EVIDENCE THAT Na^+ IN A SULFONATE ION EXCHANGE RESIN EXISTS IN Ah' ADSORBED STATE. ITS SIGNIFICANCE FOR THE INTERPRETATION OF NMR DATA IN RESINS AND CELLS

G. N. LING and Z. L. ZHAKG
Department of Molecular Biology. Pennsylvania Hospital, Eighth and Spruce Streets, Philadelphia, Pcnna 19107

• *The concenrrarions of total Na^+ and of free Na^+ were measured in solutions of No* polystyrene sulfonate (NaPSS). Free Na^+ was determined ruing a Na^+-sensitive glass electrode having a high degree of specificity for Na^+. in a 5% PSS solution, 80% or more of the No* was nor derecred by the glass electrode and was adsorbed specifically onto rhe anionic sulfonate groups. The degree of adsorption increased wirh an increase in the concenrrarion of PSS. These results were discussed in regard to the interpretation of NMR studies of Na^+ in rhe cross-linked PSS resins (Dowex 50) and in living cells. The results show that rhe quadrupolar splitting of the Na^+ NMR signal is caused by specific ionic adsorption onto fixed anionic sites and nor by a diffuse charge gradient extending over large distances.*

INTRODUCTION

There are two diametrically opposed views concerning the physical state of countercations like Na^+ in the commercial nuclear sulfonate type of cation exchange resin. In one view, proposed by H. Gregor (1948, 1951), the bulk of the countercations in the resin exist as free ions. In the opposite view, first presented by Ling in 1952 and later incorporated into the larger theme, the association-induction (AI) hypothesis, the counterions are adsorbed, i.e., one (hydrated) countercation is closely associated with and in direct contact with one fixed anion of the resin phase. The major force holding the adsorbed ion in close association is electrostatic attraction, enhanced by the phenomenon of dielectric saturation in the near-vicinity of a charged ion. (A theory similar to thar of Ling was later given by Harris and Rice in 1956.)

Our interest in ion-exchange resins originated from the resemblance between the ability of virtually all living cells to accumulate K^+ selectively over Na^+ and the ability of certain types of cation exchange resins to do the same (Ling, 1952). The suggestion was made that a similar mechanism operates in both systems. Gregor (1948, 1951), on the other hand, who had little interest in all physiology, suggested an entirely different mechanism based on the assumption of free countercations in the resin water: as a free ion, the smaller, less hydrated K^+ is preferred over the larger, more hydrated Na^+ due to the intense pressure postulated to exist in the exchange resins.

The dichotomy in these two different interpretations of the same phenomenon produced another set of divergent interpretations of the NMR of ^{23}Na in resins and in living cells. Based on the assumption of Na^+ binding in an ion-exchange resin, Cope (1967) and Ling and Cope (1969) cited the similar NMR behavior of ^{23}Na in cells and in the nuclear sulfonate ion-exchange resin, Dowex 50, as evidence for adsorption of Na^+ in living cells. Based on the assumption of full counterion dissociation in this ion-exchange resin, the similarity of NMR behavior led Berendsen and Edzes (1973) to con-

clude just the opposite — that Na* (and K*) in living cells is all free.

These widely divergent viewpoints are created by uncertainty of the physical state of the countercations, in particular Na^+, in an obviously attractive model system, the man-made ion exchange resins. To help resolve this problem, we undertook an investigation of the physical state of Na^+ in solutions of the linear polymers of Na* poly-styrene sulfonate, which exists in a solution form, easily amenable to investigation. It is well known that sulfonate ion exchange resins like Dowex 50 are simply these linear polymers (at high concentration) crosslinked into a three dimensional network with the cross-linking agent, divinylbenzene. Since increasing ion concentration decreases the free energy of dissociation and enhances ionic association, the demonstration of a significant degree of counterion association in dilute solutions of the linear polymer would confirm the adsorption concept of the AI hypothesis. On the other hand, the demonstration of complete dissociation of the countercation would offer support for Gregor's theory.

