

BIOLOGICAL ION EXCHANGER RESINS

II. QUERP WATER AND ION EXCHANGE SELECTIVITY

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ABSTRACT Biological selectivity is shown to vary with medium osmotic strength and temperature. Selectivity reversals occur at 4°C and at an external osmolality of 0.800 indicating that intracellular hydration and endosolvent (intracellular water) structure are important determinants in selectivity. Magnetic resonance measurements of line width by steady-state nuclear magnetic resonance (NMR) indicate a difference in the intracellular water signal of 16 Hz between the K form and Na form of *Escherichia coli*, providing additional evidence that changes in the ionic composition of cells are accompanied by changes in endosolvent structure. The changes were found to be consistent with the thermodynamic and magnetic resonance properties of aqueous electrolyte solutions. Calculation of the dependence of ion-pairing forces on medium dielectric reinforces the role of endosolvent structure in determining ion exchange selectivity.

INTRODUCTION¹

The association of mobile ions with counter charges on macromolecules provides an explanation for the net accumulation of ions against an "apparent concentration gradient" (1). It remains to be specified, however, what the laws of molecular interaction are that endow both biological and synthetic ion exchangers with the ability to discriminate between ions of like charge. The present study seeks to identify parameters of biological ion exchange that determine selectivity.

¹ As a result of recent magnetic resonance studies of intracellular water, the current literature now contains various references to cell water as "ice-like," "crystalline," "adsorbed," "ordered," etc., and has given rise to a degree of confusion. There is need at this point for a term for cell water that communicates the current knowledge of its state, namely its NMR behavior, while remaining non-committal with respect to the physical model for its form. We would like to suggest QUERP water (Quick Endocytic Relaxing Pulse) derived from the rapid relaxation times T_1 and T_2 observed in biological tissues. The verb participle form QUERPING has the usefulness of providing a single term for both the physiological *broadening* of the steady-state NMR spectrum of water and physiological *shortening* of the relaxation times T_1 and T_2 obtained by pulsed magnetic resonance.

Coulombic interactions dominate the ion pair forces that determine selectivity (2-5). The elementary dependence of the electrostatic force, $(1/4\pi\epsilon)(q_1q_2/r^2)$, on the macroscopic dielectric constant of the medium, ϵ , requires that biological selectivity be fundamentally dependent on the structure of the endosolvent. In synthetic ion exchanger resins, for example, dependence of selectivity on resin hydration and solvent dielectric is well established (6-23).

Calculation of the charging energy of an ion in solution in the endosolvent of

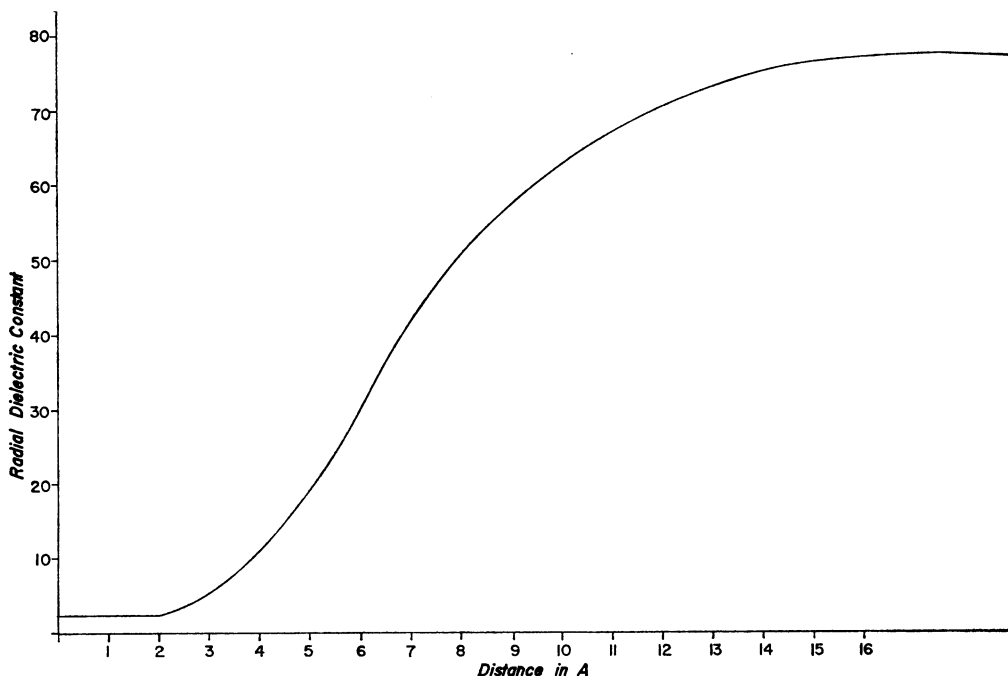


FIGURE 1 a Debye plot of variation of the dielectric constant with distance from the center of a univalent ion.

