Absorption of Cations by Roots. Effects of Hydrogen Ions and Essential Role of Calcium [1,2]

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Of all the ions to which plant roots are normally exposed, hydrogen and hydroxyl ions are the most ubiquitous, for roots can only function in aqueous media. The physiological range of H ion concentrations is from 10^{-4} M or slightly more to about 10^{-9} M. One of 2 distinct mechanisms of K absorption in barley roots is effective at K concentrations of comparably low levels (5), operating virtually at maximal velocity at a K concentration of 2×10^{-4} M. Interactions may therefore be expected between H and K ions in the process of K absorption. It has indeed long been recognized that the pH of soil solutions and the activity of H ions in the soil cation exchange system influence the absorption of ions and the growth and ecology of plants.

Experiments in the laboratory have led to 2 main hypotheses concerning the effects of pH on the absorption of ions.

I. *Competition.* Ion carriers are invoked as a cellular mechanism for transport of ions across barriers which are not readily permeable to free ions. Transport is effected through transient attachment of the ions to binding sites of carriers (3). On this view, a low pH of the external solution might reduce the rate of absorption of cations (the substrate cations) by competition between H ions and the substrate cations for available carrier sites. At high pH values, OH or HCO_3 ions might compete with substrate anions, reducing the rate of anion absorption (2).

II. *Injury.* There is evidence that H ions may cause a general derangement of, or damage to, the ion absorption mechanisms. The nature of this injury is not known, but such features as steadily declining absorption rates, leakiness, indiscriminate competition among ions, and eventual death of the tissue indicate that at low pH values progressive and ultimately irreversible alterations in cell structure and function are brought about. The following are suggested as possible mechanisms of injury induced by H ions: denaturation of proteins, nucleic acids, phospholipids, and other polymers involved in membrane structure and function; displacement by

H ions of essential cofactors from functional groups; suppression of ionization of weak acids; increase in concentration of heavy metal ions in solution within the tissue.

In the present investigation, both of the above factors, competition and injury, were found to be involved in the observed reduction of Rb absorption by barley roots induced by H ion. Ca ions govern to a large extent the response of the Rb absorption mechanisms to low pH values in the external solution. Specifically, Ca ions minimize injury due to H ions. On the other hand, Ca ions do not prevent H ions from competitively inhibiting the absorption of Rb.

Materials and Methods

Materials. Laboratory distilled water was passed through a mixed bed ion exchange column (Bantam Barnstead Demineralizer) to free it from heavy metals and other impurities. The demineralized water was stored in Pyrex glass carboys and used in all subsequent preparation of solutions. All glassware used in the experiments was rinsed in 0.5 N HCl and then rinsed repeatedly with distilled water. In experiments with Ca-free solutions, the glassware was rinsed with approximately 6 N HCl followed by repeated rinsing in distilled water and then demineralized water. All salts of the experimental solutions were chlorides, C. P. grade. The pH of the solutions was adjusted by additions of 0.1 N HCl or 0.1 N NaOH. The experimental solutions were aerated with air passed through a Koby Air Purifier. Radioactive Rb^{86} was obtained from Oak Ridge National Laboratory. The experimental solutions of RbCl were labeled to the extent of about 0.002 μc/μmole.

Handling of Root Material. The experiments were done with excised roots of barley, *Hordeum vulgare*, 'Arivat'. Preparation of excised roots and the technique of conducting the absorption experiments have been described (1,7). Samples (1 g) of excised roots were initially suspended in water or a 0.50 mM solution of $CaCl_2$, as indicated for each experiment. They were then rinsed for 1 minute with water or 0.50 mM $CaCl_2$ solution and at zero time, immersed in the vigorously aerated experimental solution. The absorption period was discontinued as described for each experiment.

Radioactive Assay. Preparation of the samples for radioactive assay and counting procedures were as described earlier (1).

Consistency of Results. Results of a given experiment were consistently obtained again in replicate experiments. Actual amounts absorbed per gram tissue under a given set of conditions varied somewhat from experiment to experiment. Differences of up to 20% were common, and occasionally the differences were larger, especially when the experiments were done at intervals of several months. These differences, however, were merely in the absolute rates, not in the relationships obtained.

Results

Since short-term absorption periods were used it was necessary to determine whether Rb uptake was maintained at a constant rate at the pH values used. Figure 1 presents the results of an experiment on Rb absorption at 2 pH values as a function of time. Rb was present at 0.10 mM, Ca at 0.50 mM, and the pH levels were 4.1 and 5.7. Absorption of Rb was a linear function of time and the linear regression of absorption vs. time extrapolated to zero absorption at zero time. There was no evidence of nonmetabolic adsorption of Rb when Ca was present (cf 6).

