Charge Density-Dependent Strength of Hydration and Biological Structure

Kim D. Collins
Department of Biochemistry and Molecular Biology, University of Maryland Medical School, Baltimore, Maryland 21201-1503 USA

ABSTRACT Small ions of high charge density (kosmotropes) bind water molecules strongly, whereas large monovalent ions of low charge density (chaotropes) bind water molecules weakly relative to the strength of water-water interactions in bulk solution. The standard heat of solution of a crystalline alkali halide is shown here to be negative (exothermic) only when one ion is a kosmotrope and the ion of opposite charge is a chaotrope; this standard heat of solution is known to become proportionally more positive as the difference between the absolute heats of hydration of the corresponding gaseous anion and cation decreases. This suggests that inner sphere ion pairs are preferentially formed between oppositely charged ions with matching absolute enthalpies of hydration, and that biological organization arises from the noncovalent association of moieties with matching absolute free energies of solution, except where free energy is expended to keep them apart. The major intracellular anions (phosphates and carboxylates) are kosmotropes, whereas the major intracellular monovalent cations (K^+; arg, his, and lys side chains) are chaotropes; together they form highly soluble, solvent-separated ion pairs that keep the contents of the cell in solution.

INTRODUCTION

Despite the considerable progress that has been made in the study of ions in aqueous solution, the origin of many ion-specific effects is still considered largely a mystery. For example, there has been no compelling systematic explanation for the ion pairing preferences manifested in the chromatographic selectivity series of ion exchange resins, or for the systematic variation in the solubility of simple salts. We have shown earlier that many of the properties of aqueous ionic solutions are a function of the charge density of the ions, that the strength of water-water interactions in bulk solution (which acts as a critical reference energy level) is comparable to the strength of ion-water interactions, and that chaotropes are monovalent ions of low charge density that bind the immediately adjacent water molecules less strongly than water binds itself (Collins and Washabaugh, 1985; Washabaugh and Collins, 1986; Collins, 1995).

Ions in solution have most often been treated with the model of continuum electrostatics. This model, framed in terms of point charges, is macroscopic (ignores microscopic structure), treats charge density effects as a "nonideality" that can be ignored to a first approximation, does not specify the strength of water-water interactions or treat this interaction as a critical energy level, and effectively assumes, among other things, that chaotropes do not exist (Perkyns and Pettitt, 1994; Collins, 1995). We offer here an alternative description of how ions behave in aqueous solution that treats charge density as a central determinant of the structure and function of biological systems. Specifically, we hypothesize that most properties of biological systems arise from the fact that small ions of high charge density bind water molecules tightly, whereas large monovalent ions of low charge density bind water molecules weakly (relative to the strength of water-water interactions in bulk solution). This hypothesis includes polar molecules, which can be represented by partial charges, and nonpolar molecules, which can be considered to be ions in the limit of very low charge density. The hypothesis is descriptive in microscopic terms and empirically based, arising directly from viscosity, NMR, thermodynamic, transport, x-ray, and neutron diffraction data (Collins and Washabaugh, 1985; Collins, 1995). A simple model of ions in aqueous solution that demonstrates how differences in ion size and thus charge density lead to the observed preferences in ion pairing is also presented.

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Address reprint requests to Dr. Kim D. Collins, Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 108 N. Greene Street, Baltimore, MD 21201-1503. Tel.: 410-706-7199; Fax: 410-706-8297; E-mail: kcollins@umabnet.ab.umd.edu.

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RESULTS AND DISCUSSION

The origin of ion pairing preferences

Fig. 1 illustrates the change in the mobility (entropy, ΔS_{II}) of water molecules caused by the presence of nearby ions as calculated from thermodynamic data; this entropy change is plotted as a function of the radius of the ions. The horizontal line $\Delta S_{II} = 0$ defines the size of cations (1.06 Å) and anions (1.78 Å) that do not change the entropy of nearby water molecules; "large" and "small" in the ensuing discussion means relative to these sizes. The small ions, which are above the horizontal line and decrease the mobility of nearby water molecules, flow through a Sephadex G-10 gel sieving column, with an apparent molecular weight greater than their anhydrous molecular weight, indicating that the immobilized water molecules are attached to the ion and move with it. These ions are called kosmotropes[1] (Collins,

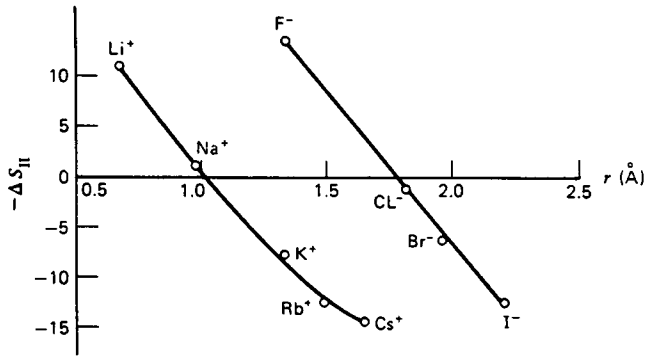

FIGURE 1 The entropy of water near an ion minus the entropy of pure water in cal·°K^{-1}·mol^{-1}. The crystal radii of the ions in angstroms are plotted along the abscissa. Positive values of ΔS_{II} (*lower portion of figure*) indicate water that is more mobile than bulk water. Negative values of ΔS_{II} (*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 (1991) as presented by Samoilov (1972). Reprinted with permission of John Wiley and Sons ©1972.

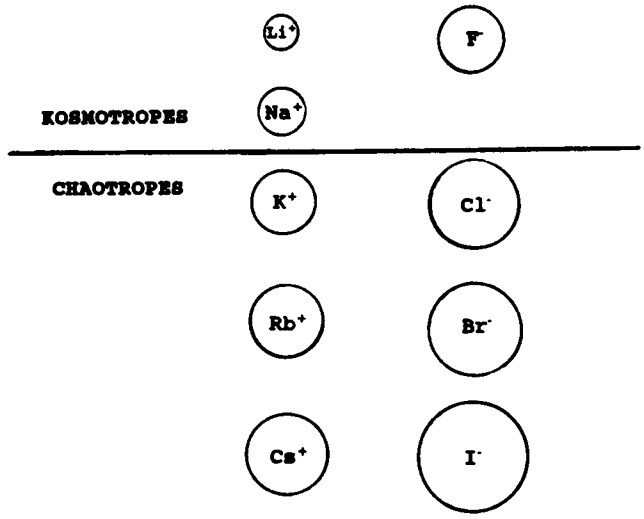

FIGURE 2 Division of the group IA cations and the halide anions into kosmotropes (water structure makers) and chaotropes (water structure breakers); the ions are drawn approximately to scale.