E. coli indicates the importance of solvent structure. Consider a solution of concentration 0.50 molal (approximate internal molality of *E. coli*) (1). This represents about 6×10^{20} ions/cm³. For a first approximation, if the hydration energies of the ions are disregarded and the water distributed equally among all ions irrespective of their hydration tendency, the solvent atmosphere for each ion will be 7.4 Å thick, the width of $2\frac{1}{2}$ water molecules. The actual Debye length, calculated for the atmosphere of a univalent ion at this concentration, would be 4.32 Å assuming a macroscopic dielectric of 80 for water. Debye's plot (24), Fig. 1 a, illustrates the marked variation of the radial dielectric constant of water over these dimensions and emphasizes the need for careful attention to the microscopic structure of the solvent.

Such variation in dielectric over this dimension has a profound effect on ion-pairing

forces. This is evident in Fig. 1 *b* where we have calculated the molar free energy (electric free energy or charging energy) over the same solvent radius. In condensed systems, where the dielectric has actually been measured, departure from the bulk macroscopic value for water of 80 has been marked. The dielectric constant, for example, of a typical resin ($\phi = 0.50$) is estimated to be 41.0 (3) and in the bio-

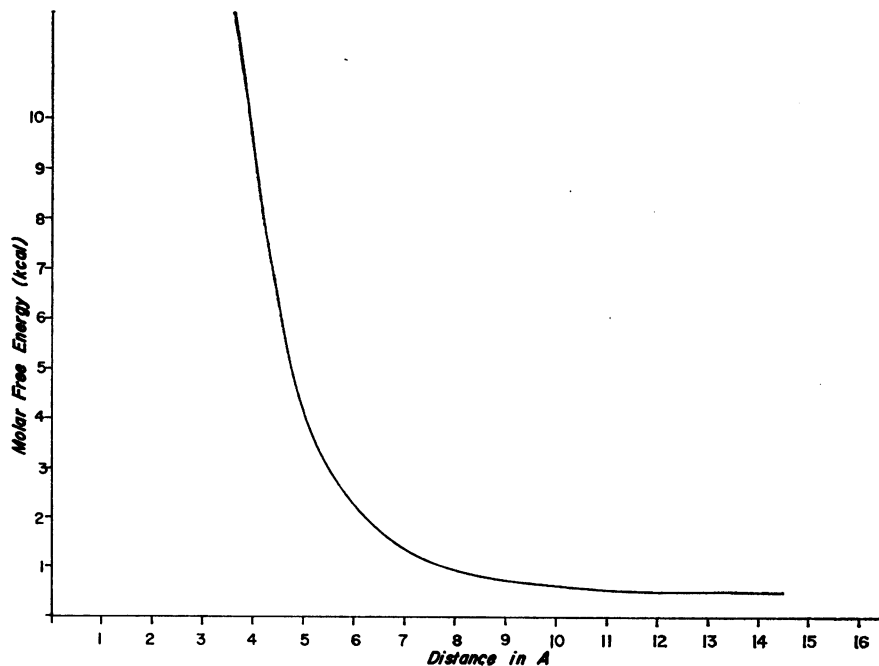


FIGURE 1 *b* Variation of ion pair interaction energy with radial distance from the ion center. The ion pair interaction energy or charging energy was calculated using the relation

$$\frac{\kappa}{2\epsilon} z^2 e^2$$

for the charging energy. κ is the Debye parameter, ϵ the dielectric, and z the ionic valence. The values for ϵ were obtained from Fig. 1 *a*.

logical tissues where it has been measured, the dielectric constant has been found to be 52–54 in muscle, 44–51 in liver, and 46–48 in skin at frequencies above 400 Mc (25). Cell water, therefore, is not a simple aqueous solution. Experimental evidence for its effect on selectivity will be given below.

