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Sticky ions in biological systems

(ion hydration/potassium ion/ammonium ion/protein structure/Hofmeister series)

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ABSTRACT **Aqueous gel sieving chromatography on Sephadex G-10 of the Group IA cations (Li^+, Na^+, K^+, Rb^+, Cs^+) plus NH_4^+ as the Cl^- salts, in combination with previous results for the halide anions (F^-, Cl^-, Br^-, I^-) as the Na^+ salts [Washabaugh, M. W. & Collins, K. D. (1986) *J. Biol. Chem.* 261, 12477–12485], leads to the following conclusions. (*i*) The small monovalent ions (Li^+, Na^+, F^-) flow through the gel with water molecules attached, whereas the large monovalent ions (K^+, Rb^+, Cs^+, Cl^-, Br^-, I^-) adsorb to the nonpolar surface of the gel, a process requiring partial dehydration of the ion and implying that these ions bind the immediately adjacent water molecules weakly. (*ii*) The transition from strong to weak hydration occurs at a radius of about 1.78 Å for the monovalent anions, compared with a radius of about 1.06 Å for the monovalent cations (using ionic radii), indicating that the anions are more strongly hydrated than the cations for a given charge density. (*iii*) The anions show larger deviations from ideal behavior (an elution position corresponding to the anhydrous molecular weight) than do the cations and dominate the chromatographic behavior of the neutral salts. These results are interpreted to mean that weakly hydrated ions (chaotropes) are "pushed" onto weakly hydrated surfaces by strong water–water interactions and that the transition from strong ionic hydration to weak ionic hydration occurs where the strength of ion–water interactions approximately equals the strength of water–water interactions in bulk solution.**

Continuum electrostatics utilizes a macroscopic dielectric constant to characterize the ability of a medium to attenuate an electric field and has been developed to a level of great sophistication in application to biological systems (1–3). But even apart from its neglect of microscopic detail and the incorporation of a number of questionable assumptions—for example, that the free energy of solvation will be the same for anions and cations of the same size and absolute charge (2)—there is a realization that continuum electrostatics gives an incomplete accounting of the forces acting on ions in aqueous solution. As a result, in calculating the free energy of processes in aqueous solution, terms must be added which reflect the cohesive force of water resulting from the strong interactions between water molecules (1, 4). This cohesive force of water may be expressed by using the macroscopic concept of a surface tension, which tends to minimize the water-exposed surface area of dissolved species or interfacial surfaces. This opposes the repulsive "image force" between an ion in a region of high dielectric constant and the surface of a nearby region of lower dielectric constant which results from the preference of the electric field of the ion to remain in the region of high dielectric constant (5–9). The image repulsion of a partially hydrated monovalent ion of radius 1.6 Å in direct contact with a nonpolar surface is about kT relative to the bulk solution (5) and decreases with increasing ion size. The surface tension "pushing" a nonpolar sphere of this size onto a nonpolar surface is of comparable magnitude, and the surface tension effect increases with increasing sphere size (1, 4). These considerations suggest that monovalent ions of about 1.6 Å and larger should adsorb to nonpolar surfaces independent of any attractive forces between the ion and the nonpolar surface, whereas those smaller than about 1.6 Å should be repelled from nonpolar surfaces.

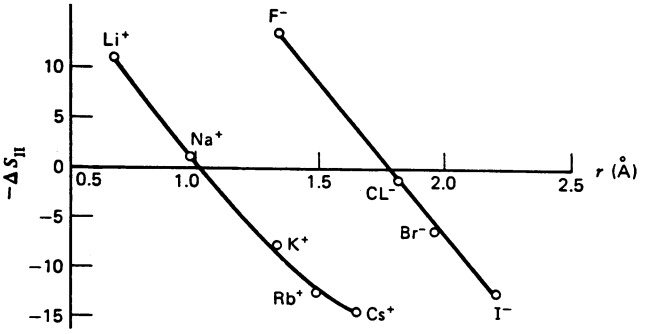

FIG. 1. The entropy of pure water minus the entropy of water near an ion in cal·K^{-1}·mol^{-1}. The crystal radii of the ions in angstroms are plotted along the abscissa. Positive values of ΔS_{II} (lower portion of figure) indicate water that is more mobile than bulk water. Negative values of ΔS_{II} (upper portion of figure) indicate water that is less mobile than bulk water. [Adaption of data of Krestov (14) as presented by Samoilov (11). Reprinted with permission of John Wiley and Sons (copyright 1972).]