1995). F^-, for example, flows through the column with 5.3 attached water molecules. The large ions, which are below the horizontal line and increase the mobility of nearby water molecules, adsorb to the nonpolar surface of a Sephadex G-10 column and chromatograph with an apparent molecular weight smaller than their anhydrous molecular weight, indicating that the water molecules of increased mobility are in contact with the ion and are easily removed from it; these ions are called chaotropes (Collins, 1995). Therefore the horizontal line $\Delta S_{II} = 0$ separates small ions that bind water strongly (above the line) from large monovalent ions that bind water weakly (below the line). [All multivalent ions are strongly hydrated (Krestov, 1991)]. Fig. 1 also shows that anions are more strongly hydrated than cations for a given charge density, because the anions begin to bind the immediately adjacent water molecules strongly at a lower charge density; this is also shown by Fig. 2, in which the ions are drawn approximately to scale. There are at least two reasons for the stronger hydration of the anions. First, quantum-mechanical calculations indicate that the anions, which interact with the hydrogen atom of water, allow intrashell hydrogen bonding of the solvating waters, whereas cations, which interact with the oxygen atom of water, do not (Combariza et al., 1994). Second, charge transfer to solvent characterizes strong hydration; because the oxygen atom of water is very electronegative, it is easier to accept negative charge from anions than positive charge from cations (Collins and Washabaugh, 1985).

The solubility of simple inorganic salts shows a striking pattern, as illustrated in Table 1 (Lee, 1991); the least soluble halide in each column has been underlined. Large ions paired with small ions give highly soluble salts (lower left and upper right corners of Table 1), whereas salts containing only large ions (lower right corner of Table 1) or small ions (upper left corner of Table 1) are only moderately soluble.[2] Although lattice enthalpies play a role in solubilities (Shriver et al., 1994), we shall focus here on the tendency of ions to form inner sphere ion pairs in solution, as illustrated in Eq. 1 (discussed below).

$$M^{n+}(\text{hydrate}) + X^{n-}(\text{hydrate}) \rightleftarrows MX(\text{hydrate}) + mH_2O. \quad (1)$$

We shall treat this process as a simple exchange reaction in which an ion replaces a water molecule in its inner coordination sphere with an ion of opposite charge. We now make two assumptions. First, we shall treat an ion as a point charge inside a sphere with a radius equal to that of the ion. Second, to illustrate charge density effects on solvation, we shall define a zwitterion of radius 1.78 Å for the anionic portion and 1.06 Å for the cationic portion to be a "virtual" water molecule (see Fig. 3).[3] Small ions are strongly hydrated because their point charge is close to the point charge of opposite sign on the water molecule, whereas large ions are weakly hydrated because their point charge is distant from the point charge of opposite sign on the water molecule (Fig. 3). It is energetically unfavorable to strip a water molecule off of a small ion, but the energy lost is more than regained when another small ion takes the place of the water molecule, because the two point charges of opposite sign in the newly formed neutral salt are closer together. Therefore, formation of small-small inner sphere ion pairs is energetically favorable. Similarly, although the interaction between the distant point charges of a large-large ion pair is weak, removal of water molecules from large ions leads to new water-water interactions that are stronger than large ion-water interactions, and thus formation of large-large inner sphere ion pairs is also energetically favorable. In contrast, the work done in stripping a water molecule off of a small ion is not regained by replacement with a large ion, because the point charge of the large ion is too distant from the point

TABLE 1 Solubilities of Group I halides

	Solubility (Molar value first, g/100 g H_2O given in brackets)			
	MF	MCl	MBr	MI
Li	0.1 (0.27)	19.6 (830)	20.4 (177)	8.8 (165)
Na	1.0 (4.22)	6.2 (36)	8.8 (91)	11.9 (179)
K	15.9 (92.3)	4.8 (34.7)	7.6 (67)	8.7 (144)
Rb	12.5 (130.6)	7.5 (91)	6.7 (110)	7.2 (152)
Cs	24.2 (367.0)	11.0 (186)	5.1 (108)	3.0 (79)

Source: Lee (1991).

charge of the small ion to interact strongly with it. Thus large-small ion pairs tend not to form inner sphere ion pairs; they remain apart in aqueous solution and are thus highly soluble. A solution consisting of ions of various sizes will tend to segregate according to size (Fig. 4): the small ions of opposite sign and comparable size will tend to pair because they form stronger interactions than those between small-large ion pairs; the medium-sized ions of opposite sign and comparable size will also tend to pair because they form stronger interactions than those between medium-large ion pairs; and the large ions of opposite sign and comparable size will also tend to pair because their formation releases water for formation of stronger water-water interactions.

