

STUDIES ON THE PHYSICAL STATE OF WATER IN LIVING CELLS AND MODEL SYSTEMS. VII. EXCLUSION OF SUGARS AND SUGAR ALCOHOLS FROM THE WATER IN SULFONATE ION EXCHANGE RESINS: THE "SIZE RULE"

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Ion exchange resins in general and the sulfonate ion exchange resin in particular have served as highly informative models toward the understanding of the living cell. Though devoid of membrane coverings, the sulfonate ion exchange resins resemble the living cell in two major attributes: they selectively accumulate K^+ over Na^+ (Ling, 1952; see also Damadian, 1971) and they contain a large amount of water which excludes hydrated ions and other solutes (Ling, 1965; Ling et al., 1973).

Contrary to once popular belief, there are now extensive evidence that the bulk of K^+ or Na^+ in sulfonate ion exchange resin, as well as in living cells, is adsorbed: singly on the sulfonate groups in the exchange resin groups (Ling and Zhang, 1983) and on β - and γ -carboxyl groups carried by aspartic and glutamic residues of cell proteins (for review see Ling, 1984; Edelmann, 1984; Ling and Ochsenfeld, to be published).

Studies of the equilibrium distribution coefficient of the pentose, D-arabinose provided evidence that the more effective exclusion from the water in the sulfonate ion exchange resin is not due to restrictive pore sizes (Helfferrich, 1962). Li^+ -loaded sulfonate ion exchange resin being the most hydrated and hence endowed with larger pores actually shows a much higher degree of exclusion of D-arabinose than the least hydrated Cs^+ -loaded resin with smaller pores (Ling and Sobel, 1975).

In this communication, I report results of further studies of distribution of neutral solutes of various sizes in sulfonate ion exchange resin and at different temperatures.

MATERIALS AND METHODS

The materials and methods follow those established and reported by Ling and Sobel (1975). Polystyrene sulfonate ion-exchange resin was obtained from Fisher Scientific Co. under the tradename Rexyn 101 (H^+ form) mesh 50 to 100. Conversion to different ionic forms was achieved by incubation in repeated changes of solutions of the hydroxides or chlorides of the cations (K^+ , Na^+ , Li^+ , Rb^+ , Cs^+) until no further pH change occurred.

Resin loaded with the ion of interest is incubated in an equal volume (usually 3 ml) of a solution containing radioactively labeled solute and shaken in a constant temperature bath accurate to $\pm 0.05^\circ C$. Time course studies indicated that it takes about 1 hr. to achieve equilibrium (Ling and Sobel, 1975). A standard procedure established is a 4 hr. incubation. The resin is then separated from the incubation solution by centrifugation at 1000 g. for 4 minutes with the aid of Gelman filter holders (Gelman Instrument Co., Ann Arbor, Mich.). The water contents of the resin beads before and after incubation were determined by heating in a $100^\circ C$ oven till constant weights were reached. The concen-

TABLE I. Apparent equilibrium distribution coefficient (p) of D-arabinose and water content in sulfonate ion exchange resins loaded with different monovalent cations (25°C). Data represent means \pm error of the means. Each set of data were from at least 4 (mostly more than 4) independent assays.

Counterions	H ₂ O Contents (%)	$p_{\text{arab.}}$
H ⁺	49.32 \pm 0.049	0.423 \pm 0.007
Li ⁺	49.27 \pm 0.723	0.432 \pm 0.006
Na ⁺	48.84 \pm 0.381	0.544 \pm 0.023
K ⁺	47.37 \pm 0.348	0.612 \pm 0.021
Rb ⁺	40.04 \pm 0.337	0.735 \pm 0.038
Cs ⁺	32.12 \pm 0.530	1.013 \pm 0.010
NH ₄ ⁺	43.66 \pm 0.308	0.702 \pm 0.088
TEA	30.02 \pm 0.500	0.276 \pm 0.026
TBA	29.17 \pm 4.48	0.224 \pm 0.041

tration of labeled solute in the resin water is calculated from the change in concentration of radioactivity in the incubation solution before and after equilibration and from the known volume of resin water and incubation solution at the beginning and the end of incubation. Radioactivity was assayed on a Packard 3330 β -scintillation counter using Bray's scintillation fluid (Bray, 1960).

All chemicals used were of c.p. grade. The radioactively labeled chemicals used were as follows: D-arabinose-1-¹⁴C (ICN Lot 904300); D-arabinose-5-³H (ICN Lot 619265; New England Nuclear, Lot 241-084); D-glycerol U.L.-¹⁴C (ICN Lot 7314-49); Inulin (carboxyl-¹⁴C) (ICN Lot 591163); D-Ribose-5-³H (Amersham, Lot LB-3236); D-sorbitol-¹⁴C (ICN Lot 628971).