Similar predictions can be made from a microscopic perspective by comparing the entropy of water molecules near monovalent ions to that of water molecules in bulk solution as determined by thermodynamic (10, 11, 14) and transport (12, 13) data or dynamic measures such as NMR (15). Fig. 1 plots the entropy of water near monovalent ions as calculated from the entropy of hydration of the ion (from dissolving the ion in water) versus the ionic radius of the ion (11). A negative ΔS_{II} (upper portion of Fig. 1) indicates tightly bound water that is less mobile than bulk water, whereas a positive ΔS_{II} (lower portion of Fig. 1) indicates loosely held water that is more mobile than bulk water. Increasing ion size (decreasing ion charge density) is associated with increasing mobility of nearby water molecules. If this mobile, loosely held water is immediately adjacent to the ion, as suggested by x-ray and neutron diffraction data (16), then the horizontal line in Fig. 1 indicating $\Delta S_{II} = 0$ separates strongly hydrated ions (above the line) from weakly hydrated ions (below the line). Since this transition from weak to strong hydration occurs at a larger size for anions than for cations, the anions must be more strongly hydrated than the cations since anions begin to immobilize adjacent water molecules at a lower charge density than do cations. This picture also suggests that ions associated with a positive ΔS_{II} should adsorb to weakly hydrated surfaces, driven by the release of weakly bound water molecules to interact strongly in bulk solution; this adsorption should occur even in the absence of any attractive forces between the adsorbed ion and the surface.

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Sephadex G-10 is epichlorohydrin-crosslinked dextran in beaded form which is meant to separate solutes up to a molecular weight of about 700 by gel sieving: large molecules are excluded by the small pore size of the beads, taking a short path through the column and emerging early, while small molecules penetrate the beads, taking a long path through the column and emerging late. The surface of Sephadex G-10 is very non-polar, containing structures such as that shown in Fig. 2 (which was isolated from the less highly crosslinked Sephadex G-25) but no aromatic residues (17). Sephadex G-10 is unusual in having different, effective mechanisms for the simultaneous separation of both strongly hydrated (via gel sieving) and weakly hydrated (via adsorption) solutes and clearly delineating the boundary between the two behaviors (18). Previous work has shown that Cl^- (radius, 1.81 Å) and larger monovalent anions adsorb to the nonpolar surface of Sephadex G-10, whereas F^- (radius, 1.33 Å) and other strongly hydrated anions do not (18, 19). The present study examines the chromatographic behavior of the Group IA cations (Li^+, Na^+, K^+, Rb^+, Cs^+) and NH_4^+ on Sephadex G-10.