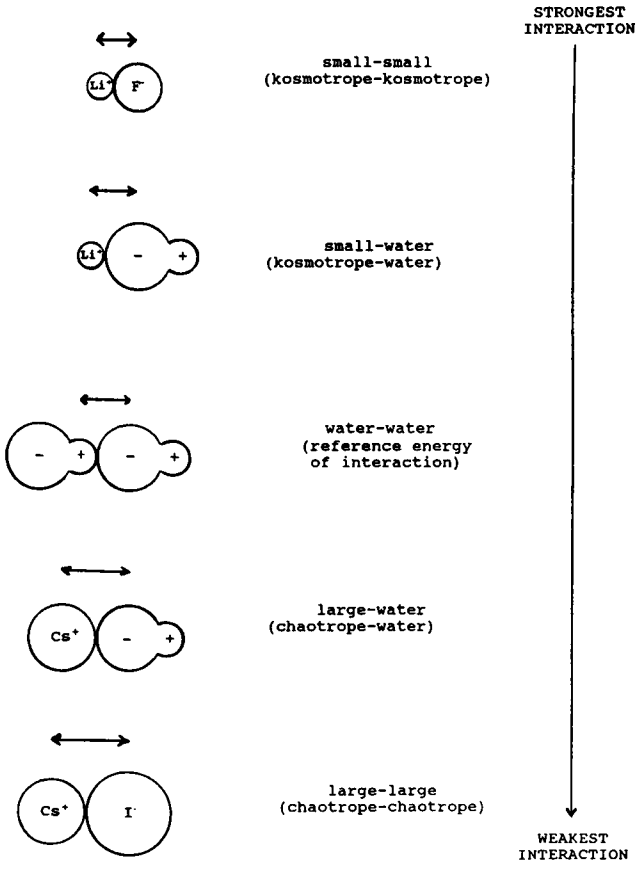

FIGURE 3 Rank-ordering of the interactions in aqueous salt solutions as a function of ion size from strongest (*top*) to weakest (*bottom*). The ions (and water) are modeled as spheres of the appropriate size with a point charge at the center. The strongest interactions occur when point charges of opposite sign are closest together. The "virtual" water molecule used to demonstrate charge density effects is modeled as a zwitterion with a radius of 1.06 Å for the positive portion and a radius of 1.78 Å for the negative portion.

Thermodynamic properties of ion pairs

Fig. 5 (Morris, 1969, after Fajans, 1921) shows that the standard heat of solution (at infinite dilution) of a crystalline alkali halide kosmotrope plus chaotrope neutral salt is negative (exothermic), whereas the standard heat of solution of a crystalline alkali halide kosmotrope plus kosmotrope or chaotrope plus chaotrope salt is positive (endothermic), suggesting that kosmotrope plus chaotrope neutral salts dissociate extensively upon dissolution, and that the kosmotropic ion of this salt acquires stronger interactions in solution than in the crystal.[4] This is supported by neutron diffraction solution data of kosmotrope plus chaotrope salts such as Cr(III) perchlorate, which shows six water molecules in the inner hydration sphere of the strongly hydrated Cr(III), with no penetration by the weakly hydrated perchlorate (Broadbent et al., 1992). This appears to be generally true of kosmotrope plus chaotrope salts (Neilson et al., 1995), although concentration and charge density affect their behavior; for example, at moderate concentrations the marginally chaotropic Cl^- penetrates the inner hydration sphere of the kosmotropic Cu^{2+} and Zn^{2+} ions (Neilson et al., 1995; Powell et al., 1990).

"The absolute heat of hydration of an ion ... may be defined as the increase of enthalpy accompanying the solution of one mole of the gaseous ion in a very large excess of water at 1 atm and 298.2° K" (Morris, 1968). Because the strength of interaction of an ion with water is a good measure of the strength of interaction of the same ion with other ions of opposite charge, those crystalline salts containing ions of opposite charge with the most closely matched absolute enthalpies of solution will form inner sphere ion pairs in solution most readily and will thus have the most positive (most endothermic) enthalpies of solution, producing the "volcano relationship" of Fig. 5 (Morris, 1969, after Fajans, 1921). Strong interactions form at the expense of weaker interactions in solution, and the system as a whole maximizes the number of strong interactions. There is also interaction between the ligands of an ion. When water molecules are added one at a time to an ion in the gas phase, each succeeding water molecule binds less tightly than the one before until the inner hydration sphere is filled, and thus the average ion-water molecule energy of interaction decreases until the inner hydration sphere is filled (Arshadi et al., 1970; Dzidic and Kebarle, 1970; Blades et al., 1995). One can think of an ion paired with an oppositely charged ion of very low charge density almost as having a vacant coordination position, thus strengthening its interactions with its other ligands, usually water. Taking into consideration the effects of concentration and of releasing water molecules upon inner sphere ion pair formation (i.e., taking into consideration the effects of entropy), we

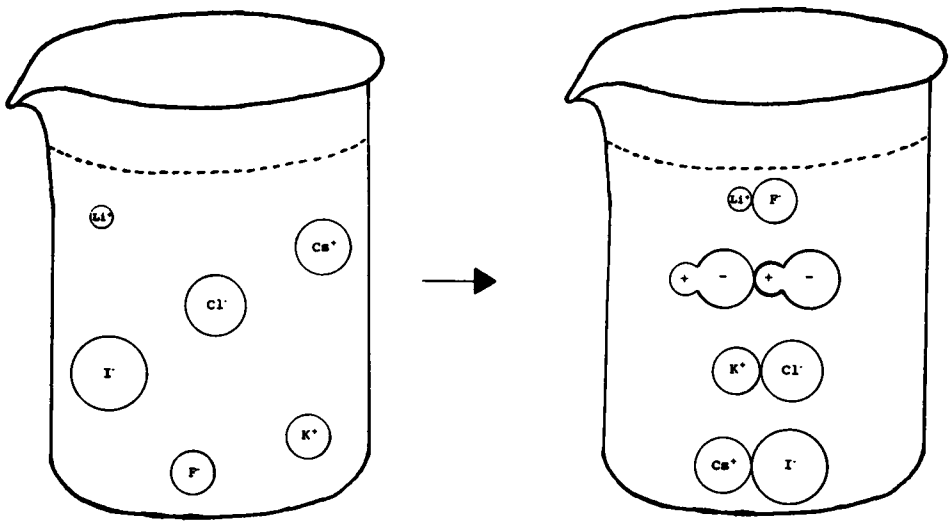

FIGURE 4 Segregation of the ions in an aqueous salt solution according to charge density and thus size; oppositely charged ions of similar charge density and thus size tend to form inner sphere ion pairs. Chaotropes are shown to ion pair with chaotropes, and kosmotropes are shown to ion pair with kosmotropes. The model assumes that the least soluble salts are the most likely to form inner sphere ion pairs.

see from Fig. 6 (Morris, 1969, after Fajans, 1921) that the least soluble salts (those composed of ions that associate most readily) are those whose constituent ions have the most closely matched absolute free energies of solution. Because polar molecules can be represented with partial charges and nonpolar molecules can be considered to be ions in the limit of very low charge density, it appears that biological structure in general reflects the tendency of moieties with matching absolute free energies of solution to associate. This can be considered an extension of the well-known phenomenon that "like dissolves like" (Israelachvili, 1992, sections 6.7–6.8, pp. 99–105), and that liquid species segregate according to their molar cohesion energies (Israelachvili, 1992, section 9.1, pp. 139–143). Thus protein folding results from a tendency of nonpolar amino acid side chains to associate and amide groups to polymerize, consistent with the demands of close-packed interiors, hydrogen bond formation of polar side chains, and keeping ionized groups in contact with water.