MATERIALS AND METHODS

The polymers studied were poly-styrene sulfonate (PSS) obtained as several batches of gifts under the commercial name Versa-T1® 400 and Versa-T1® *500* from Proctor Chemical Company, a subsidiary of National Starch and Chemical Corp., Bridgewater, N. J. The approximate molecular weight of the Versa-T1® *500* is 500,000 daltons; that of Versa-T1® 400,400,000 daltons.

All the chemical; used were of the reagent grade. Guanidine HCl and choline chloride were from Eastman, Rochester, N. Y. Arginine HCl and lysine HCl were from Sigma Chemical Co., St. Louis, Mo.

The Na electrode (Corning 476-210) used in the early part of this work was a gift from Corning Glass Works, Medfield, MA; this fine electrode is now commercially available. Made with glass of NAS 11-18 composition, this electrode has high specificity for Na^+. Thus at pH 7, the Na^+/K^+ selectivity is about 1000; that of Na^+/Li^+, 250. For maximum reproducibility we kept the electrode bulbs immersed in 0.1 N NaCl at all times when not in use. The reference electrode used was a single junction calomel electrode (Model 90-01, Orion Res. lnc.) connected to the

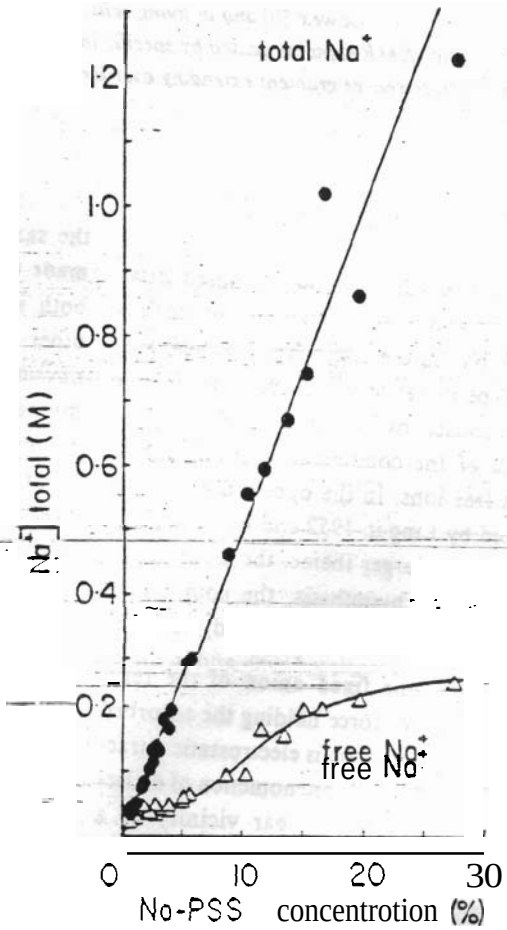

FIGURE 1. The concentrations of total Na^+ and free Na^+ in solutions of Na^+ polystyrene sulfonate (NaPSS). The abscissa represents the concentration of NaPSS expressed as percentage (W/V). The ordinate represents the concentration of total Na^+ and fret Na^+ both in molaritv. The free Na^+ represents that which was detected by the Na^+ sensitive electrode.

measuring vessel by a salt bridge of heavy wall capillary tubing filled with 0.1 N KCl and 2% agar. The end of this salt bridge in the solution to be measured is drawn to a very small diameter with the tip inner bore of less than 1 mm. The electrodes wcre coupled to either a Fisher pH meter Model 620, or a Beckman pH meter Model 4500. The output of the meter was fed into a Linseis multi-channel strip-chart recorder and readings were taken after steady levels were reached. This usually took 2 to 3 minutes. As a rule new standard curves were constructed each day. However, reading of an unknown sample was always sandwiched between a pair of readings of standards above and below the reading of the unknown sample.