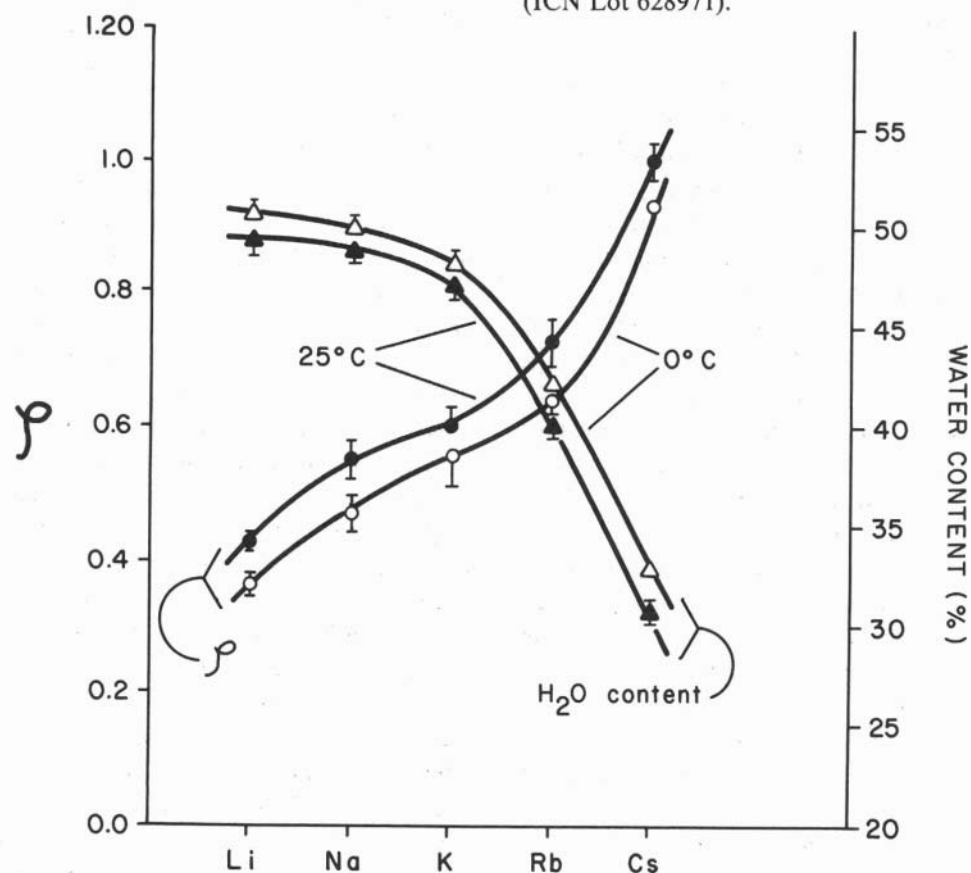


FIGURE 1. The apparent equilibrium distribution coefficients (p) of D-arabinose in the water of sulfonate ion exchange resin loaded with Li⁺, Na⁺, K⁺, Rb⁺, or Cs⁺ at 0°C and 25°C and its water content. Concentration of D-arabinose was 20 mM in all experiments.

RESULTS

The apparent equilibrium distribution coefficient (p) of D-arabinose in sulfonate ion exchange resin loaded with different monovalent cations (25°C). Table I affirms and extends earlier reported results of Ling and Sobel (1975) in which the p-values and water contents varied with the five alkali metal ions as counterions. Thus H⁺ is equally as effective as Li⁺ in increasing the water contents and depressing the p-value for D-arabinose. NH₄⁺ is less effective than K⁺ but more effective than Rb⁺ in both changes.

Cs⁺, tetraethylammonium (TEA) and tetra-n-butyl-ammonium (TBA) ions all caused extensive shrinkage; yet in the Cs⁺ form the resin has a very high p-value for D-arabinose while in the TEA or TBA form the p-value is very low.

The effect of temperature on the p-value of D-arabinose in the different ion forms.

Figure 1 shows how lowering the temperature from 25°C to 0°C uniformly increased the water contents but decreased the p-value for D-arabinose. These data offer additional evidence against the pore size interpretation of different p-values for solutes of various sizes (Helfferich, 1962), which, if true, would have predicted a higher p-value for D-arabinose for resins at 0°C than at 25°C.