Properties of Ca^{2+} and Mg^{2+}

A good illustration of charge density effects may be found in the comparison of Mg^{2+} (radius = 0.72 Å) and Ca^{2+}

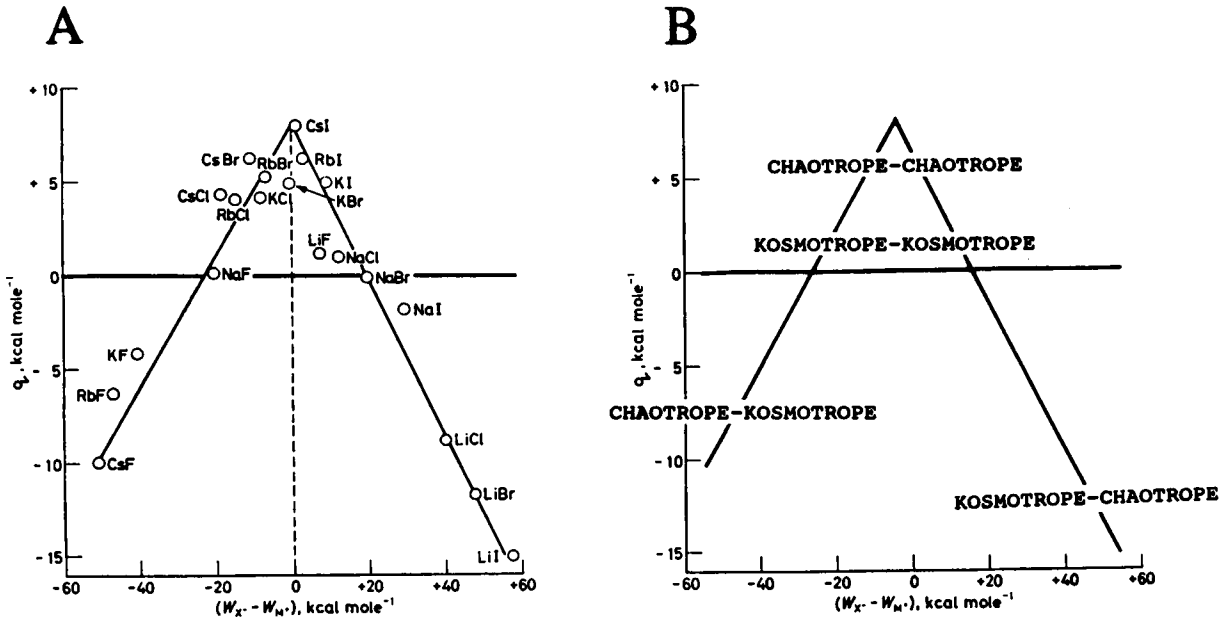

FIGURE 5 (*A*) Relationship between the standard heat of solution of a crystalline alkali halide (at infinite dilution) in kcal mol^{-1} and the difference between the absolute heats of hydration of the corresponding gaseous anion and cation, also in kcal mol^{-1}. (Source: Morris (1969). Copyright Springer-Verlag. 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 is positive (takes up heat), whereas the enthalpy of solution of chaotrope-kosmotrope and kosmotrope-chaotrope salts is negative (gives off heat).

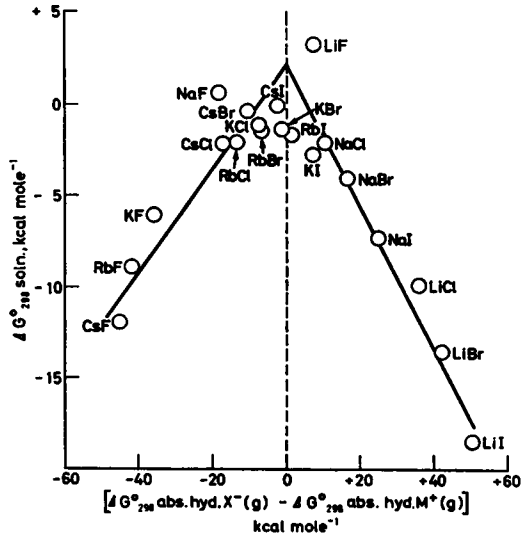

FIGURE 6 Relationship between the standard free energies of solution (at infinite dilution) of alkali halides ($\Delta G°_{298}$ soln.) in kcal mol^{-1} and the difference between the absolute free energies of hydration of the corresponding gaseous anion and cation, also in kcal mol^{-1}. Source: Morris (1969). Copyright Springer-Verlag. Reprinted with permission.

(radius = 1.00 Å) (Sharpe, 1992). The surface charge densities of Mg^{2+}, Ca^{2+}, Na^+, and K^+ are presented in Table 2; the surface charge density of Mg^{2+} is 7.3 times greater than that of K^+. At high surface charge densities, ion-water interactions become partially covalent, there is substantial charge transfer to the solvent, and the bound water molecules are partially ionized. For example, the net charge on the central Mg^{2+} of $Mg[H_2O]_6^{2+}$ has been calculated to be only 1.18 (Bock et al., 1994). Under these circumstances, representing the hydrating water molecule as a net-neutral zwitterion of fixed size is obviously an oversimplification. Ca^{2+}, with a charge density apparently well matched to that of phosphate, forms only sparingly soluble salts with phosphate (Talmage, 1996), whereas the smaller Mg^{2+}, with its "excess" charge density, forms moderately soluble salts with phosphate (Cowan, 1995; Martin, 1995; Williams, 1993) and typically remains partially hydrated. Mg^{2+} in biological systems interacts with cellular components via both inner and outer sphere complexes (Porschke, 1995), whereas Ca^{2+} apparently favors inner sphere complexes. Ca^{2+} also readily forms insoluble carboxylate salts, and Ca^{2+} binding sites on proteins typically involve carboxylate groups (Forsen and Kordel, 1994; Frausto da Silva and Williams, 1991). We postulate that a principal function of ion pumps is to separate ionic species such as calcium and phosphate, which have a strong tendency to form inner sphere ion pairs, the first step in forming a crystalline solid; most biological molecules must be in solution to function.