MATERIALS AND METHODS

Bacteria and Growth Media

A histidine auxotroph of *E. coli* B was used in these studies (26). The organism was routinely cultivated in medium KA (26) supplemented with 0.05% Vitamin-Free Casamino Acids

(Difco Laboratories, Detroit, Mich.) and 1% dextrose. The culture was incubated at 37°C in a reciprocating shaker and the cells were harvested when the turbidity of the culture reached 0.400 OD 620. Bacteria in the K form² consisted of cells harvested at this turbidity and washed twice with 0.4 M sucrose to remove growth medium. The Na form² of the bacteria was prepared by methods previously described (1) and then washed two times with 0.4 M sucrose.

Equilibrium Dialysis

All equilibrium dialyses were performed on cells initially in the Na form. The Na form of the bacteria was washed twice with 0.4 M sucrose, resuspended in dialyzing medium not containing alkali cations, and adjusted by dilution with dialysis medium until the cell density was 30 mg dry weight/ml. Aliquots of suspension, 0.5 ml, were tied in 10 mm VisKing tubing (Armour Instrument Co., Inc., Copiague, N.Y.) and dialyzed to equilibrium at 22°C. All selectivity coefficients were measured in dialyzing medium that was equimolar (3 mM) in NaCl and KCl. Care was taken to assign a position to the equilibrium that excluded kinetic differences due solely to temperature or osmotic effects on the dialyzing membrane.

Temperature Regulation

The experimental arrangement consisted of glass containers filled with water and immersed in a tray of crushed ice jacketed with a 115 v heating mat (5 w/square inch) that was connected in series with a mercury thermoregulator (VersiTherm, Inc., Farmingdale, N.Y.; Cole-Parmer Instrument Co., Chicago, Ill.). Conical centrifuge tubes containing 1 ml aliquots of cell suspension were clamped in a Burrell wrist action shaker (Burrell Corp., Pittsburgh, Pa.) and the sample end of the tube immersed in the temperature bath. The experiments were carried out in a cold room maintained constant at 4°C. The temperature regulation achieved with this apparatus was $\pm 0.2^\circ\text{C}$.

Rational Selectivity Coefficient $K_{K/Na}$ and Cation Analysis

Bacterial dry weight, cell cation content (K and Na), and molality were determined with techniques described previously (26). The selectivity coefficient $K_{A/B}$ ($K_{K/Na}$) was determined as defined in paper I of this series (1).

NMR Measurements

Steady-state NMR measurements were made on a Varian HA 100 MHz spectrometer utilizing a 15-inch Varian electromagnet (Varian, Palo Alto, Calif.) operating at a field of approximately 23,400 gauss. Line widths at $\frac{1}{2}$ height, $\nu_{1/2}$, were determined from spectra of suspensions of *E. coli* in 0.4 M sucrose. Concentrated H_2SO_4 was used as the external reference.

The *spin-echo NMR* measurements of T_1 in aqueous electrolyte solutions were made using a PS-60 AW pulse spectrometer (Nuclear Magnetic Resonance Specialties Inc., New Kensington, Pa.), a Varian electromagnet 12 inches in diameter operating at approximately 5610 gauss, and a probe of cross-coil design operating at 24 MHz. T_1 was measured by the method of Carr and Purcell (27) which employs a sequence of two pulses set to produce a 180° nutation followed by one of 90°. Once the two pulses were phased and pulse widths set for the proper nutation angle, a Fairchild 766 H/F (25 and 50 MHz) oscilloscope (Fairchild Camera and Instrument Corporation, Mountain View, Calif.) was synchronized to trigger on the second

² See definition of K form and Na form in paper I of this series (1).

pulse and the pulse interval adjusted until the null free induction decay was obtained. The interval between the two pulses was obtained from a Computer Measurements Co. 200 CN frequency counter (San Fernando, Calif.) interfaced with the output of the PS-60 spectrometer programmer.

RESULTS AND DISCUSSION

Osmotic Strength and Selectivity

Alkali cation selectivity was found to be osmotic strength dependent. Dependence of the ion exchange equilibrium on solute strength was evident when the rational selectivity coefficient for $K \leftrightarrow Na$ exchange was unity unless the dialysis medium contained added sucrose (Fig. 2). Optimum selectivity was not obtained until a sucrose concentration of 0.4 M was reached. Furthermore at sucrose concentrations in excess of 0.800 M sucrose, selectivity reversal and preference for Na was observed. The same results were obtained with other solutes (Table I) irrespective of the nature of the solute or its charge, indicating with certainty that the solution strength or osmolarity rather than the chemistry of the solute governs the ion exchange equilibrium.