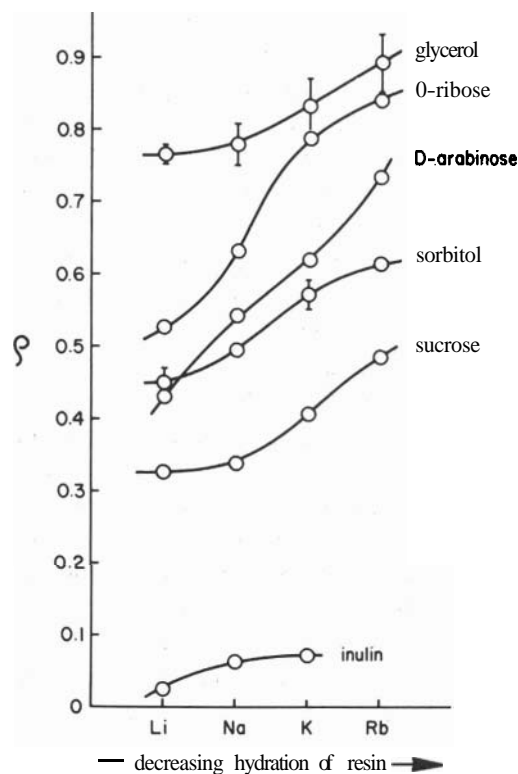


FIGURE 2. The apparent equilibrium distribution coefficients (p) of various sugars, sugar alcohols, and inulin in the water of sulfonate ion exchange resins containing as counterion to the anionic sulfonate groups Li⁺, Na⁺, K⁺, or Rb⁺ (25°C). The concentration of glycerol and all other solutes studied was 20 mM.

TABLE II. The apparent equilibrium distribution coefficients of some sugars, sugar alcohols, and inulin in the water of sulfonate ion exchange resin loaded with Li⁺, Na⁺, K⁺ or Rb⁺ (25°C). Percentage figures given in parentheses represent the average water contents of the resin.

	Glycerol	D-ribose	D-arabinose	D-sorbitol	Sucrose	Inulin	r
Li ⁺	0.766±0.019(50.8%)	0.529(51.3%)	0.432±0.006(49.3%)	0.462±0.019(50.5%)	0.325±0.003(50.8%)	0.0213(50.9%)	-0.934
Na ⁺	0.772±0.031(45.5%)	0.631(49.9%)	0.544±0.023(48.8%)	0.496±0.007(46%)	0.337 (50.0%)	0.0621(46.2%)	-0.923
K ⁺	0.831±0.042(40.8%)	0.794(47.1%)	0.612±0.02(47.4%)	0.574±0.017(40.7%)	0.409±0.01(48.2%)	0.0740(41.0%)	-0.932
Rb ⁺	0.893±0.054(36.6%)	0.849(40.7%)	0.735±0.04(40.0%)	0.614±0.002(36.4%)	0.482±0.01(40.7%)		-0.026
Molecular Weights (daltons)	92.09	150.13	150.13	191.18	342.3	990.96	

The p-values of different sugars and sugar alcohols in sulfonate ion exchange resin in various ionic forms. Figure 2 illustrates how the p-values for sugars and polyols vary with the nature of the counter cations of the resin. The trend of decreasing p-value with decreasing atomic weight of the ions seen in D-arabinose distribution (Figure 1 and Table I) is seen for all solutes investigated. This uniformity in the trend of p-value changes affirms our assumption that D-arabinose (and the other sugars and polyols) are found exclusively, or virtually exclusively, in the resin water and are not bound or complexed to the resin matrix. For this reason, they are genuine probes reporting by their distribution patterns the physical state of water in the model systems (see Ling, 1987, p. 170). One would not see this uniformity otherwise.

A somewhat unexpected finding is the rather large differences seen between the p -value of the two pentoses: D-arabinose and D-ribose.

Another feature of note is the different ways the p -value changes when one goes from the Na^+ to the Li^+ form of resin. For D-ribose, D-arabinose and even inulin, there are sharp decreases of the p -value. In contrast, glycerol, sorbitol, and sucrose remained relatively constant. The possibility that this might indicate a trend of glycerol, sorbitol, and sucrose to fit into the dynamic structure of polarized water and thus help to stabilize cell water structure will be discussed at greater length in a forthcoming paper.

The data presented in Figure 2 are given in greater detail and with the water contents in Table II. The possibility that there is a negative correlation between the size of the solutes and their respective p -values will be dealt with under Discussion.