The intracellular Ca^{2+} concentration is about 10^{-7} M (Hille, 1992, p. 84), and extracellular Ca^{2+} is about 2.5 mM, whereas intracellular Mg^{2+} is 40 mM (0.5 mM of which is free) and extracellular Mg^{2+} is 1 mM (West, 1990).

TABLE 2 Characteristics of the four biologically relevant alkali and alkaline earth metal cations

Property	K^+	"H_2O" (+ portion)	Na^+	Ca^{2+}	Mg^{2+}
Ionic radius r (pm)	138	106	102	100	72
Surface area of spherical ion (in pm^2) $O = 4\pi r^2$	239,300	141,100	130,700	125,700	65,100
Surface charge density q/O (relative value)	0.59	1.0	1.06	2.24	4.3

Ionic radii from Sharpe (1992); table revised from Kaim and Schwederski (1994).

The Jones-Dole viscosity B coefficient

Individual ions may be systematically classified as chaotropes or kosmotropes by the sign of the Jones-Dole (Jones and Dole, 1929) viscosity B coefficient (negative and positive, respectively), as shown by the pioneering work of Cox and Wolfenden (1934), which was later extended by Kaminsky (1957), Stokes (Robinson and Stokes, 1959; Stokes and Mills, 1965), and others (Robinson et al., 1981). The viscosity B coefficient correlates with charge density and is defined by the expression

$$\eta/\eta_o = 1 + Ac^{1/2} + Bc,$$

which is valid at concentrations (c) up to about 0.1 M for binary strong electrolytes. η is the viscosity of an aqueous salt solution; η_o is the viscosity of water at the same temperature; A is an electrostatic term that can be neglected at moderate concentrations; and B is a measure of ion-water interactions. Viscosity B coefficients for a number of relevant ions are given in Table 3. The viscosity B coefficients of Table 3 are linearly related to the ΔS_{II} values of Fig. 1 after suitable approximations (Krestov, 1991, p. 178).

TABLE 3 Jones-Dole viscosity B coefficients

Cations	B	Anions	B
Mg^{2+}	0.385	PO_4^{3-}	0.590
Ca^{2+}	0.285	$CH_3CO_2^-$	0.250
Ba^{2+}	0.22	SO_4^{2-}	0.208
Li^+	0.150	F^-	0.10
Na^+	0.086	HCO_2^-	0.052
K^+	−0.007	Cl^-	−0.007
NH_4^+	−0.007	Br^-	−0.032
Rb^+	−0.030	NO_3^-	−0.046
Cs^+	−0.045	ClO_4^-	−0.061
		I^-	−0.068
		SCN^-	−0.103

Sources: Phosphate, formate, and perchlorate from Krestov (1991); all others from Robinson et al. (1981).

Intracellular ions

All of the major intracellular anions [phosphates (Washabaugh and Collins, 1986), sulfates, and carboxylates (Table 3)] are kosmotropes, and all of the major intracellular monovalent cations (K^+; and the nitrogen-based lysine ϵ-ammonium, arginine δ-guanidinium, and histidine imidazolium) are chaotropes.[5] These oppositely charged kosmotropes and chaotropes should form highly soluble, solvent-separated ion pairs, keeping the contents of the cell in solution. The negatively charged Cl^-, which is in low intracellular concentration (3 mM, as compared to an extracellular concentration of 115 mM; West, 1990), is marginally a chaotrope (see Fig. 1). This rough separation of intracellular ions into positively charged monovalent chaotropes and negatively charged kosmotropes, which together form highly soluble, solvent-separated ion pairs, also implies that inner sphere intramolecular ion pairs between nitrogen-based chaotropes[5] and carboxylate kosmotropes (Table 3) on proteins are not strongly favored and are thus unlikely to be the major source of protein stabilization; indeed, a study of systematic substitution with both alanine and glycine of all the charged residues (including salt bridges and charged hydrogen bonds) in staphylococcal nuclease concludes that "ionizable amino acids contribute to stability predominantly through packing and bonding interactions that do not depend on their electrostatic charge" (Meeker et al., 1996). Hofmeister interactions are dominated by the anions (Collins and Washabaugh, 1985), and an important effect of the kosmotropic polyphosphates in the cell is to produce an environment that is highly stabilizing for native protein structure; these polyphosphates appear to exist as (presumably solvent separated) K^+ salts in vivo (Guttman et al., 1995).

Ion pumps and solubility products

We postulate that the principal original role of the continuously operating (Ca^{+2})- and (Na^+-K^+)-ATPase membrane pumps may have been to keep the kosmotropic Ca^{+2} and Na^+ ions, respectively, away from the kosmotropic intracellular phosphates, and that the resulting ionic gradients have been subsequently adapted for signaling and transport purposes. Intracellular Ca^{2+} concentrations are about 10^{-7} M, whereas extracellular Ca^{2+} is about 2.5×10^{-3} M, or some 10,000-fold higher. "Primary calcium phosphate ($Ca(H_2PO_4)_2$) is extremely soluble. Secondary calcium phosphate ($CaHPO_4$) has a solubility of around 2×10^{-3} M. Tertiary calcium phosphate ($Ca_3(PO_4)_2$) has a solubility only slightly greater than 10^{-4}M" (Talmage, 1996). About 85% of the Ca^{2+} in solution in the extracellular fluid of mammals is secondary calcium phosphate; this is not a supersaturated solution with respect to secondary calcium phosphate. However, the extracellular fluid of mammals is supersaturated with respect to bone [tertiary calcium phosphate ($Ca_3(PO_4)_2$)], and Ca^{2+} must be continually returned to fluids from bone through the utilization of metabolic energy (Talmage, 1996). Thus the fundamental role of Ca^{2+} pumps may be to oppose the natural tendency of Ca^{2+} to form inner sphere ion pairs with intracellular and bone phosphate.[6] The high extracellular Ca^{2+} concentration allows it to enter cells rapidly for signaling purposes.