The experiment shown in figure 2 presents the results of an experiment on Rb absorption as a function of the Rb concentration in solutions of pH 4.1 and 5.7. The absorption time was 10 minutes; rates are calculated on a 60-minute basis. The concentration of Rb varied 100-fold, from 0.002 to 0.20 mM, and Ca was present at 0.50 mM. In figure 2A, the results are plotted conventionally, the rate of absorption, v, on the ordinate as a function of the concen-
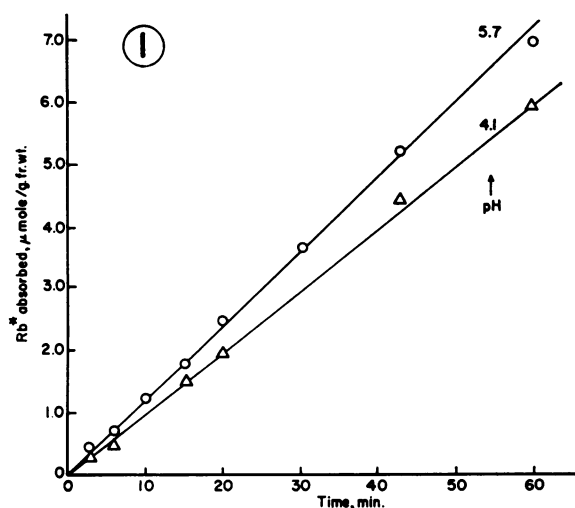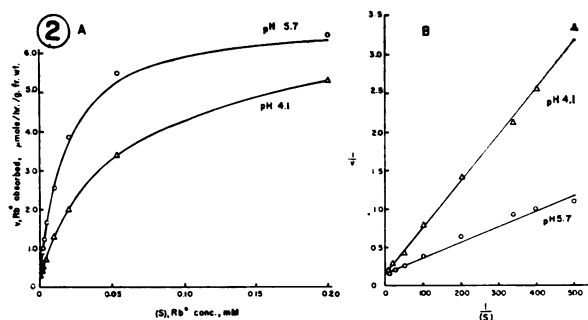

FIG. 2. Absorption of Rb* as a function of the concentration of Rb*Cl, over the range 0.002 to 0.20 mM, at pH 4.1 and 5.7. Ca, 0.50 mM. Tissue in 0.50 mM $CaCl_2$ before the absorption period; absorption discontinued by water rinses. A, conventional plot; B, double-reciprocal plot of the same data.

tration of Rb in the external solution, (S), on the abscissa. Figure 2B is a Lineweaver-Burk plot of the same data, $1/v$, vs. $1/(S)$. The interference with Rb absorption caused by H ions has the characteristics of competitive inhibition. The calculated values of the kinetic analysis are as follows: V_{max}, 7.2 μmole/hr g fr wt; K_M for Rb 0.016 mM; K_I for H, 0.038 mM.

Competition by H ion with Rb in the absorption mechanism is not the only way, however, in which H ions may diminish the absorption of Rb by the roots. At pH 3.9, the rate of Rb absorption from a 0.10 mM solution declined with time in the absence of Ca, whereas in its presence at 0.50 mM it remained constant, as shown in figure 3.

The decrease in the rate of Rb absorption at the low pH levels in the absence of Ca was interpreted to reflect injury due to H ions. The progressive impairment can be almost completely reversed by the addition of 0.50 mM Ca, as demonstrated in figure 4. Rb was present at a concentration of 0.10 mM, Ca when added, 0.50 mM, and the pH was 3.9. Rb absorption in the presence of Ca was a linear function of time for 120 minutes. The —Ca samples show a progressive decline in the rate of Rb absorption. The recovery of the absorption rate did not appear to be complete, i.e., H ion seemed to cause some irreversible injury.

The next 3 figures show the effect of varying Ca concentrations on the absorption of Rb from 0.10 mM solutions of RbCl. The pH was either 5.7 or 4.1 and the absorption time was either 10 or 60 minutes.

At pH 5.7, the rate of Rb absorption was independent of the $CaCl_2$ concentration over the range of 0 to 1 mM $CaCl_2$ and was increased slightly at the higher $CaCl_2$ concentrations (fig 5). At pH 4.1 (fig 6), the rate of Rb absorption increased significantly over the 0 to 2 mM range of $CaCl_2$ concentrations but not beyond. The absorption period in both these experiments was 10 minutes.