For the determination of total Na* concentration of the polymer solutions, we relied on atomic adsorption spectrophotometry (Perkin-Elmer Model 103). Extreme care was exercised in diluting the viscous samples to assure complete homogeneity. The samples diluted to contain Na^+ concentration in the range, 5 to 100 μM, were read in the presence of a constant concentration of LiCl (97 mM) and $NH_4H_2PO_4$ (3.0 mM) which served as radiation buffers.

We chose two methods to convert the polystyrene sulfonate (PSS) into the Na^+ form: (i) extensive dialysis with repeated changes of solutions of 1 M NaCl and (ii) conversion o PSS first into a H^+ form after overnight exposure of PSS in a dialysis bag to 1 N HCl at 4° C and subsequent dialysis against repeated changes of 1 M NaCl until neutrality was reached. The results are similar; both methods produced pure Na^+ polymers. PSS in the Na^+ form thus obtained is then further dialyzed against distilled water adjusted to pH 11 with NaOH. The dry weight of aliquots of the stock solution was determined by heating in vacuo at 100° C. Some aliquots were diluted with similar basic distilled water to the various desired concentrations before their free Na^+ concentrations were measured with the Corning 476-210 Na^+ electrode. Other aliquots were diluted to the proper concentration range for the determination of total Na^+ with the aid of atomic absorption spectrometry.

RESULTS

Determination of Free and Bound Na^+. Figure 1 shows the result of a series of measurements of total and free Na^+. From the total Na^+ one estimates a concentration of 5 mmoles of Na^+ per gram of dry NaPSS, which is slightly higher than that predicted on the assumption of a uniform monomer formula $(-CH_2-CH\ SO_3^-Na^+-)_n$, which has a monomer weight of 206.20 and a predicted Na^+ content of 4.85 mmoles/g. NaPSS.

The free Na^+ conantration for each of these samples as indicated in Figure 1 fell far below the level of total Na^+ concentrations measured. By subtraction, one obtains the

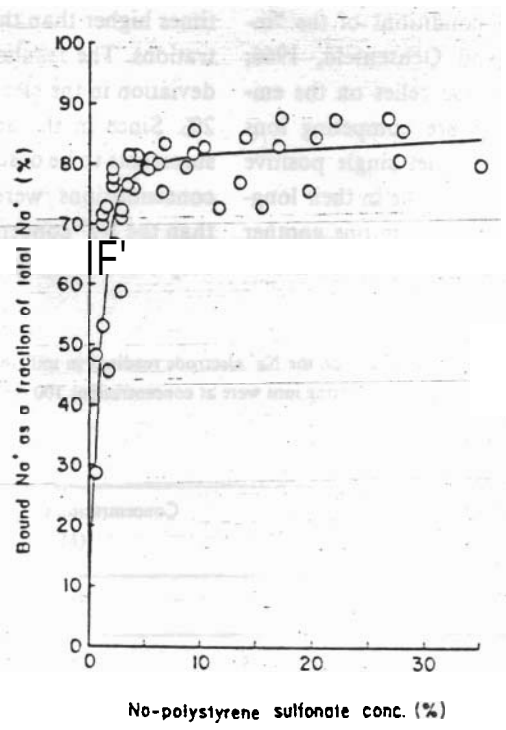

FIGURE 2. The fraction of Na^+ that exists in a bound form not detected by the Na^+ electrode in different concentrations of Na^+ polystyrene sulfonate.

bound fraction of Na^+. Figure 2 combines the results of data given in Figure 1 and another set of data. The ordinate represents adsorbed Na^+ expressed as a percentage of total Na*. To be noted is that the percentage of adsorbed Na^+ was low in very dilute solutions of NaPSS but it increased sharply with increase of NaPSS concentration until at about 4% NaPSS, the adsorbed Na' makes up 80% of the total Na*.

