THE MECHANISM FOR THE EXCLUSION OF SUGARS FROM THE WATER IN A MODEL OF THE LIVING CELL: THE ION-EXCHANGE RESIN: PORE SIZE OR WATER STRUCTURE?

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SUMMARY

The equilibrium distribution coefficients (p-value) of **D-arabinose** between the water in sulfonate ion-exchange resin and the external aqueous solution vary with the nature of the five alkali metal counterions studied. The strongest exclusion (lowest p-value) is found in the Li^+ resin and the least exclusion (highest p-value) in the Cs^+ resin. The p-value decreases with the increasing atomic weights for the alkali-metal ions: $\rho Cs^+ \geqslant \rho Rb^+ \geqslant \rho K^+ > \rho Na^+ > \rho Li^+$. The water contents of these resins, on the other hand, vary in the opposite direction, being highest for the Li^+ resin and lowest for the Cs^+ resin. These data disprove the pore size theory but fully substantiate the predictions of the **association-**induction hypothesis.

INTRODUCTION

According to the association-induction hypothesis, the exclusion of sugars and ions from the water in living cells arises from the unfavorable free-energy change in moving these solutes from an external medium, comprised largely of normal liquid water, into cell water, which exists in a state of polarized multilayers (ref. 1, chap. 12; refs. 2-10; see also, Troschin[11]). In support of this theory we have frequently cited the similar exclusion of sugars and other solutes from the water in sulfonate ion-exchange resin (H^+ form).[1,2,7,8,12] It has been shown that, in agreement with this theory, the exclusion of nonelectrolytes is not absolute (as is, for example, implicit in the different concept of "non-solvent" water). Rather, the degree of exclusion, measured as the q-value or equilibrium distribution coefficient between resin (or cell) water and the external media, varies with the size or number of H-bonding groups of the solute. Thus, the q-value should follow the rank order: methanol>ethylene glycol>glycerol>pentoses, hexoses>disaccharide.[10] This trend has been shown to hold for distribution in frog muscles at $0°C$ as well as for distribution in sulfonate ion-exchange resin (H^+ form) at $0°C$.[10]

However, the strength of the ion-exchange resin analogy is weakened by the existence of an alternative interpretation: the pore size or sieve size concept.[13] In this concept, ion-exchange resin may be seen as consisting of pores of varying sizes, all of the pores being accessible to small solute molecules such as methanol but only some of the larger pores being accessible to larger solutes, this accessibility becoming more and more limited as one proceeds from methanol to disaccharides.

In this communication new experimental findings will be presented which show that in sulfonate ion-exchange resin, sugar exclusion cannot be explained by the pore size theory. These data are in full accord with the theory proposed in the association-induction hypothesis.

MATERIALS AND METHODS

All experiments were carried out on samples or a polystyrene-sulfonate ion-exchange resin obtained from the Fisher Scientific Co. under the trade name Rexyn 101 (H^+ form), mesh 50 to 100. The resin was converted to different ionic forms by equilibrating in repeated changes of solution of the hydroxides or chlorides of the cation until further incubation in fresh solution produced no further pH changes. The resin was then separated from the liquid by centrifugation for 4 min at 1000 g with the aid of a Gelman centrifugal filter holder (Gelman Instrument Co., Ann Arbor, Mich.); a small circle of Whatman No. 1 filter paper was placed on the stainless steel wire screen to insure no loss of resin particles. Before use, the resin was kept in a sealed glass jar in a desiccator containing water.

To study the equilibrium distribution of D-arabinose in the water of ion-exchange resin, duplicate samples of the resin in the desired ion form were carefully weighed and their (initial) water contents determined by drying in a vacuum oven at $100^\circ C$ for more than 24 hours. Three milliliters of 20 mM D-arabinose solution labeled with H^3-D-arabinose were then introduced into glass tubes containing 3 g samples of the same batch of resin. After equilibration by shaking in either a $O^\circ C$ constant-temperature bath (Aminco, temperature accurate to $\pm 0.05^\circ C$) or in a constant temperature room maintained at $25^\circ C \pm 1^\circ C$, the solution of the resin was rapidly separated from the bathing solution by centrifugation with the Gelman filter holder (1000 g, 4 min). The radioactivity of the solution before and after equilibration with the resin was assayed on a Packard 3330 β-scintillation counter using Bray's scintillation fluid.

Samples of the resin separated from the incubation solution were again weighed and their water contents determined by drying under vacuum at $100^\circ C$.

The equilibrium distribution coefficient of D-arabinose between the resin water and the external medium was calculated from the water contents of the resin before and after incubation; the radioactivity of the external solution was also assayed before and after

incubation.