The solubility of K_2HPO_4 is about 8.6 M (Dean, 1992), and that of $Na_2HPO_4 \cdot 7H_2O$ is about 0.93 M (Dean, 1992). Li_2HPO_4 is only slightly soluble, precipitating readily in the presence of K^+ (Spector et al., 1957), and RNA is precipitated by 4 M LiCl (Cathala et al., 1983). The body fluid concentrations of K^+ are 159 mM (intracellular) and 4 mM (extracellular); for Na^+ they are 10 mM (intracellular) and 150 mM (extracellular) (West, 1990). Dog erythrocytes, which do not have nuclei, do not maintain Na^+ or K^+ gradients across their membrane (Parker et al., 1995), supporting the argument that the original purpose of the Na^+,K^+ ATPase was to replace inner sphere Na^+-nucleic acid phosphate oxyanion ion pairs with solvent separated K^+-nucleic acid phosphate oxyanion ion pairs.

Charge neutralization on proteins

The effect of salts at pH 7 on proteins and amino acids can be divided roughly into two separate concentration ranges. Up to a concentration of about 0.1–0.3 M salt, the effects are due mostly to the neutralization of charges on the protein (i.e., formation of inner sphere ion pairs) (Melander and Horvath, 1977). These effects can lead to *salting in* (increased solubility) when intramolecular ion pairs (ion pairs within or between protein subunits) are replaced by protein-small ion pairs (Fox and Foster, 1957; Green and Hughes, 1952; Cohn, 1936 and 1943; Edsall and Wyman, 1958; Ries-Kautt and Ducruix, 1989 and 1991) or *salting out* (decreased solubility) (Fink, 1995; Ries-Kautt and Ducruix, 1989 and 1991) when the formation of protein-small ion pairs abolishes net charge on the protein. Above 0.1–0.3 M salt, the effects are largely on the neutral parts of the protein, producing a *salting out* effect (decreasing solubility) because of a salt-induced increase in surface tension when kosmotropic ions are used (e.g., SO_4^{-2}) (Melander and Horvath, 1977) or a *salting in* effect when chaotropic ions are used (e.g., guanidinium) because of direct interactions with the protein (Lin and Timasheff, 1996, and references therein). We have thoroughly reviewed ion-specific effects on proteins in the high-concentration region (Collins and Washabaugh, 1985), and one major conclusion is that kosmotropes at high concentration stabilize proteins, but chaotropes at high concentrations destabilize proteins. Neutralization of the chaotropic cations of positively charged proteins with chaotropic anions in the low concentration region leads to more compact or crystalline structures (Goto and Nishikiori, 1991; Fink, 1995; Ries-Kautt and Ducruix, 1989 and 1991; Barkley et al., 1981). These chaotropic anions are known to adsorb to nonpolar surfaces (Washabaugh and Collins, 1986) and thus are also expected to adsorb to the nonpolar portions of arg, his, and lys side

chains; the chaotropic anions may therefore not be well matched in charge density to the protein cations being neutralized. Although no mechanism has been established, chaotropic anions have also been shown to shift the insulin hexamer from the T to the R state (Brzovic et al., 1994); and the chaotropic anion ClO_4^- at 10 mM potentiates excitation-contraction coupling in mammalian skeletal muscles (Gallant et al., 1993).

The special role of chloride

Because Cl^- is the only chaotropic anion in physiological fluids, it appears to have a particularly intimate relationship with the chaotropic cation groups on proteins. K^+ and Cl^- have a strong tendency to form inner sphere ion pairs, as shown by the moderate solubility of KCl (Table 1) and the positive standard enthalpy of solution of KCl (Fig. 5). NH_4^+ and K^+ have similar charge density characteristics, as shown by their viscosity B coefficients (Table 2). Neutron diffraction (Walker et al., 1989; Adya and Neilson, 1991), NMR (Perrin and Gipe, 1987), and Monte Carlo techniques all indicate that NH_4^+ is weakly hydrated. Thus the weakly hydrated Cl^- should readily form inner sphere ion pairs with nitrogen-based protein cationic side chains, as neutron diffraction studies show that it does with cationic polyethylene imine (Bieze et al., 1994). As the pH is lowered below 7 and more positively charged imidazolium groups are created on proteins, Cl^- binding increases (Carr, 1953), stabilizing the protein (Johnson et al., 1995). Lysozyme, with its net positive charge, crystallizes most readily from solutions containing Cl^- or other chaotropic anions (Ries-Kautt and Ducruix, 1989 and 1991), and acid-denatured proteins are more compact when Cl^- or other chaotropic ions are present (Goto and Nishikiori, 1991; Fink, 1995; Oliveberg et al., 1994). Cl^- has a much bigger salting in effect than does sulfate (Green, 1932), and lysine and arginine residues have been implicated in the Cl^- channel of the cystic fibrosis transmembrane conductance regulator (Gadsby et al., 1995). Cl^- binding to a cluster of positive residues on hemoglobin is linked to O_2 release (Colombo et al., 1994), and the introduction of a new arginine at $\beta 37$ creates a new Cl^- binding site (Kelley et al., 1994). Cl^--arginine interactions have been identified in thermolysin (Yang et al., 1994) and shown to be the basis of Cl^- transport by halorhodopsin (Braiman et al., 1994; Rudiger et al., 1995). Chloride channels are generally permeable to many small chaotropic anions such as Br^-, I^-, NO_3^-, and SCN^-, as might be expected for a channel containing a chaotropic cationic binding site for Cl^- (Hille, 1992, p. 136). A Cl^--Schiff's base interaction has also been detected in halorhodopsin from two different bacteria (Walter and Braiman, 1994; Scharf and Engelhard, 1994). When the asp of an asp-arg salt link in an *Escherichia coli* phosphate-binding protein was mutated to a gly or thr, a Cl^- was found to have replaced the asp carboxylate (Yao et al., 1996). Less well characterized requirements for Cl^- have been reported for several proteins (Scott et al., 1994; Tachibanaki et al., 1995; Orii et al., 1995; Lamhasni et al., 1995; van Vliet et al., 1994).