Since cell water can be expected to distribute between the intracellular phase and external medium until osmotic equilibrium is achieved, the results of these experiments imply that there exists a critical hydration state within the biological exchanger for maximum selectivity between the alkali cations K and Na. Variation of the selectivity coefficient with hydration indicates that alkali cation selectivity varies

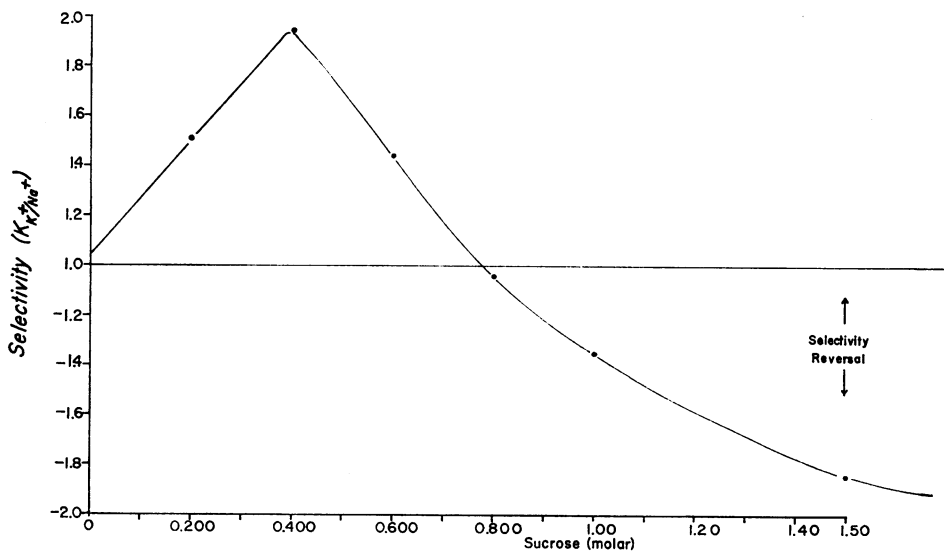


FIGURE 2 Variation of selectivity with osmolar strength. $K_{K/Na}$ is defined in paper I of this series (1). Values of $K_{K/Na}$ below 0 are reciprocals of the equilibrium constant arbitrarily given negative assignments.

TABLE I
 VARIATION OF THE SELECTIVITY COEFFICIENT
 $K_{K/Na}$ WITH OSMOTIC STRENGTH FOR SEVERAL
 IONIC AND NONIONIC SOLUTES

Molarity	Sucrose	Tris-Cl	NaCl
0.0	1.0	1.0	1.0
0.1	1.20	3.3	2.24
0.2	1.54	4.04	2.31
0.4	1.90	2.50	1.60
0.6	1.45	1.71	1.40
0.8	-1.04	-1.09	1.20
1.0	-1.40	-1.46	1.11
1.5	-1.80		

with changes in endosolvent structure, such as the degree of ordering of cell water molecules or the size of the "free water" fraction not committed to cell polar groups.

Temperature and Selectivity

An independent test of the dependence of biological selectivity on endosolvent structure is possible by making use of the well-known alterations in water structure that accompany change in temperature.

It will be remembered that as the temperature of water falls from 100 to 0°C, the density profile for water increases steadily, passing through a maximum at 4°C where it obtains a value for 1.00000, and then decreases to 0.99987 at 0°C. Various models have been proposed to account for the minimum in molar volume at 4°C (28, 29). Of the more recent, Samoilov (30) has presented convincing evidence deduced principally from radial distribution curves of X-ray diffraction data of water that the density maximum is the result of an increase in molecular packing.

Ion exchange equilibria in *E. coli* proved to have substantial temperature dependence (Fig. 3). Between 0 and 24°C (five repetitions of experiment), the selectivity coefficient for the exchange $BR \cdot Na^3 + K \rightleftharpoons BR \cdot K + Na$ varied from -1.7 to +1.7, the preference for K reversing to Na at the position for the characteristic anomaly of water, 4°C. Changes in water structure are known to exert direct influence on biological phenomena, although the role of water structure in biological ion exchange selectivity has not been considered. Oppenheimer and Drost-Hanson (31) have reported pronounced changes in bacterial growth at temperatures that correspond to abrupt changes or "kinks" in a number of properties of pure water (viscosity, index of refraction, vapor pressure, specific heat, solubilities of numerous substances, and thermal expansivity) that occur at 15, 30, 45, and 60°C. Matches and Liston reported that growth ceases in five strains of *Salmonella* between 5.5 and

³ BR refers to bacterial resin exchanger phase.