It is to be noted that the equilibrium distribution coefficient is represented by the notation p-value, which encompasses possible. adsorption of D-arabinose onto the resin in addition to pure interstitial D-arabinose dissolved in the resin water. We reserve the q-value for the case where it is certain that all of the solute is in the aqueous phases of the resin.

All chemicals used were of C.P. grade. Rubidium and cesium hydroxides and chlorides were obtained as 99.9% pure from Kewecki Chemical Co. (Boyertown, Pa.). D-Arabinose was from Sigma Chemical Co. and tritium-labeled D-arabinose from ICN Pharmaceuticals, Inc. (Cleveland, Ohio).

RESULTS

The Time Course of Equilibration of D-Arabinose

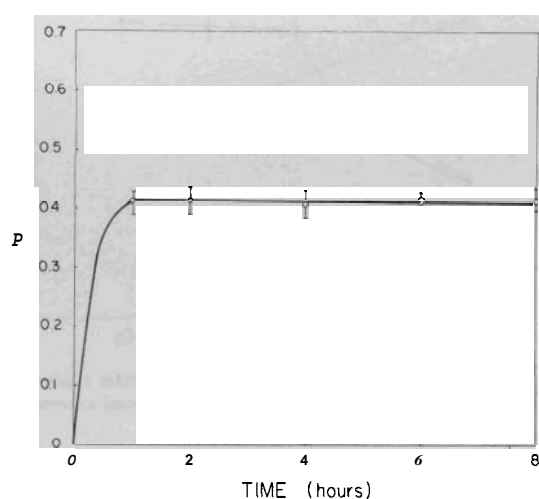Figure 1 shows that D-arabinose reaches equilibrium between the resin water and the

Figure 1. Time course of D-arabinose uptake by sulfonate ion-exchange resin in the Li+ form. Nine samples from three experiments were averaged for each point.

external medium after 1 hour of incubation. Other experiments showed that equilibration was, in fact, reached even earlier than this. In all subsequent experiments 4 hours of incubation was accepted as a standard procedure.

The Equilibrium Distribution of D-Arabinose in Resin with Different Counterions

Figure 2 summarizes the data on the p-value of D-arabinose in sulfonate ion-exchange resin in Li^+, Na^+, K^+, Rb^+, and Cs^+ form. These data show quite clearly that the p-value for a solute in the resin water is not uniquely defined by the nature and degree of swelling of the resin matrix. Indeed, the p-value is strongly dependent on the nature of the counterion. Thus, the p-value rises from 0.46 for resin in the Li^+ form to a value near 1.0 for resin in the K^+, Rb^+ or Cs^+ form.

Figure 2 also shows the equilibrium water contents of the different forms of resins. The water contents, being highest in the Li^+ resin, decline until a minimum value is reached in the Cs^+ resin
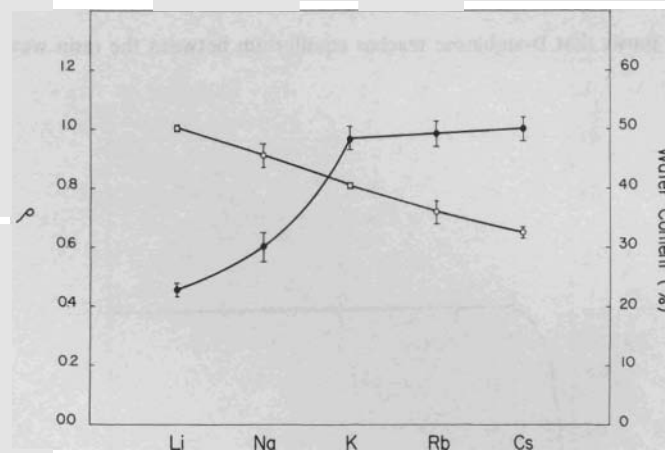

Figure 2. **Variation of the equilibrium distribution coefficient and water contents of sulfonate ion-exchange resin with different counterions. Twenty-seven samples from nine experiments were averaged for each point.**

DISCUSSION AND CONCLUSIONS

Disproof of the Pore Size Model

If the pore sizes are responsible for the low p-value for D-arabinose in, say, the Li^+ form, there should be more-or-less the same p-value for D-arabinose regardless of the nature of the counterion as long as the total resin volume remains constant. On the other hand, if the resin shrinks, the p-value can only decrease as the pores shrink and the pores inaccessible to D-arabinose increase. This is a mandatory prediction of the pore size theory

Data on the p-values and water contents of the same resins in different alkali metal ionic forms, shown in Figure 2, demonstrate the opposite. The most severely shrunken resin (Cs^+ form) has the highest ρ-value of unity. This contradiction rules out the pore size theory as an explanation for the exclusion of D-arabinose.