Demonstration of Specificity in Cation Adsorption. The results presented in Figures 1 and 2 show that the bulk of Na^+ in solutions of NaPSS is not "seen" by the Na* electrode. The simplest interpretation is that the bulk of Na^+ is adsorbed. However, there is also the theoretical possibility that the "invisible" Na* reflects not Na^+ adsorbed onto sulfonate anionic sites, but rather fully dissociated Na^+ hovering around in the vicinity of the anionic sites in spaces not accessible to the electrode.

There is a method to distinguish between these two alternative conditions of the "invisible. Na^+ (Ling and Ochsenfeld, 1966; Ling, 1977). This method relies on the employment of two or more competing ions which, like Na^+, carry a net single positive charge and are thus quite alike in their long-range attributes but differ from one another in short-range attributes. If the "invisible" Na^+ is not in direct contact with the anionic site, then the displacement effect of a pair of these competing cations would depend only on their long-range attributes (i.e., their valency) which are identical and are thus indistinguishable. If on the other hand, the "invisible" Na^+ is in close contact with the anionic sites, then the degree of displacement by two such ions with different short-range attributes may be quite different.

As competing ions, we chose the chloride salts of four "cations" that carry a single net positive charge: arginine HCl, quanidine HCl, choline chloride, and lysine HCl.

Before testing their effects on displacing Na^+ from PSS, we must first establish that these cations do not by themselves significantly alter the ability of the Na* electrode to register only the free Na^+ concentration in a solution. To do so, we determined the Na* activity at concentrations of 10^{-5}, 10^{-4}, and 10^{-3} M in the absence and presence of each of these competing ions at concentrations 100 times higher than the respective Na^+ concentrations. The results (Table I) show that the deviation in the electrode reading is less than 2%. Since in the actual experimental measurements to be described, the competing ion concentrations were not 100 times higher than the Na^+ concentration but were at most only 5 times higher than the Na^+ concentra-

TABLE I. Effect of competing ions on the Na^+ electrode readings in millivolts in solutions containing 10^{-3}, 10^{-4}, and 10^{-5} M free Na^+. Competing ions were at concentrations 100 times higher than the concentration of Na^+ present.

Competing Ion ($100 \times C_{Na}$) (M)	Concentration of Na^+ (C_{Na}) (M)		
	10^{-3}	10^{-4}	10^{-5}
None	−81.6	−140.3	−195.1
Arginine	−81.9	−139.7	−192.8
Choline	−83.4	−140.3	−195.2
Guanidine	−83.7	−141.3	−196.1
Lysine	−82.5	−141.5	−203.5

tion, there was in essence no direct significant interference of these ions on the ability of the Na^+ electrode to faithfully monitor only the free Na^+ concentration.

Figure 3 presents a typical experiment showing the effects of varying concentrations of the competing ions (indicated on the abscissa) on the percentage of bound Na^+ that is displaced by them. Note that the bulk of bound Na^+ (i.e., 77%) is displaced by 300 mM guanidine. However, at 100 mM concentration, the most effective displacement was not by guanidine but by arginine. (Due to its lower solubility, we could not study the effect of arginine at higher concentrations.) Lysine is ineffective in displacing bound Na^+. This was not due to its possession of the additional α-amino and α-carbonyl groups, since this feature is shared by both lysine and arginine. The data presented in Figure 3 show that there was a high degree of specificity in the effectiveness in displacing the Na^+ even though all of these substances carry a single net positive electric charge. Thus, their short-range attributes played major roles in the displacement of the Na^+, thereby proving that the "invisible" Na^+ is indeed adsorbed in the sense that the Na^+ is in close contact with the anionic sites in a one Na^+-one sulfonate group relationship.

Since guanidine can displace 77% of the bound Na^+ while lysine at equal concentration can only replace 8% of the bound Na^+, at least 77 − 8% = 69% of the "invisible" Na^+ is in the close-contact adsorbed state. However, the slope of the guanidine curve at 300 mM is considerably higher than that of the lysine curve. Thus one may safely conclude that more than 69% of the "invisible" Na^+ is in the direct-contact adsorbed state in the PSS solution.

