, A. Nilsson, L.G.M. Pettersson, P. Wernet, D.C. Edwards, M. Sandstrom, S. Myneni, Direct evidence of orbital mixing between water and solvated transition-metal ions: an oxygen 1s XAS and DFT study of aqueous systems, J. Phys. Chem., A 107 (2003) 6869–6876.

[40] O.Y. Samoilov, in: R.A. Horne (Ed.), Residence Times of Ionic Hydration, Water and Aqueous Solutions: Structure, Thermodynamics, and Transport Processes, Wiley-Interscience, New York, 1972, pp. 597–612.

[41] J.E. Combariza, N.R. Kestner, J. Jortner, Energy–structure relationships for microscopic solvation of anions in water clusters, J. Chem. Phys. 100 (1994) 2851–2864.

[42] D. Asthagiri, L.R. Pratt, M.E. Paulaitis, S.B. Rempe, Hydration structure and free energy of biomolecularly specific aqueous dications, including $Zn2^+$ and first transition row metals, J. Am. Chem. Soc. 126 (2004) 1285–1289.

[43] G. Nadig, L.C. Van Zant, S.L. Dixon, K.M. Merz Jr., Charge–transfer interactions in macromolecular systems: a new view of the protein/water interface, J. Am. Chem. Soc. 120 (1998) 5593–5594.

[44] D.F.C. Morris, Ionic radii and enthalpies of hydration of ions, Struct. Bond. 6 (1969) 157–159.

[45] S. Ramos, G.W. Neilson, A.C. Barnes, P. Buchanan, An anomalous X-ray diffraction study of the hydration structures of Cs^+ and I− in concentrated solutions, J. Chem. Phys. 123 (2005).

[46] S. Forsen, J. Kordel, in: I. Bertini, H.B. Gray, S.J. Lippard, J.S. Valentine (Eds.), Calcium in Biological Systems, Bioinorganic Chemistry, University Science Books, Mill Valley, CA, 1994, pp. 107–166.

[47] L. Vrbka, J. Vondrasek, B. Jagoda-Cwiklik, R. Vacha, P. Jungwirth, Quantification and rationalization of the higher affinity of sodium over potassium to protein surfaces, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 15440–15444.

[48] A.S. Woods, S. Ferre, Amazing stability of the arginine-phosphate electrostatic interaction, J. Proteome Res. 4 (2005) 1397–1402.

[49] N. Sakai, S. Futaki, S. Matile, Anion hopping of (and on) functional oligoarginines: from chloroform to cells, Soft Matter 2 (2006) 636–641.

[50] E. Di Cera, A structural perspective on enzymes activated by monovalent cations, J. Biol. Chem. 281 (2006) 1305–1308.

[51] M.J. Page, E. Di Cera, Role of Na^+ and K^+ in enzyme function, Physiol. Rev. 86 (2006) 1049–1092.

[52] P.S. Ledvina, A.L. Tsai, Z. Wang, E. Koehl, F.A. Quiocho, Dominant role of local dipolar interactions in phosphate binding to a receptor cleft with an electronegative charge surface: equilibrium, kinetic, and crystallographic studies, Protein Sci. 7 (1998) 2550–2559.

[53] W.W. Cleland, Low-barrier hydrogen bonds and enzymatic catalysis, Arch. Biochem. Biophys. 382 (2000) 1–5.

[54] A.S. Mildvan, M.A. Massiah, T.K. Harris, G.T. Marks, D.H.T. Harrison, C. Viragh, P.M. Reddy, I.M. Kovach, Short, strong hydrogen bonds on enzymes: NMR and mechanistic studies, J. Mol. Struct. 615 (2002) 163–175.

[55] P.A. Frey, Strong hydrogen bonding in chymotrypsin and other serine proteases, J. Phys. Org. Chem. 17 (2004) 511–520.