When the absorption period lasted 60 minutes

FIG. 1. Absorption of Rb* as a function of time, at pH 4.1 and 5.7. Rb*, 0.10 mM; Ca, 0.50 mM. Tissue in 0.50 mM $CaCl_2$ before the absorption period; absorption discontinued by water rinses.
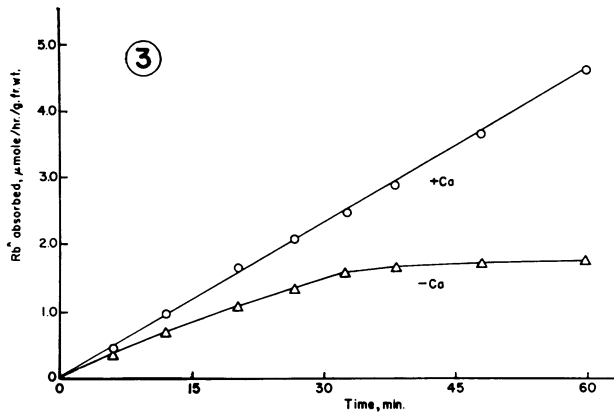
FIG. 3. Absorption of Rb* as a function of time, at pH 3.9.Rb*, 0.10 mM, with and without Ca at 0.50 mM. Samples of –Ca run in water before the absorption period; +Ca samples in 0.50 mM CaCl₂; absorption discontinued by a 30 minute desorption period in cold (5°) 2.0 mM RbCl, 0.50 mM CaCl₂.
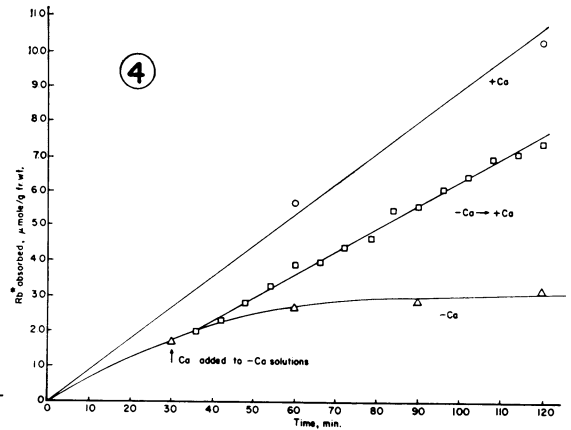
FIG. 4. Absorption of Rb* as a function of time, at pH 3.9, and the effect of adding Ca at 0.50 mM to –Ca solutions. Rb, 0.10 mM. Samples of –Ca run in water before the absorption period, +Ca samples in 0.50 mM CaCl₂; absorption discontinued by a 30 minute desorption period in cold (5°) 2.0 mM RbCl, 0.50 mM CaCl₂.

(fig 7), addition of CaCl₂ considerably increased the rate of Rb absorption over that of the –Ca controls, the effect being more pronounced and elicited over a wider range of CaCl₂ concentrations at pH 4.1 than at pH 5.7.

Discussion

Use of Rb. The experiments reported here were all done with Rb as the substrate ion. Rb is not normally an ion of physiological significance but there is ample evidence that Rb and K are absorbed via identical mechanisms by barley roots (1, 3, 5, 8) and other plant tissues (2). Absorption of Rb closely simulates K absorption, and the findings on Rb absorption presented here are believed to apply equally to absorption of K. The choice of Rb was dictated by the convenience of working with Rb⁸⁶, half-life 18.6 days, instead of with 12.5-hour K⁴².

Uptake by Nonmetabolic Cation Exchange. Values of Rb absorbed reported in this study do not

include measurable nonmetabolic cation exchange components. It is recognized that cations may be taken up by such nonmetabolic, nonselective, reversible cation exchange (4,7). In the present study, however, this fraction was minimized by 2 factors. A. Whenever Ca is included in the experimental solutions at a concentration of 0.50 mM, there is no appreciable uptake of Rb by exchange, provided the Rb concentration in the external solution is kept well below this concentration. Under these conditions, the general, nonselective cation exchange capacity of the tissue is satisfied by Ca ions which keep exchange adsorption of Rb to a minimum (6). [At higher concentrations of Rb this is not so, (7)]. B. In experiments which included treatments without Ca present during the absorption period, Rb ions re-
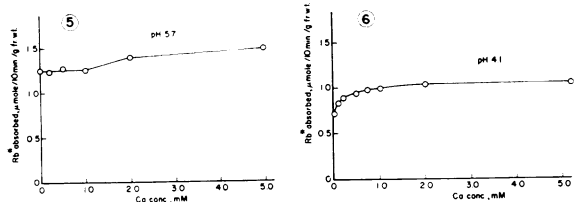