DNA-protein interactions

Record and co-workers (Ha et al., 1992) have shown that binding of the *lac* repressor to DNA in vitro is 40- to 300-fold stronger when Cl^- is replaced by glu^- (the physiologically important anion in *E. coli*), and similar findings have been reported for at least six other DNA-binding proteins (cited in Ha et al., 1992). Only the kosmotrope F^- has an effect comparable to that of glu^- (Kowalczykowski et al., 1981; Overman et al., 1988). Record and co-workers (Ha et al., 1992), conclude that glu^- is an "inert" anion, whereas Cl^- competes with DNA phosphate groups in binding to the *lac* repressor. We are now in a position to explain why this should be true. Whereas Cl^- is a small chaotrope that readily forms inner sphere ion pairs with chaotropic positive groups on proteins, glu^- is much larger and contains two kosmotropic carboxylates, making it very unlikely to form inner sphere ion pairs with protein cations; the kosmotrope F^- is also very unlikely to form inner sphere ion pairs with the chaotropic cations on proteins, explaining its behavior in this system. This explanation is also consistent with the observation that the apparent enthalpy of binding of *E. coli* single-stranded binding protein to poly(U) is a strong function of Cl^- concentration but not of F^- concentration (Lohman et al., 1996). It is interesting to note that the nitrogen-based cations of proteins are chaotropes,[5] whereas the DNA phosphate groups with which they interact are kosmotropes,[7] suggesting that other kinds of DNA-protein interactions are the important ones. This must be true for sequence-specific DNA-binding molecules, as has been shown for proteins (Ha et al., 1989; Thayer et al., 1995) and small molecules (Geierstanger and Wemmer, 1995; Krishnamurthy et al., 1995). Replacement of glu^- by Cl^- reduces nonspecific binding more than specific binding (Ha et al., 1992), indicating that interaction between DNA phosphate oxyanions and protein cations is relatively more important in nonspecific binding. DNA-protein complex formation is driven partly by release of the cations associated with the DNA, as can be shown by the salt dependence of the binding constant; this subject has been elegantly reviewed by Record and co-workers (Ha et al., 1992; Record et al., 1991; Anderson and Record, 1995) and by Lohman and Mascotti (1992). Whereas the binding constant for DNA-protein interactions is sensitive to the charge density of the anion of the salt (because only chaotropic anions compete with the DNA for the chaotropic cations on the protein), it is not very sensitive to the charge density of the monovalent cations, all of which appear to remain in the vicinity of the DNA and thus must be competed off by proteins that bind to the DNA (Barkley et al., 1981; Lohman and Mascotti, 1992; Record et al., 1991). This is because the high axial charge density of DNA causes it to act as a strongly hydrated polyelectrolyte (Record et al., 1991; Pod-

gornik et al., 1994), presumably with much charge transfer to solvent. The cations in the vicinity remain fully hydrated, with both electrostatic and hydrogen bonding contributions to their interaction with the polynucleotide, as has been shown by various physical techniques (Black and Cowan, 1994). However, increasing the charge density of the cation in the series Cs^+, K^+, Na^+, Li^+ does seem to produce effects on *lac* repressor binding to DNA (Barkley et al., 1981) and on the forces between DNA double helices (Podgornik et al., 1994) that are consistent with the highest charge density cation (Li^+) forming the most inner sphere complexes with DNA phosphate oxyanions.

It is presumably because Cl^- competes with DNA for cationic binding sites on proteins that the intracellular Cl^- concentration (3 mM) is kept low compared to the extracellular concentration (115 mM) (West, 1990).

Salt-specific effects on the amide bond

Chaotropic anions but not fluoride are retarded on polyacrylamide columns (von Hippel et al., 1973); chaotropic anions but not fluoride or sulfate increase the solubility of formamide (Nandi and Robinson, 1972); and high concentrations of chloride but not sulfate denature a neutral water-soluble α-helical peptide (Schlotz et al., 1991). This suggests that amide groups have substantial zwitterion character in aqueous solution, with the amide nitrogen acting as a chaotropic cation, which readily forms inner sphere ion pairs with chaotropic anions.

However, most salts increase the solubility of the peptide group (Nandi and Robinson, 1972), and salt-peptide interactions appear to be complex, with more than one mode of interaction likely, probably including both direct and indirect (mediated through water molecules) interactions. The effect of Hofmeister ions on peptides and proteins has recently been reviewed (Baldwin, 1996).

Overview of the model

The simple idea presented here—that chaotropes form inner sphere ion pairs with chaotropes and kosmotropes form inner sphere ion pairs with kosmotropes, always with a preference for a counterion with a matching absolute free energy of hydration—provides a generally useful way to think about biological organization. Certainly it provides a simple explanation for the observed types and arrangements of ion pumps. Presumably transmembrane ion gradients of many types could be used by cells for purposes of signaling, transport, and the maintenance of osmotic balance, but the observed ion gradients achieve the additional purpose of keeping the intracellular phosphates and carboxylates in solution, and in the case of chloride, of avoiding interference with DNA-protein interactions. It also highlights the tendency for calcium to rapidly form inner sphere ion complexes with carboxylates (Bertini et al., 1995; Forsen and Kordel, 1994) and phosphates (Porschke, 1995), which makes calcium an ideal signaling molecule but also makes it dangerous at high concentrations because of the ready formation of insoluble complexes.

The matching of absolute free energies of hydration for chaotrope-chaotrope and kosmotrope-kosmotrope inner sphere ion pair formation also provides a plausible explanation for the selectivity series of ion exchange resins (Wheaton and Bauman, 1951; Bregman, 1954) and is at least seemingly consistent with the self-association of amide groups to form structures such as peptide nanotubes (Clark and Ghadiri, 1995). In addition, neutralizing the chaotropic cations of subtilisin with wet kosmotropic phosphate stabilizes the enzyme in dimethlyformamide (presumably because solvent-separated ion pairs that are poorly soluble in this solvent have been formed) in comparison to wet chaotropic chloride (presumably because inner sphere ion pairs that are more soluble in dimethylformamide have been formed) (Sears et al., 1994); other interpretations have not been ruled out, because anions also interact strongly with polar aprotic solvents (Davidson and Kebarle, 1976; Magnera et al., 1984).

