Sodium Absorption by Barley Roots: Its Mediation by Mechanism 2 of Alkali Cation Transport

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Summary. When barley roots absorb Na⁺ at concentrations ranging from 1 to 50 mM, in the presence of low concentrations of Ca²⁺ and K⁺, absorption of Na⁺ is mediated by carrier mechanism 2 of alkali cation transport, mechanism 1 being unavailable for Na⁺ transport under these conditions. The absorption isotherm depicting the rate of Na⁺ absorption as a function of the external Na⁺ concentration, over the 1 to 50 mM range of concentrations, shows several inflections. This stepwise response occurs whether Cl⁻ or SO₄²⁻ is the counterion, but actual rates of Na⁺ absorption are lower in the latter case.

When the concentration of Na⁺ is 50 mM, and the concentration of either K⁺ or Ca²⁺ is increased from nil to 50 mM, the rate of absorption of Na⁺ is diminished not as a smooth function of increasing concentrations of the interfering ions but stepwise. Similarly, when the concentration of K⁺ is 50 mM, and the concentration of either Na⁺ or Ca²⁺ is increased from nil to 50 mM, the rate of absorption of K⁺ is diminished not as a smooth function of increasing concentrations of the interfering ions but stepwise.

Together, this evidence supports the previous conclusion to the effect that mechanism 2 of alkali cation transport possesses a spectrum of carrier sites with different ion affinities.

When both K⁺ and Na⁺ are presented at equivalent concentrations over the 1 to 50 mM range, mechanism 2 transports Na⁺ almost exclusively, and mechanism 1 K⁺ almost exclusively. These findings support previous conclusions to the effect that the active sites of mechanism 2 have higher affinity for Na⁺ than for K⁺, whereas the reverse is true for mechanism 1.

Two mechanisms effect the absorption of alkali cations by plant roots and other plant tissues (see ref 5 for a comprehensive review). The 2 differ drastically in many features relating to ionic affinities and selectivities, effects of counterions, and kinetics. One of the outstanding differences between them is that the high-affinity mechanism, mechanism 1 of alkali cation transport, which is operative even at very low concentrations, is highly selective for K⁺ as against Na⁺, whereas the low-affinity mechanism 2, which only comes into play at concentrations of 0.5 to 1 mM and above, transports Na⁺ as well as K⁺ (see 11 for references). In the preceding paper (11), Na⁺ transport in barley roots has been examined over a wide range of concentrations, in terms of the activities of both mechanisms 1 and 2. In the present paper, the transport of Na⁺ and K⁺ by mechanism 2 is scrutinized in detail.

Materials and Methods

Excised roots of barley, Hordeum vulgare, 'Arivat,' were the experimental material. The methods throughout were those described in the preceding paper. Seedlings were grown as described earlier (3), and the technique of conducting the absorption experiments with excised roots was as outlined before (8). Sodium was labeled with ²²Na. Potassium was labeled with ⁴²K in the experiments shown in figures 3 and 5, and with ⁴³K in the experiment of figure 6.

Results

It has been shown before that mechanism 2 of alkali cation transport in barley roots shows
a certain heterogeneity (6). When the rate of absorption of K+ or Na+ is examined over the high range of concentrations where mechanism 2 contributes to absorption, i.e., from 0.5 or 1.0 to 50 mM, the absorption isotherm depicting the rate of absorption as a function of the external concentration has several inflections (6). In those experiments K+ and Na+ were furnished as the Cl− salts. Figure 1 shows that the kinetics of Na+ absorption over the high range of concentrations exhibit the same complex pattern with SO₄²⁻ as the anion. The rate of Na+ absorption rises not as a smooth function of increasing Na₂SO₄ concentrations in the solution but stepwise. The inflections in the isotherm occur at similar if not identical concentrations, whether Cl− (6) or SO₄²⁻ (fig 1) is the counter-ion, viz., at about 10, 18 to 20, and 30 to 35 mM Na+.

Figures 2 to 5 present the results of experiments in which this heterogeneity was demonstrated in a novel way. In each of these experiments, the concentration of the ion whose absorption was to be measured (the substrate ion) was 50 mM, while the concentration of another cation (the interfering ion) varied from nil to 50 mM. The rate of absorption of the substrate ion was examined as a function of the concentration of the interfering ion.

In the experiment shown in figure 2, the substrate ion was Na+, present at 50 mM. When the concentration of K+, the interfering ion, was varied, the rate of Na+ absorption decreased not as a smooth function of increasing concentrations of KCl but stepwise. The reverse of this experiment is shown in figure 3 in which K+ was the substrate ion, present at 50 mM, and the concentration of Na+ varied from nil to 50 mM.