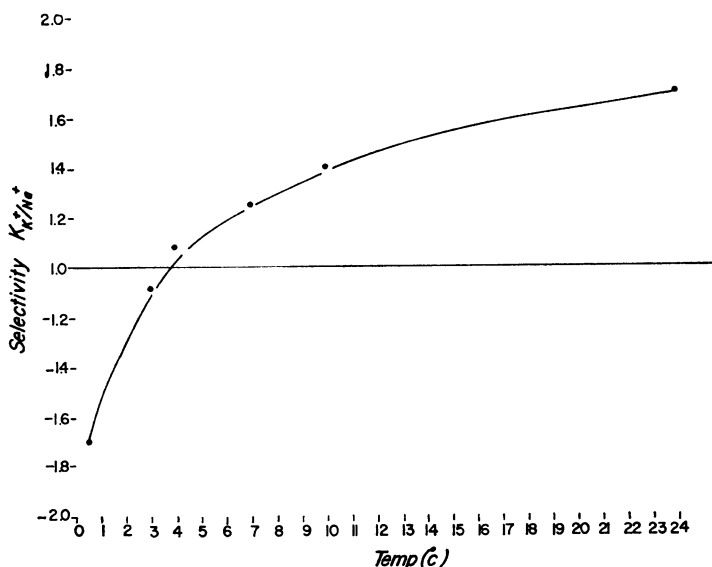


FIGURE 3 Variation of selectivity and temperature.

6.1°C (32) and Foter and Rahn (33) reported that *Streptococcus lactis* and *Lactobacillus acidophilus* stopped growing at 5°C, closely approximating the temperature of maximum density for water. Foter and Rahn pointed out that few, if any, mesophilic bacteria (bacteria with growth optima near physiologic temperature) exhibit growth below 4°C even though they remain unfrozen.

NMR

Additional evidence for the dependence of ion exchange equilibria in the biological ion exchanger resins on endosolvent structure can be adduced from NMR measurements of water in *E. coli*.

Magnetic resonance has provided evidence that water molecules in the region of macromolecules are ordered and nonrandom (34, 35). Recently, Cope (36) has reported NMR relaxation measurements (T_1 and T_2) of D_2O in tissues (muscle and brain), and Hazelwood et al. (37) have reported steady-state NMR measurements in skeletal muscle that support the conclusion that water molecules in biological tissue experience restriction in their motional freedoms either because of adsorption on the surface of macromolecules or because of an ordered crystallinity in cell water structure.

Empirically, this conclusion is derived from broadening of the line width measurements of NMR spectra of tissue water and from direct measurement of the Bloch "relaxation" parameters (38) for dissipation of the macroscopic nuclear moment of a sample by thermal perturbation (T_1) and by internuclear interaction (T_2). After excluding local magnetic fields from paramagnetic impurities and microscopic in-

homogeneities (36, 37), broadening of the line width of the water signal and shortening of T_1 and T_2 relaxation time (QUERPING)¹ was attributed to the presence of one or more fractions of intracellular water with correlation times significantly greater than the Larmor period.

Quantitative consideration of the paramagnetic ion concentrations in *E. coli* indicates that broadening of the water signal, as in the tissues studied by Cope (36) and Hazlewood et al. (37), cannot be attributed to the presence of a significant concentration of these molecules. The measured concentration of Fe, and the reported concentrations of Cu and Mn, the paramagnetic metals of physiological importance in microbes, are respectively 2×10^{-3} M (1), 0.504×10^{-3} M, and 0.145×10^{-3} M (39). The combined paramagnetic molecule concentration is 2.65×10^{-3} M