METHODS AND MATERIALS

All methods and materials were those of Washabaugh and Collins (18) unless otherwise indicated. A jacketed silanized glass column 1.5 cm in diameter and 95 cm tall was packed with a slurry of about 85 g of Sephadex G-10 (lot number KA33825; Pharmacia) in 0.1 M NaCl and equilibrated with 0.1 M NaCl (filtered through 0.45-μm polyamide filters) by gravity flow at 0.5–2.0 ml/min and 30°C for several months until the column gave reproducible results. The packed column bed was 1.5 cm in diameter by 90 cm tall. The column was washed in succession with 1.0 M pyridine, 0.2 M acetic acid, and 10 mM sodium phosphate (pH 7.0), always with 0.1 M NaCl. To suppress any ion exchange effects, the column eluant for all the experiments reported in this paper was 0.1 M NaCl that had been deaerated (by water aspiration) and filtered. For quantitative work, the column was fed at 0.5 ml/min from a heavy 4-liter flask containing 1–2.5 liters of 0.1 M NaCl; 0.65-ml (14-drop) fractions were collected. Each salt analyzed was run as a 1-ml sample of a 0.1 M solution. Each chemical species shown in Fig. 4 was determined by a specific assay for that substance. $^{22}NaCl$, $^{86}RbCl$, and $^{137}CsCl$ were purchased from Amersham and detected by liquid scintillation counting. Trace amounts of each isotope [2 μCi (74 kBq) in 2 μl] were added to 1 ml of 0.1 M salt and then layered onto the top of the column. K^+ was determined by an Orion model 93-19 K^+ electrode and an Orion model 90-02 double-junction reference electrode used with a Radiometer PHM 64 pH meter. Li^+ was determined with a Ciba-Corning 654 clinical Li^+-specific electrode system in the University of Maryland Hospital clinical laboratory. NH_4^+ was detected by ninhydrin plus hydrindantin (20). The excluded volume of the column ($K_D = 0$) was measured with a 0.5-ml sample of dextran (molecular weight, 40,000) at 0.3 mg/ml and detected colorimetrically with the anthrone reaction (21). The $H_2{}^{18}O$ included volume of the column ($K_D = 1$) was determined by measuring the ^3HOH included volume and multiplying by 0.917 (22). The calibration standards for the column were polymers of glycine, ranging from glycine ($n = 1$) to pentaglycine ($n = 5$) and were detected with ninhydrin (20). Results are reported as the distribution coefficient, K_D, defined as $K_D = (V_e - V_o)/(V_i - V_o)$, where V_o is the excluded (void) volume, V_i is the included volume, and V_e is the elution volume for a given solute. The fractionating volume of this column is 129 fractions or 83.8 ml. Each sample or standard was determined three to eight times except for $^{137}CsCl$, which was determined twice, and LiCl, which was determined once. The elution position of any species varied by no more than ±1 fraction.

The ionic radii are taken from Sharpe (23), except for those reprinted in Fig. 1.

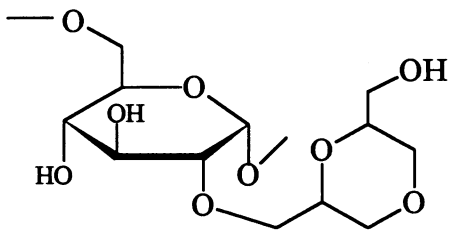

FIG. 2. A typical structure isolated from Sephadex G-25 (17), which is less highly crosslinked than Sephadex G-10. The epichlorohydrin-crosslinked dextrans contain no aromatic residues.

RESULTS AND DISCUSSION

Fig. 3 shows the calibration curve for this Sephadex G-10 column, using H_2O to measure the included volume ($K_D = 1$; see *Methods and Materials*), a dextran polymer of molecular weight 40,000 to measure the excluded volume ($K_D = 0$), and polymers of glycine (glycine, $n = 1$; pentaglycine, $n = 5$) to determine the slope of the line. There is a linear correlation between elution position and the logarithm of the anhydrous molecular weight for compounds on the calibration curve (which is drawn as a straight line in Fig. 3). The slight systematic deviation in the position of the standards is probably due to their changing shape in going from glycine ($n = 1$) to pentaglycine ($n = 5$), since the column actually measures an effective hydrated radius of the solutes (24). Previous work has shown that species which flow through the column with an apparent molecular weight higher than their anhydrous molecular weight are eluted from the column in a temperature-independent manner, are subject to only small eluant salt effects, and stabilize proteins (18). In contrast, species which flow through the column with an apparent molecular weight lower than their anhydrous molecular weight are eluted from the column in a temperature-dependent manner (indicating adsorption), are subject to large salting-in and salting-out effects, and destabilize proteins (18).