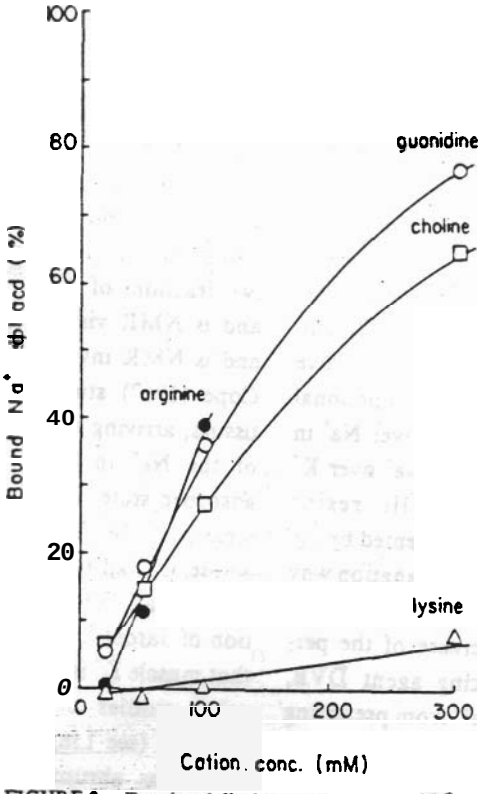

FIGURE 3. Fractional displacement of bound Na^+ in solutions of NaPSS (5%). The ordinate represents the percentage of bound Na^+ that has been displaced by the competing ions. The abscissa represents the concentration of the competing ions.

DISCUSSION

The results of our investigation left no doubt that the bulk of Na^+ in a solution of Na PSS at concentrations higher than 2% is in a close-contact adsorbed state. As a rule, with the increase of the concentration of Na PSS, the fraction of Na^+ existing in this adsorbed state increases. Commercial nuclear sulfonic acid ion exchange resins like Dowex 50 are cross-linked polystyrene sulfonate just like the Versa-Tl we studied. The total solid content of Dowex 50 and similar resins are in the 40% to 50% range and are higher than the highest concentration of Na PSS we studied (i.e., 35%, see Figure 1). Therefore we have M e doubt that virtually all Na^+ in a Dowex-50 resin exists in a close-contact adsorbed form.

These findings therefore disprove Gregor's theory of total counterion dissociation in sulfonate ion exchange resin and confirm the prediction of the AI hypothesis.

As mentioned earlier, the present finding that the counterions in Dowex 50 as well as other types of ion exchange resins are in an adsorbed state offers simple explanations for much of the characteristics of ion exchange resin properties that Gregor's theory could not explain, including the much higher preference for Ag^+ and Tl^+ over Cs^+ and other monovalent cations of equal size (Helfferich, 1962) and the diametrically opposite selective preference of Na' vs. K^+ when the functional groups differ. preference for K^+ over Na^+ in sulfonate resins; preference for Na^+ over K^+ in phosphoric and carboxylic resins. (Bregman, 1953). The model presented by the AI hypothesis also offers an explanation why in resins bearing the same functional groups (nuclear sulfonate), an increase of the percentage of the cross-linking agent DVB, causes a 'selectivity reversal" from preferring K^+ over Na^+ to one favoring Na^+ over K^+ (Reichenberg, 1951, 1955; Bregman, 1953), a finding that cannot be explained by Gregor's theory (see Hellferich, 1962, p. 159). This observation agrees with the theoretically calculated relations between the electron density at the acidic group (i.e., the c-value) and the relative adsorption energies of K^+ and Na^+, with the assumption that incorporation of DVB changes this electron density (see Reichenberg, 1966; also Ling, 1981).