DISCUSSION

According to the polarized multilayer theory of cell water and model systems order-

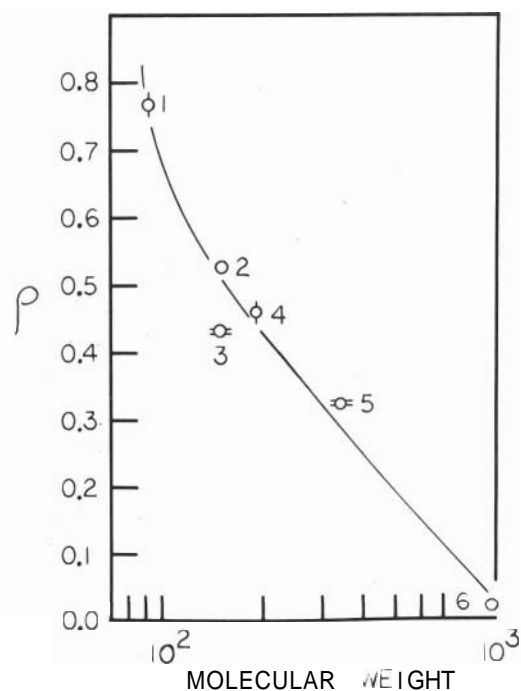


FIGURE 3. The relation between the apparent equilibrium distribution coefficients (p) and the molecular weights of six sugars, sugar alcohols and inulin in Li^+ -loaded sulfonate ion exchange resins. The concentrations of all the solutes were 20 mM.

ly arrays of positively charged (P) sites and negatively charged (N) sites whether in the form of juxtaposed two dimensional checkerboard arrangements (NP-NP system) or as a matrix of one dimensional chains (NP-NP-NP system) can polarize water in multilayers (Ling, 1984). Experimental studies of synthetic polymers like poly(ethylene oxide) ($-\text{CH}_2-\text{O}-\text{CH}_2-$)_n give reason to believe that properly spaced N sites separated from vacant or neutral (O) sites can be equally effective (NO-NO-NO system) (Ling, 1984, p. 167).

The sulfonate groups of a sulfonate type ion exchange resin occur at high density and may thus be expected to behave like an NO-NO-NO system. There are, however, reasons to believe that when sulfonate ion exchange resin (especially when it exists in the H^+ , Li^+ , or Na^+ form), acts as an NP-NP-NP system with the P sites provided by the counter

cations, and are separated from the N sites by an appropriate number of water molecules. This agrees with the strongly acidic nature of the sulfonate groups with low pK_a values — corresponding to what in the association-induction hypothesis is called low c-value sites. Theoretical calculation presented in 1962 (Ling, 1962) shows that at such low c-value, energetically favorable configurations of H, Li^+ and Na^+ (but less so in K, Rb, and especially in Cs^+) involves one or two water molecules intervening between the sulfonate singly charged oxygen atoms and the cations. These intervening water molecules may thus make the sulfonate oxygen- H_2O-H , Li^+ , or Na^+ a more effective NP-NP-NP system than say a sulfonate oxygen- Cs^+ system since in the last case the required distance of separation between the NP and P sites will be absent or inadequate.

The basic mechanism offered by the polarized multilayer theory of cell water to exclude solutes is twofold: enthalpic and entropic. Both mechanisms lead to the prediction of what has been called the "size rule", i.e., lower (true) equilibrium distribution coefficient (or q value) for larger solutes of a common molecular structure. The enthalpic mechanism is primarily the consequence of the greater energy needed to excavate a hole in the polarized water in order to accommodate the solute molecules than the enthalpy gained in filling the hole left behind in the surrounding normal aqueous medium. The entropic mechanism arises from the greater loss of rotational (and translational) motional freedom in the more "sticky" polarized water than in normal liquid water (for further discussion, see Ling, 1984, p. 170).

In earlier publications, I have shown how solute distribution in both living cells and model systems follow the "size rule". The data presented in this paper allows another chance to test this rule quantitatively. Thus in Figure 3 I have taken the p-values of six solutes in the sulfonate ion exchange resin in

the Li^+ form given in Table II and plotted them against the solutes' respective weights. The general trend of increasing p-value with decreasing molecular weight is evident.

In the last column of Table II I have presented the linear correlation coefficients (r) between solute molecular weights and their p-values. These data show that regardless of whether the counter cation was Li^+ , Na^+ , K, or Rb^+ , essentially the same r value of -0.923 to -0.934 were observed.

SUMMARY

The equilibrium distribution of D-arabino-rose, glycerol, D-ribose, sorbitol, sucrose or inulin in the water of sulfonate ion exchange resins (containing as counterions to the sulfonate anions, H, Li^+ , Na^+ , K, Rb^+ , Cs^+ , NH_4^+ , tetraethylammonium or tetra-n-butylammonium) was studied, usually at one temperature (25°C) but sometimes at two (25°C, 0°C). The apparent equilibrium distribution (p-value) changes with the nature of the counterion, temperature and the molecular weights of the sugar, sugar alcohols, and derivative in question. Linear correlation coefficients between the molecular weights of the solutes and their respective p-values in the range of -0.92 to -0.93 were obtained when the resins were in the Li^+ , Na, K, or Rb form.

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