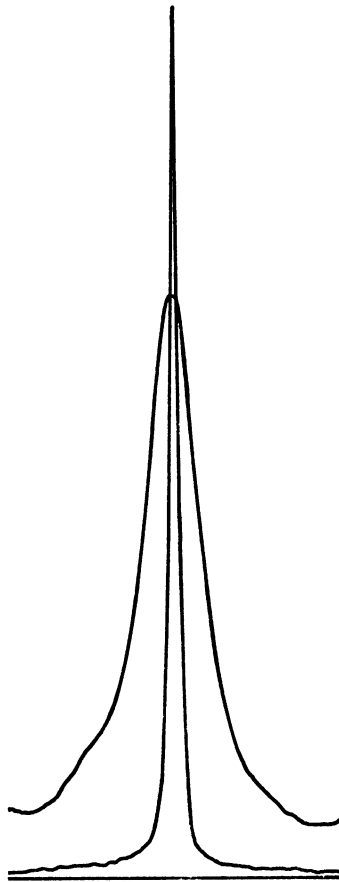


FIGURE 4a Relative line width of steady-state NMR spectra of an aqueous suspension of *E. coli* (156 mg/ml in 0.4 M sucrose) and a coaxially located H_2SO_4 reference. Measured line widths at one-half height of the *E. coli* suspension and H_2SO_4 reference at 100 MHz are 15 Hz and 2 Hz respectively.

and similar in magnitude to the paramagnetic molecule concentration which Cope concluded from pulsed magnetic resonance measurements was "approximately 200 times too small" to account for QUERPING. A coaxial tube located concentric with the bacterial sample excluded magnetic inhomogeneities in the macroscopic magnetic field and in the sample as the cause of the line broadening observed in bacteria. The concentrated sulfuric acid sample used as external reference had a line width of 2 Hz at 100 MHz (Fig. 4 a).

The line width differences between a bacterial ion exchanger, such as *E. coli* in the K form and in the Na form (Fig. 4 b), provide still another kind of experimental evidence that important perturbations of endosolvent structure accompany the biological ion exchange event. NMR line widths of water for *E. coli* in the K form were less than for *E. coli* in the Na form and varied linearly with the mole fraction of cell water in bacterial suspensions. Values extrapolated to mole fraction unity can be regarded as the line widths of pure bacterial cell water in *E. coli*. The line width of the water signal in K form *E. coli* was 30.4 Hz and 46 Hz in the Na form of the bacterium. The differences in the line width of cell water in the Na and in the K form of the bacterium can be ascribed to differences in endosolvent structure. The observed difference, in fact, is precisely what one would predict from knowledge of the aqueous behavior of these two electrolytes. Two distinctly different approaches to the theory of ionic hydration by O. Ya. Samoilov and by G. A. Krestov illustrate this point.

Samoilov has calculated the energy increment, ΔE_i , required for a water molecule to escape the hydration atmosphere of an ion in solution, and this energy increment

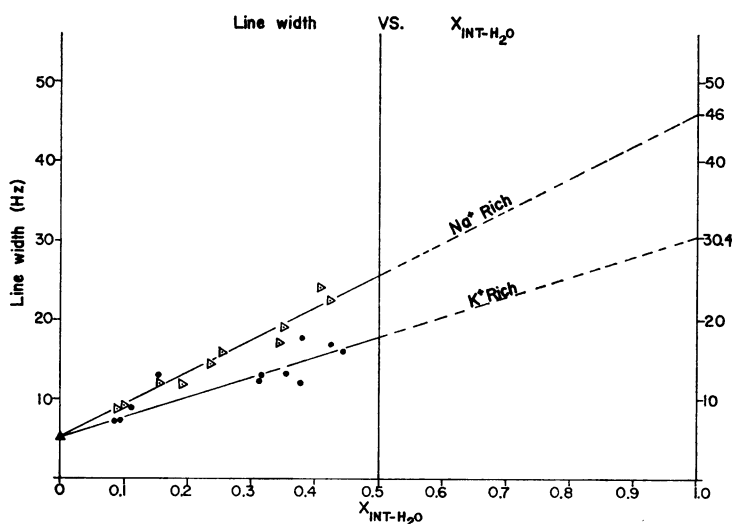


FIGURE 4 b Line width of steady-state NMR signal for water in K-rich and Na-rich bacteria. X_{INT-H_2O} is the mole fraction of cell water in the aqueous bacterial suspensions. The lines were calculated from the method of least squares.