The charge density-dependent rule for association of biological moieties presented here uses the affinity of these species for water as a guide for their tendency to associate; this assumes that associating moieties do not interact via strong interactions not available to the interaction of these moieties with water; it may be that interactions such as short strong hydrogen bonds (Cleland and Kreevoy, 1994; Tobin et al., 1995) or those between highly polarizable moieties (Nair et al., 1994) such as aromatic groups may be limited exceptions to this assumption. Nor should the rule presented here be construed as implying that water does not participate in dispersion (Luck, 1980; Wolfenden and Radzicka, 1994) or partially covalent (Bock et al., 1994) interactions. Because gaseous ions have affinities for polar organic moieties such as acetonitrile and dimethylsulfoxide that are similar to their affinities for water (Davidson and Kebarle, 1976; Magnera et al., 1984), the interaction of ions with macromolecules is potentially complex.

Relationship between the Hofmeister series and ionic strength

The series

$$SCN^-, I^-, Br^-, Cl^-, \| F^-$$

(Table 3, Figs. 1 and 2) can be considered an abbreviated Hofmeister series (Collins and Washabaugh, 1985). The charge density increases from left to right, as does the ratio of the strength of ion-water interactions to water-water interactions.

The double slash marks the monovalent anion size (1.78 Å) for which the strength of ion-water interactions is equal to the strength of water-water interactions (the position at which the Jones-Dole viscosity B coefficient changes sign).

Weakly hydrated ions (chaotropes) are to the left of the double slash; strongly hyrated ions (polar kosmotropes) are to the right.

Continuum electrostatics specifies the net charge on an ion but does not address issues of charge density, treating ions as point charges. Continuum electrostatics also assumes that the strength of ion-water interactions is large compared to the strength of water-water interactions—i.e., that chaotropes do not exist (Collins, 1995). Because of this, even if charges are given a realistic size by fixing a point charge at the center of a sphere of appropriate size, the adsorption of ionic chaotropes onto nonpolar surfaces containing no aromatic residues cannot be modeled with continuum electrostatics (Collins, 1995), nor can the tendency of chaotropes to self-pair and thus have lower solubility than kosmotrope plus chaotropes salts, as shown in Table 1 (Perkyns and Pettitt, 1994). The variation of a specified parameter as a function of ionic strength is typically measured experimentally, using several salts that vary in net charge; NaCl and KCl (which are in the middle of the Hofmeister series and are thus approximately neutral in the Hofmeister sense) are the common monovalent salts used to generate ionic strength. And because multivalent salts are all strongly hydrated, only salts from the middle to the strongly hydrated side of the Hofmeister series are commonly used to generate ionic strength. When a monovalent ionic chaotrope such as thiocyanate is examined, for example in the solubility of lysozyme as a function of ionic strength at pH 4.5, the salt-specific effects are found to be quite large—varying over two orders of magnitude (Ries-Kautt and Ducruix, 1989), undercutting the assumption that ionic strength exists independently of the charge density of the ionic species producing it. And there is sometimes a discontinuity in behavior where the Jones-Dole viscosity B coefficient changes sign—for example, in anion effects on DNA-binding proteins (see above) that cannot be explained by continuum electrostatics. The assumptions of continuum electrostatics restrict its applicability to strongly hydrated salts; the distinctive behaviors of chaotropes cannot be reproduced with this model. Even though continuum electrostatics models may predict the dependence of some parameter on "ionic strength" with no reference to the position of the salt in the Hofmeister series, chaotropic salts cannot be assumed to produce the same effect as kosmotropic salts; this must be demonstrated experimentally.

NOTES

1. Kosmotropes are "water structure makers" and include both nonpolar solutes, which bind water molecules very weakly, and polar solutes, which bind water molecules tightly, particularly small ions of high charge density (Collins and Washabaugh, 1985). "Polar kosmotropes" refer to polar solutes that bind water molecules tightly relative to water-water interactions in bulk solution; in the context of discussing ions, it is appropriate to use the shorter appellation "kosmotrope" instead of the more accurate term "polar kosmotrope."

2. The solubility patterns of Table 1 can be generated by microscopic electrostatic models, but the decreased solubility of chaotrope-chaotrope ion pairs is not shown by continuum electrostatic models (Perkyns and Pettitt, 1994).

3. The radius of a water molecule is 1.38 Å (Lonsdale, 1958), illustrating that this zwitterionic model of a "virtual" water molecule incorporating an effective charge density does not accurately represent many other geometry-dependent properties of the water molecule.

4. NaCl has a slightly positive standard heat of solution, but both of these ions are nearly neutral in the Hofmeister sense; that is, Na^+ is only marginally a kosmotrope and Cl^- is only marginally a chaotrope. See Fig. 1.

5. The nitrogen-based cations are judged to be chaotropes on the basis of the chromatographic behavior of the guanidinium ion on Sephadex G-10 (Washabaugh and Collins, 1986), and the following evidence that NH_4^+ is weakly hydrated: a negative Jones-Dole viscosity B coefficient (Table 3); neutron diffraction studies (Adya and Neilson, 1991; Walker et al., 1989), NMR studies (Perrin and Gipe, 1987), and Monte Carlo simulations (Noto et al., 1991).

6. Extracellular calcium also crystallizes in the synovial fluid of the joints as calcium pyrophosphate, causing arthritis (Hoffman and Reginato, 1994), whereas calcium oxalate and calcium phosphate comprise 80% of the stones that form in the urine collecting system of the kidney (Coe and Favus, 1994).

7. The phosphodiester oxyanions of nucleic acids are presumed to be kosmotropes, because RNA is precipitated by the kosmotrope Li^+ (Cathala et al., 1983) but not by the chaotrope Cs^+ (Chirgwin et al., 1979), and because HPO_4^{2-} (Washabaugh and Collins, 1986) and PO_4^{3-} (Table 3) are kosmotropes.

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