From Fig. 4 we draw the following conclusions. K^+ (radius, 1.38 Å), which is a chaotrope (water-structure breaker) as judged by thermodynamic (10, 11, 14), transport (12, 13), viscosity (25), NMR (26), infrared spectroscopy (27), and neutron diffraction (16) data, adsorbs to the nonpolar surface of Sephadex G-10, as do Rb^+ (radius, 1.49 Å) and Cs^+ (radius, 1.70 Å), with decreasing ion charge density correlating with stronger adsorption. Na^+ (radius, 1.02 Å), which is marginally

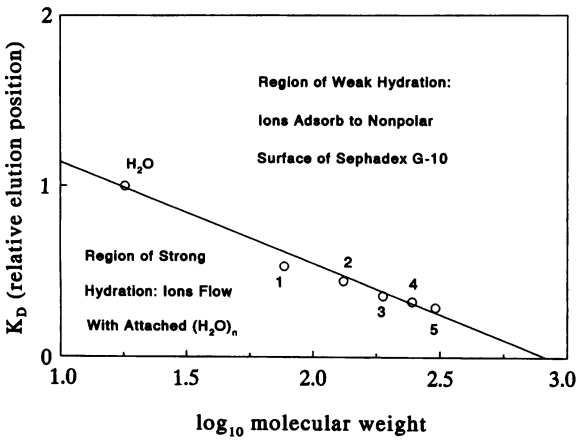

FIG. 3. Calibration curve for the Sephadex G-10 column eluted with 0.1 M NaCl at 30°C. H_2O indicates the calculated $H_2{}^{18}O$ included volume ($K_D = 1$) (see *Methods and Materials*). The glycine polymer standards range from glycine ($n = 1$) to pentaglycine ($n = 5$).

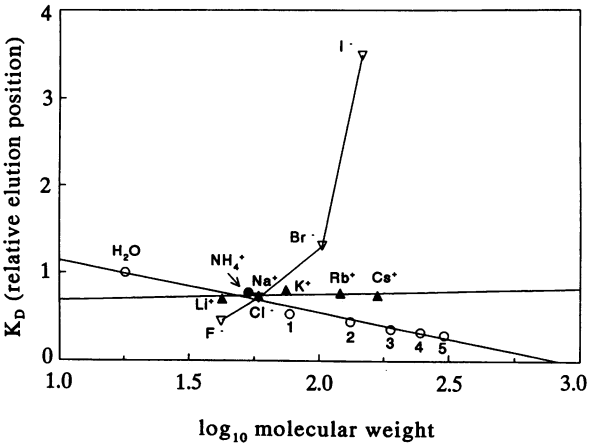

FIG. 4. Aqueous gel sieving chromatography on Sephadex G-10 of the Group IA cations (Li^+, Na^+, K^+, Rb^+, Cs^+) plus NH_4^+ as the Cl^- salts in combination with previous results for the halide anions (F^-, Cl^-, Br^-, I^-) as the Na^+ salts (18). Eluant was 0.1 M NaCl; temperature was 30°C.

a polar kosmotrope [polar water-structure maker (18, 19)] as judged by thermodynamic (10, 11, 14), transport (12, 13), viscosity (25), infrared spectroscopy (27), and x-ray diffraction (16) data, when chromatographed as the Cl^- salt is eluted in the position expected for Cl^- (which adsorbs weakly to the nonpolar surface of Sephadex G-10), thus confirming that the effect of Na^+ on water structure is small and demonstrating that the anion dominates the chromatographic behavior of the neutral salt. Li^+ (radius, 0.74 Å), in contrast, which is a polar kosmotrope as judged by thermodynamic (10, 11, 14), transport (12, 13), viscosity (25), infrared spectroscopy (27), and neutron diffraction (16) data and has a substantial effect on water structure for a monovalent cation, does not adsorb to the nonpolar surface of Sephadex G-10. The neutral salt LiCl has an apparent molecular weight slightly larger than its anhydrous molecular weight, indicating that Li^+ flows through the column with some water molecules attached. NH_4^+, which is marginally a chaotrope as judged by thermodynamic (14) and viscosity (25) data when chromatographed as the Cl^- salt also is eluted in the position expected for Cl^-, confirming that its effect on water structure is small and that the properties of the anion dominate the chromatographic behavior of the neutral salt.