[56] P.A. Frey, in: A. Kohen, H.-H. Limbach (Eds.), Isotope Effects in the Characterization of Low Barrier Hydrogen Bonds, Isotope Effects In Chemistry and Biology, CRC, Boca Raton, 2005, pp. 975–993.

[57] P.S. Ledvina, N. Yao, A. Choudhary, F.A. Quiocho, Negative electrostatic surface potential of protein sites specific for anionic ligands, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 6786–6791.

[58] F.A. Quiocho, P.S. Ledvina, Atomic structure and specificity of bacterial periplasmic receptors for active transport and chemotaxis: variation of common themes, Mol. Microbiol. 20 (1996) 17–25.

[59] N.K. Vyas, M.N. Vyas, F.A. Quiocho, Crystal structure of M tuberculosis ABC phosphate transport receptor: specificity and charge compensation dominated by ion-dipole interactions, Structure 11 (2003) 765–774.

[60] Z. Wang, A. Choudhary, P.S. Ledvina, F.A. Quiocho, Fine tuning the specificity of the periplasmic phosphate transport receptor. Site-directed mutagenesis, ligand binding, and crystallographic studies, J. Biol. Chem. 269 (1994) 25091–25094.

[61] A.K. Meeker, B. Garcia-Moreno, D. Shortle, Contributions of the ionizable amino acids to the stability of staphylococcal nuclease, Biochemistry 35 (1996) 6443–6449.

[62] A.V. Gribenko, G.I. Makhatadze, Role of the charge–charge interactions in defining stability and halophilicity of the CspB proteins, J. Mol. Biol. 366 (2007) 842–856.

[63] J. Antosiewicz, J.A. McCammon, M.K. Gilson, Prediction of pH-dependent properties of proteins, J. Mol. Biol. 238 (1994) 415–436.

[64] V. Dillet, H.J. Dyson, D. Bashford, Calculations of electrostatic interactions and p*K*as in the active site of *Escherichia coli* thioredoxin, Biochemistry 37 (1998) 10298–10306.

[65] Y.Y. Sham, I. Muegge, A. Warshel, The effect of protein relaxation on charge-charge interactions and dielectric constants of proteins, Biophys. J. 74 (1998) 1744–1753.

[66] D.A. Karp, A.G. Gittis, M.A. Stahley, C.A. Fitch, W.E. Stites, B. Garcia-Moreno, High apparent dielectric constant inside a protein reflects structural reorganization coupled to the ionization of an internal asp, Biophys. J. 92 (2007) 2041–2053.

[67] R.L. Thurlkill, G.R. Grimsley, J.M. Scholtz, C.N. Pace, Hydrogen bonding markedly reduces the p*K* of buried carboxyl groups in proteins, J. Mol. Biol. 362 (2006) 594–604.

[68] G.N. Lewis, M. Randall, The activity coefficient of strong electrolytes, J. Am. Chem. Soc. 43 (1921) 1112–1154.

[69] E. Di Stasio, C. Nagaswami, J.W. Weisel, E. Di Cera, Cl− regulates the structure of the fibrin clot, Biophys. J. 75 (1998) 1973–1979.

[70] M. Ries-Kautt, A. Ducruix, Inferences drawn from physicochemical studies of crystallogenesis and precrystalline state, Methods Enzymol. 276 (1997) 23–59.

[71] I. Jelesarov, E. Durr, R.M. Thomas, H.R. Bosshard, Salt effects on hydrophobic interaction and charge screening in the folding of a negatively charged peptide to a coiled coil (leucine zipper), Biochemistry 37 (1998) 7539–7550.

[72] J. Sunner, P. Kebarle, Ion-solvent molecule interactions in the gas phase. The potassium ion and Me2SO, DMA, DMF and acetone, J. Am. Chem. Soc. 106 (1984) 6135–6139.

[73] T. Arakawa, S.N. Timasheff, Mechanism of protein salting in and salting out by divalent cation salts: balance between hydration and salt binding, Biochemistry 23 (1984) 5912–5923.