The conclusion derived from the present studies also reaffirms the parallel seen thirty years ago between the mechanism of selective K^+ accumulation in ion-exchange resins and in living cells (Ling, 1952, 1962, 1983). It is now firmly established that K^+ in living muscle cells also exists in an adsorbed state, by three laboratories using a total of four independent methods: autoradiography (Ling, 1977, Edelmann, 1980); transmission electron microscopy (Edelmann, 1977); dispersive x-ray microprobe analysis (Edelmann, 1978; Trombitas and Tigyi-Sebes, 1979); and laser mass spectrometer microprobe analysis (LAMMA) (Edelmann, 1981).

In the fifties, Jardetsky (1956) and Jardetsky and Wertz (1956a, b) studied Na^+ complexing in various solutions and other systems including the sulfonate ion exchange resins, Dowex 50. They found that only about half of the Na^+ in Dowex 50 resin was NMR-visible. They suggested that there are two fractions of Na^+ in the resin: one is free and is NMR visible, the other is adsorbed and is KMR invisible. Accepting this logic, Cope (1967) studied Na' NMR of living tissues, arriving at the conclusion that much of the Na^+ in living tissues exists in an adsorbed state. Later Ling and Cope (1969) measured the Na^+ NMR of frog muscle whose K^+ had been replaced stoichiometrically by Na^+. They also used the same assumption of Jardetsky and Wertz, and concluded that muscle K^+ is in an adsorbed state. Many other studies of a similar type rapidly followed (see Ling, 1984) until in 1972 this trend was abruptly brought to a stop by Berendsen and Edzes (1973).

Berendsen and Edzes (1973) pointed out that the disappearance of part of the Na^+ signal is not due to partial binding, but reflects a 40-60 splitting of the Na^+ signal when the Na' nuclei (which have a quadrupolarmoment) is in an electric field gradient They then postulated that the electric field gradient in living cells is a diffuse one, spread over domains of 100 Å or wider. To support their view, they showed that in deteriorated muscle (deteriorated to such an extent that "it smelled") and in Dowex 50 ion exchange resin charged with Na^+, the Na^+ signals show a similar 40-60 splitting as well as other characteristics. They based their argument on the assumption that all Na^+ in Dowex 50 resin exists in a free-form, much as the widely known theory of Gregor had predicted. They did not explain how a diffuse gradient over a space of 100 Å can be penerated in Dowex 50. Thus with a total anionic site density of 4 M in these resins the average charge-to-charge distance should be closer to $10/(4 \times$

$6.06 \times 10^{23})^{1/3}$ charges/cm, which is one charge every 7.4 Å rather than 100 Å.

Proof that the bulk of Na^+ in Dowex 50 is adsorbed shows that the NMR observations cannot be interpreted on the basis of a diffuse electric field gradient. The similarity Berendsen and Edzes demonstrated in the NMR behavior of deteriorating muscle and Dowex 50 suggests that Na* in the deteriorating muscle also existed in an adsorbed state. Whether this Na^+ was adsorbed on the proteins of the dead muscle or perhaps even in the bacteria growing in the sample that had already produced the bad smell, remains to be determined.

It is also appropriate to point out that in 1978 Chang and Woessner reached the conclusion from purely theoretical grounds that the electric field gradient required to produce the NMR behavior must be a great deal steeper than Berendsen and Edzes envisaged. In fact, they argued that a reasonable estimate gives a gradient over such a short distance that it is quite compatible with that of a Na^+ adsorbed on a negatively charged site. Lindblom (1971) had reached a similar conclusion, and, Edzes, et al. (1977, p. 733) themselves suggested that the 40–60 split of % and other nuclei in an electric field gradient can be the result of "binding of an ion in a specific site."

These more recent developments have fully restored our confidence that NMR study of Na^+ and K^+ remains an exciting and useful approach to understanding the cell physiology of ions. Past conclusions concerning the adsorbed state of Na^+ in living cells and model systems derived from NMR studies by Cope and others are qualitatively quite correct even though quantitatively, the amount of adsorbed Na^+ might have been underestimated (see Ling, 1984).

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