NaF (anhydrous molecular weight, 41.99) flows through the column with an apparent molecular weight of 137. If we assume that any nonideal behavior of Na^+ arising from hydration effects makes an insignificant contribution to the apparent molecular weight of NaF in water, we may conclude that F^- flows through the column with 5.3 attached water molecules.

The radii of isoenergetic hydration ($\Delta S_{II} = 0$) were estimated from Fig. 1 by using 1.02 Å as the radius of Na^+ and 1.81 Å as the radius of Cl^- (23) and interpolating. The larger anion size at the point of isoenergetic hydration, a radius of about 1.78 Å for the anions, as compared with a radius of about 1.06 Å for the cations, indicates that the anions are more strongly hydrated than the cations for a given charge density. This is consistent with the estimated single ion enthalpies of hydration of the approximately isosteric K^+ (radius, 1.38 Å; $\Delta H^o_{hyd} = -330$ kJ/mol) and F^- (radius, 1.33 Å; $\Delta H^o_{hyd} = -513$ kJ/mol) (23). The stronger hydration of anions results from the asymmetric charge distribution of water as shown by computer simulations which incorporate the microscopic structure of the water molecule (28–30); it has also been rationalized in terms of charge transfer to solvent (19).

The anions show larger deviations from ideal behavior than the cations and dominate the chromatographic behavior of the neutral salts. In this system, ideal behavior is defined as having an elution position that corresponds to the anhydrous molecular weight. Much of this difference between anions and cations is presumably due to the larger size of the anions. For example, if the driving force for adsorption of weakly hydrated ions onto the weakly hydrated nonpolar surface of Sephadex G-10 is derived largely from the release of weakly bound water molecules, the anions should displace more water molecules than the cations. Similar arguments can be made for the preferential accumulation of anions *near* interfaces, by treating Hofmeister phenomena as solvation-layer edge effects (19). These direct (adsorptive) and indirect (mediated through water molecules) effects explain how pure hydrocarbon surfaces in electrolyte solutions can behave as though they were charged (31).

GENERAL DISCUSSION

A Model for the Interpretation of the Results. Consider each of the ions in Fig. 4 to be a point charge at the center of a sphere of the appropriate size. As the sphere becomes larger, the point charge eventually becomes distant enough from the water molecules at the surface of the sphere that the ion–water interactions are weaker than water–water interactions in bulk solution. At this point, ΔS_{II} becomes positive (as in the lower portion of Fig. 1), the water immediately adjacent to the ion becomes more mobile than bulk water (i.e., weakly held), and the ion becomes sticky. (This is the simplest description of a chaotrope.) The validity of this picture may be appreciated by considering the limiting case of ions as they expand to infinite size and thus zero charge density; their hydration properties are now like those of nonpolar hydrocarbons and they adsorb to nonpolar surfaces driven by strong water–water interactions. The adsorption of weakly hydrated anions onto nonpolar surfaces has also been measured by NMR (32, 33) and other (19) techniques.

Although almost all small ions increase the surface free energy (surface tension) near an air–water interface by increasing the geometric complexity of the space available for water molecules (therefore restricting the ability of these water molecules to interact strongly); weakly hydrated ions increase the surface tension at an air–water interface less than do strongly hydrated ions (34). Thus the surface tension at an air–water interface (the more global measure of the interfacial region) does not change sign upon going from strongly hydrated to weakly hydrated ions in the water, even though the surface potential difference does (19, 34).