Figures 4 and 5 show the results of experiments similarly designed, in which the effect of Ca²⁺...
on the rate of absorption of Na\(^+\) and K\(^+\), respectively, was examined.

In the experiments reported so far, the absorption of either Na\(^+\) or K\(^+\) was measured. Figure 6 presents the results of an experiment in which the concentrations of both Na\(^+\) and K\(^+\) varied from 1 to 50 mM. Any given value on the abscissa denotes the concentration of each ion. On the ordinate are plotted the rates of absorption of Na\(^+\), K\(^+\), and the sum of these rates. These values were obtained by first counting the total radioactivity, then letting the 12.4 hour \(^{40}\)K activity decay (5 days) and finally counting the \(^{22}\)Na activity alone. The rates of K\(^+\) absorption were obtained by subtracting the values for \(^{22}\)Na absorption from the total.

Figure 6 shows that under these conditions, with Na\(^+\) and K\(^+\) present in equivalent concentrations, the rate of absorption of K\(^+\) barely rises over this range (1-50 mM), and the total rate of absorption of K\(^+\) + Na\(^+\) (top curve) mainly reflects the absorption characteristics of Na\(^+\) (bottom curve).

Discussion

Earlier findings (6) were to the effect that mechanism 2 of alkali cation transport, which becomes operative at concentrations of about 0.5 or 1.0 mM and above, is heterogeneous. When the concentration of K\(^+\) or Na\(^+\) was varied from 1 to 50 mM, the isotherm describing the rate of absorption as a function of the external concentration of the substrate ion showed a number of inflections. The interpretation offered was that carrier mechanism 2 possesses a spectrum of active sites which differ somewhat, though not greatly, in their affinity for a given ion. At concentrations in the range of a few millimoles per liter, the mechanism 2 site with the highest affinity for the substrate ion mainly effects the transport of the ion by this mechanism. At progressively higher concentrations, additional carrier sites of mechanism 2 come into play until, at 50 mM, a total of 4 such sites participate in the transport of the ion via this mechanism (6).

If this interpretation is sound, 2 consequences are predictable. First, the role of the counterion should be secondary. That this is indeed so is shown in figure 1. The absorption of Na\(^+\) over the high concentration range (1-50 mM) shows the same pattern when SO\(_4^{2-}\) is the anion as when the counterion is Cl\(^-\) (fig 2 of ref 6). The pattern of the process of absorption is thus mainly a function of the concentration of Na\(^+\).

Second, if the inflections in the absorption isotherm over the range of mechanism 2 (fig 1) denote the progressive occupation of additional carrier sites by the substrate ion in response to increasing external concentrations, then at a high external concentration of the substrate ion, increasing the concentration of a competing ion should result in a stepwise reduction of the rate of absorption of the substrate ion, each step or inflection denoting the displacement of substrate ion from 1 of the carrier sites.

Figures 2 to 5 show that this prediction, based on the hypothesis of a spectrum of active sites in carrier mechanism 2, is borne out by the evidence. Increasing concentrations of K\(^+\) cause a stepwise diminution of the rate of Na\(^+\) absorption, when Na\(^+\) is present at 50 mM (fig 2), and conversely, increasing concentrations of Na\(^+\) reduce the rate of absorption of K\(^+\), present at 50 mM, in stepwise fashion (fig 3). Similar findings are shown with Ca\(^{2+}\) as the interfering ion for absorption of Na\(^+\) (fig 4) and K\(^+\) (fig 5).

The addition of the lowest concentration of Ca\(^{2+}\) used (1 mM) caused a slight increase in the rate
of K⁺ absorption over that of the control lacking Ca²⁺ (fig 5). However, it would probably be more proper to say that in the complete absence of Ca²⁺, the rate of K⁺ absorption was somewhat less than in the presence of a low concentration of Ca²⁺. As documented elsewhere (see 11 for references) the presence of Ca²⁺ at a low concentration is essential for unimpaired membrane transport of ions, and this condition, rather than the absence of Ca²⁺, should be looked upon as the physiological control. Omission of Ca²⁺ quickly results in unphysiological responses denoting damage to membrane and transport processes (3, 11). In the present case, reversal by 1.0 mM Ca²⁺ of the negative effect of its omission outweighed its inhibitory effect, resulting in the slight net increase apparent in figure 5 at 1 mM.