Confirmation of the Association-Induction Model

In a theoretical model presented in 1962, one of us (G.L.) showed that in a fixed charge system where the fixed anion is strongly acidic (i.e., a low c-value site) the number of water molecules intervening between the fixed anion and the countercation decreases in the general order: $Li^+>Na^+>K^+>Rb^+>Cs^+$.[1] As illustrated in Figure 3, a substantial part of the resin water will be oriented in the same general direction in the case where the counterion is Li^+. On the other hand, because of the proximity between the fixed anion and Cs^+, there will be relatively less of an effect on the water structuring, since the electrostatic fields due to the anion are largely cancelled by the closely placed cation. Therefore, this theoretical model implies that there will be more intensive water structuring when the counterion is Li^+.' In terms of the terminology introduced in 1972, the polarizing matrix is of the N-P type where the neighboring rows of water molecules are oriented essentially in the same direction when the counterion is Li^+ or Na^+, and this N-P orientation effect will decrease with the increasing atomic weight of the alkali metal ion, reaching a minimum in the Cs^+ form of sulfonate ion-exchange resin.
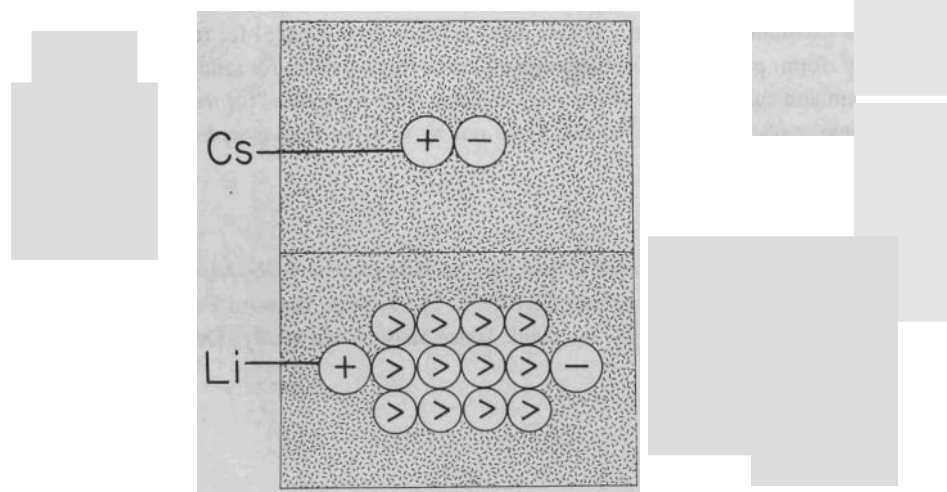

Figure 3. Diagrammatic illustration of the orientations of water in the vicinity of fixed anion-counterion pair, showing more monopolar orientation of water molecules in the Li^+-fixed anion pair than in the Cs^+-fixed anion pair. Shaded area represents less oriented water molecules.

In 1965, the association-induction hypothesis further suggested that oriented water may exclude large multiatomic solutes for both enthalpic and **entropic** reasons.' The enthalpy mechanism is twofold: (1) the energy needed to produce the cavity that must be created in the polarized and oriented water to accommodate the solute molecule may exceed the energy gained in filling up the cavity left behind in the free solution from which the solute molecules come; (2) the H-donating and H-accepting sites on the solute molecules may not match the H-accepting and H-donating ends of the surrounding oriented water molecules in the resin.

The **entropic** mechanism arises from the solute's restricted rotational and other motional freedom in the polarized and oriented water.

In previous preliminary work, evidence has shown that the enthalpic reasons seem to dominate in the exclusion of sugars from water in the H^+ form of sulfonate ion-exchange resin.[2]

It should be emphasized that, as we have demonstrated, both the enthalpic and entropic reasons are such that the predicted q-value is always lowest for larger molecules and highest for the smaller solute molecules.

In the above discussion, the p-value of D-arabinose distribution rather than the q-value was used because we have no definitive proof that some of the sugar may be adsorbed. However, as the p-value is generally more-or-less constant over a fairly wide concentration range (unpublished), such adsorption is at most a minor cause of error if one assumes p and q to be equal.

The variation in the degree of water polarization and orientation when combined with the exclusion mechanism, predicts high exclusion (low q-value) for resin in the Li^+ and Na^+ form, progressing to a minimum exclusion (high q-value) for resin in the K^+ and Rb^+ form and culminating in the lowest exclusion (highest q-value) for resin in the Cs^+ form. These predictions are, of course, borne out by what we have observed.

ACKNOWLEDGMENT

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