Attractive Forces Between a Neutral Surface and an Adsorbed Ion. Nair *et al.* (35) have provided evidence that a tryptophan residue at the active site of acetylcholinesterase interacts strongly with the substituted $^+N(CH_3)_3$ group [$^+N(CH_3)_4$ (radius, 3.47 Å) is known to be a chaotrope (36–40)] of inhibitors via short-range London dispersion interactions, which are directly proportional to the polarizabilities and the ionization potentials of the interacting moieties; the polarizabilities, in turn, are a function of molecular volume. While the energy of interaction of two highly polarizable species can be substantial (41), since the surface of Sephadex G-10 contains no highly polarizable aromatic residues (Fig. 2) and the size at which monovalent ions become sticky is predictable by their entropies of hydration (Fig. 1), we conclude that the ions of low charge density examined here are "pushed" onto the weakly hydrated neutral surface of Sephadex G-10 by strong water–water interactions [these being about 44.0 kJ/mol at 20°C as measured by the heat of evaporation (42)] rather than being "pulled" onto the surface by favorable ion–surface interactions.

The Problem of Ion Size. There has been much discussion as to the appropriate radii to use in calculating ionic hydration energies with continuum electrostatics (43–49). Typically the

radii used in these calculations are larger than the ionic radii, particularly for the cations, to get agreement between theory and experiment. In presenting the results in this paper, I have used a self-consistent set of ionic radii (23) for simplicity and ease of interpretation; these values differ slightly from those reprinted in Fig. 1.

Properties of K^+. Voltage-gated K^+ channels are known to have relatively nonpolar walls (50–52), and this has led some investigators to argue for favorable chemical bonding between K^+ and the π bonds of aromatic residues lining the pore (51, 53) [the cation–π interaction (54–58)]. However, the results with Sephadex G-10 reported here show that aromatic residues are not necessary; for any nonpolar surface, it appears that K^+ is sticky and Na^+ is not. K^+ (radius, 1.38 Å), whose partial dehydration is favorable, should slip down a narrow greasy hole while the smaller Na^+ (radius, 1.02 Å), whose partial dehydration is unfavorable, should not—explaining the 100-fold or larger selectivity of K^+ channels for K^+ over Na^+ (59). [The radius of H_2O is 1.38 Å (60).] In fact, K^+ channels show a maximum permeability for Tl^+ [also weakly hydrated (14)], which is slightly larger than K^+. The ionic permeability ratio P_x/P_K of K^+ channels is $\leq$0.01 for Na^+ (radius, 1.02 Å), 1 for K^+ (radius, 1.38 Å), about 2 for Tl^+ (radius, 1.45 Å), about 0.9 for Rb^+ (radius, 1.49 Å), and about 0.1 for NH_4^+ (radius, 1.50 Å), and Cs^+ (radius, 1.70 Å) actually blocks the channel (50, 59).

Properties of NH_4^+. NH_4^+ has an ionic radius of 1.50 Å, about the same as that of Rb^+ (1.49 Å) (23). Krestov (14) has calculated that ΔS_{II} is slightly positive for NH_4^+ (that NH_4^+ is weakly hydrated). Viscosity B coefficients (25, 61–63), the selectivity series of sulfonated polystyrene–divinylbenzene copolymers (64–66), and the high rotational mobility of NH_4^+ (67) all indicate that NH_4^+ is weakly hydrated. Burley and Petsko (68), in surveying 33 refined high-resolution protein crystal structures, found that about 25% of the protonated lysine ε-amino groups interacted with aromatic groups. The two groups were preferentially separated by distances of between 3.4 Å and about 6 Å, and the preferred interaction geometry placed the amino group adjacent to the face of the aromatic ring. While the geometric relationship of the two groups in protein structures, gas-phase experiments (69), and simple energy calculations (70) suggest that there may be a favorable electrostatic interaction between the ammonium group and aromatic π bonds, the hydration properties of NH_4^+ indicate that adsorption onto any nonpolar surface should be a favorable process even in the absence of any attractive forces between NH_4^+ and the surface.

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