TABLE II
 VARIATION OF THE ACTIVATION ENERGY OF EX-
 CHANGE BETWEEN NEIGHBORING WATER MOLE-
 CULES WITH IONIC RADIUS*

Ion	ΔE_i	r_i
	<i>kcal/mole</i>	<i>A</i>
Li ⁺	0.39	0.68
Na ⁺	0.17	0.98
K ⁺	-0.20	1.33
Rb ⁺	-0.30	1.49
Cs ⁺	-0.34	1.65

* From O. Ya. Samoilov (30).

is related to the lifetime of this molecule in the vicinity of the ion, τ_i , by the approximate relation,

$$\frac{\tau_i}{\tau} \cong e^{-\frac{\Delta E_i}{RT}}, \quad (1)$$

where τ is the mean lifetime for a molecule in bulk water. Values of ΔE_i tabulated by Samoilov for ions of various crystal radius appear in Table II and it is evident from equation 1 that for $\Delta E_i > 0$ (Li, Na) and $\tau_i/\tau < 1$ the exchange between water molecules in the near vicinity of the ions and the pure water phase is less frequent than the exchange between neighboring molecules in pure water; whereas, for K, Rb, and Cs, $\Delta E_i < 0$ and $\tau_i/\tau > 1$, so that exchange between water molecules in the vicinity of the ion and the pure water phase is actually greater than the exchange between neighboring water molecules outside the hydration atmosphere. The transition from positive to negative values of ΔE_i occurs at an ionic radius of 1.1 Å. Calculation of the entropy changes that accompany ionic hydration have been made by Krestov (40) and confirm the hydration transition as occurring between sodium and potassium. The occurrence of the transition between Na and K corresponds to the difference in the macroscopic effects of these two ions on the viscosity of water. Na and Li increase the viscosity of water (30) and accordingly have been referred to as "structure making" agents, whereas K, Rb, and Cs decrease the bulk viscosity of water and are considered "structure breaking."

The results of Samoilov and Krestov correspond to our NMR measurements of spin-lattice relaxation time (T_1) in aqueous solutions of the alkali halides LiCl, NaCl, KCl, and RbCl. The transition in T_1 between Na and K is apparent in Table III and indicates that Na and Li increase the average correlation time (i.e., increase viscosity) for the rotation or translation of a water molecule in the solution whereas K and Rb have either no effect on the mean correlation time for a molecule in solution or slightly increase it.

TABLE III
 T_1 RELAXATION TIME OF AQUEOUS SOLUTIONS OF
 ALKALI HALIDES*

Molarity	LiCl	NaCl	KCl	RbCl	Distilled H ₂ O (twice distilled)
	T_1	T_1	T_1	T_1	T_1
	<i>sec</i>	<i>sec</i>	<i>sec</i>	<i>sec</i>	<i>sec</i>
4	1.96	2.12	3.06	2.71	2.691
3	2.13	2.29	2.74	2.70	2.690
2	2.25	2.34	2.74	—	2.640
1	2.72	2.37	2.84	2.60	2.677 ± 0.020

* Measurements made at 24°C.

It follows from the NMR measurements of T_1 in aqueous alkali halide solutions and from the investigations of Samoilov and of Krestov that consistency requires that cells in the Na form should be characterized by NMR signals with greater line width (shorter relaxation time) than the spectral line for cells in the K form. Furthermore, the line width differences were expected from the published data for ion exchanger resin beads in which the free water content of the bead increases with the cation form of the bead according to the sequence Li, Na, K, Rb, Cs (41–43). This was the observation made for the Na and K forms of *E. coli* (Fig. 4 b). Accordingly, cells in the K form contain an endosolvent in which the average restriction in the motional freedom of the water molecules is less (i.e., more “free water”) than it is for cells in the Na form.⁴

The general conclusion of these studies is that biological ion exchange equilibria (i.e., selectivity), like the ion exchange equilibria of ion exchanger resin beads, depend to a considerable extent on the detailed nature of the endosolvent, and that changes in the state of the endosolvent have profound effects on selectivity.

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⁴ It is tempting, in fact, to comment on the basis of this observation and the aqueous properties of potassium that potassium may preside as the dominant intracellular alkali cation in biology largely because it least perturbs endosolvent structure, thereby optimizing the amount of “free water” within the cell that is available to participate in intracellular catalytic events. Many biological catalytic reactions, for example, pyruvate kinase, carbonic anhydrase, peptidases, etc., are hydrolytic and require direct participation of a molecule of water in the over-all reaction.

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