It has been shown that barley roots absorb Cl⁻ present at concentrations ranging from 1 to 50 mM via a mechanism, halide carrier mechanism 2, which shows a heterogeneity similar to that of mechanism 2 of alkali cation transport (2). These findings have since been confirmed for roots of 2 species of wheatgrass, Agropyron (1), and for corn roots (9,12). It might thus be surmised that the transport of the anion is the primary act, and that the kinetics of the transport of alkali cations shown here and in references 1, 6, and 9 merely parallel those of chloride transport. However, like the absorption isotherm of the experiment shown in figure 1, in which SO₄²⁻ was the anion, the competition experiments shown in figures 2 to 5 reemphasize a point made earlier (7). It is that the absorption of alkali cations at high concentrations is effected by a cation carrying mechanism in the operation of which the anion plays only a subsidiary role. In each of these experiments, the interfering cation was added as the chloride. Despite the progressive increase in Cl⁻ concentration, the effects of the added salts on the rate of absorption of the substrate cations were negative. Furthermore, there were marked differences in the fine structure of these isotherms which were functions of the cations involved. These results therefore reflect cationic affinities for the active sites of carrier mechanism 2.

However, one point concerning the role of the anion requires comment. The transport of K⁺-Rb⁺ at high concentrations is quite markedly influenced by the identity of the anion (4,7,9). Specifically, substitution of SO₄²⁻ for Cl⁻ as the anion severely inhibits the operation of mechanism 2 with these substrate cations, their rates of absorption rising only slightly above the maximal rate due to the operation of mechanism 1 (fig 8 of ref 4, fig 2 of ref 7, fig 10 of ref 9). Sodium on the other hand, is transported readily by mechanism 2 whether the anion is Cl⁻ or SO₄²⁻ (figs 4 to 6 of ref 11, fig 1 of ref 10), although the rate of absorption of Na⁺ is consistently lower with SO₄²⁻ than with Cl⁻ as the anion. That the rate of Na⁺ absorption via mechanism 2 is depressed so much less severely than is that of K⁺-Rb⁺ by this mechanism is probably related to the considerably higher affinity of mechanism 2 for Na⁺ (7, 11 and see below), and possibly to other factors also.

The results of the experiment shown in figure 6 lead to the conclusion that with both Na⁺ and K⁺ present at equivalent concentrations, over the high range (1-50 mM), mechanism 2 transports Na⁺ almost exclusively, i.e., its active sites have a higher affinity for Na⁺ than for K⁺. The argument is as follows.

Over the range 1 to 10 mM the rate of K⁺ absorption in this experiment averaged 12.8 μmole/g/hr, close to the maximal velocity of K⁺ absorption via mechanism 1 in these roots (11.9 μmole/g/hr in ref 7, and 11.8 in ref 6). Since the rate of absorption of K⁺ (or Rb⁺) via this mechanism is not appreciably affected by Na⁺ (3,4,7) it is concluded that this absorption of K⁺ is almost entirely through the operation of mechanism 1. Over the entire concentration range explored, the rate of K⁺ absorption rose by only 4.5 μmole/g/hr above this value. This small additional uptake must indeed be considered due to the operation of mechanism 2, inasmuch as the rate of K⁺ absorption via mechanism 1 is essentially at the theoretical maximum at 1 mM K⁺ (7).

The absorption of Na⁺ via mechanism 1 of alkali cation transport (see figs 2, 3 and the left side of figs 4 and 5 of ref 11) is virtually abolished in the presence of Ca²⁺ and K⁺ (see fig 1 of ref 10 and fig 6 of ref 11). The absorption of Na⁺ shown in figure 6 of the present paper is therefore due almost entirely to the operation of mechanism 2 of alkali cation transport. The stepwise nature of the Na⁺ absorption isotherm in this figure supports this conclusion. This, when both Na⁺ and K⁺ ions are present at equivalent concentrations in the high range where mechanism 2 contributes to absorption, absorption of K⁺ is almost entirely effected by mechanism 1 and absorption of Na⁺ almost entirely by mechanism 2. This evidence for preferential Na⁺ transport by mechanism 2 confirms the previous conclusion (7) to the effect that mechanism 2 has considerably higher affinity for Na⁺ than for K⁺.

The dual pattern of ion transport discussed in this and the preceding paper (11) in terms of the absorption of Na⁺ has been shown to apply widely, for various ions, for many species of plants, and for different types of tissue (5). Specifically, the complex fine structure of the absorption isotherm characteristic of absorption via the type 2 mechanisms, discovered in experiments with barley roots (2,6), has recently been confirmed in work with roots of Agropyron elongatum and A. intermedium (1) and of Zea mays (9,12). It is therefore likely that the pattern which has emerged is quite general, possibly universal in higher plants. It appears to
offer a rationale for phenomena of specificity and mutual ion effects ("antagonisms"), and in particular, for both the preferential absorption of K\(^+\) and the frequently observed interference by other ions, Na\(^+\) included, with the absorption of K\(^+\). The former phenomenon, of highly selective absorption, is due to the operation of the type 1 mechanisms, whereas the latter, viz. fairly indiscriminate competition, is a characteristic of the type 2 mechanisms. That this generalization holds for other ions as well is brought out in a recent review (5).

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**Literature Cited**