FIG. 5. Absorption of Rb* as a function of the concentration of Ca, at pH 5.7. Rb*, 0.10 mM. Absorption period, 10 minutes. Tissue in water before the absorption period; absorption discontinued by a 30 minute desorption period in cold (5°) 2.0 mM RbCl, 0.50 mM CaCl₂.
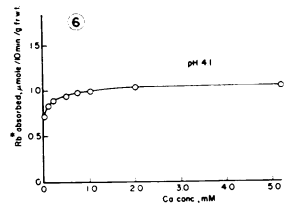
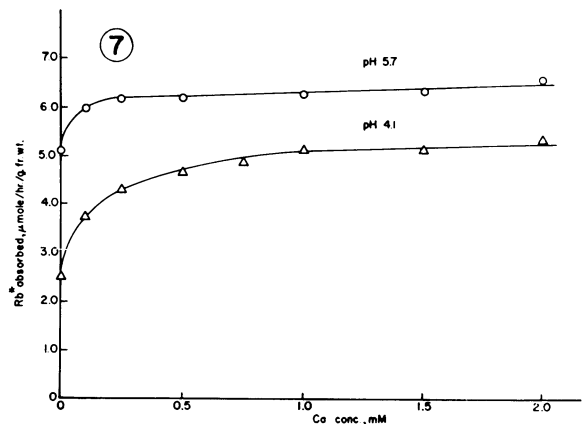FIG. 6. Absorption of Rb* as a function of the concentration of Ca, at pH 4.1. Other conditions as for figure 5.

FIG. 7. Absorption of Rb* as a function of the concentration of Ca, at pH 4.1 and 5.7. Absorption period, 60 minutes. Other conditions as for figure 5.

tained by the tissue by reversible cation exchange were desorbed at the end of the absorption period by exposure of the roots to a cold (5°) solution 2.0 mM in respect to RbCl (nonradioactive) and 0.50 mM CaCl₂ (cf 7).

Absorption Kinetics. The experiment shown in figure 2B demonstrates that over the concentration range 0.002 to 0.20 mM Rb, a single absorption isotherm describes the relation between the external concentration, (S), and the rate of Rb absorption, *v*, at each pH used. The relationship is given by the equation:

$$v = \frac{V_{max} \ (S) \ K_I}{K_M K_I + K_M \ (I) + K_I \ (S)} \qquad I$$

This equation is the Michaelis-Menten equation with inclusion of terms representing a competitive inhibitor or competing substrate present at concentration (I). V_{max} is the maximal rate of absorption of substrate ion at nonlimiting external concentration, (S) the concentration of substrate ion resulting in absorption rate *v*, and K_M and K_I are the Michaelis constants for substrate and competing ion, respectively. If this equation is cast into the reciprocal form and the data are plotted accordingly, $1/v$ vs. $1/(S)$, the slope of the straight line obtained in the presence of the inhibitor is greater than the slope of the control line (no inhibitor) by the factor $1 + [(I) / K_I](3)$.

At pH 5.7 competition by H ion is negligible. Absorption rates are independent of pH over the pH range 5.2 to above 7. At these pH values the term $1 + [(I) / K_I]$ so closely approaches the value of 1 that the solution of equation I becomes numerically equal to that of the simple equation not containing inhibitor terms, viz.,

$$v = \frac{V_{max} \ (S)}{K_M + (S)} \qquad II$$

Not only is competition by H ion negligible at pH 5.7 but so is injury, at least in very short absorption periods. At pH 5.7 absorption in the absence of Ca is not perceptibly different from absorption in its presence, in 10-minute experiments.

Experimental coverage of the relation between *v* and (S) described by this equation was nearly complete. At the lowest concentration used, 0.002 mM Rb, the rate, *v*, of Rb absorption at pH 5.7 was 11% of the theoretical maximal rate, while at the highest concentration, 0.20 mM, *v* was 86% of V_{max}. The 100-fold range of concentrations used, then, covered the response in terms of *v* from a low value to near the theoretical maximum.

The mechanism of absorption operating over this range of concentrations is one of two mechanisms of K-Rb absorption in barley roots (5). It has a high affinity for K and Rb, a low affinity for Na, and is indifferent to the counterion being Cl or SO₄. Another mechanism comes into play at higher concentrations of K or Rb in the external solution. This second mechanism has a much lower affinity for K-Rb than the first, has considerable affinity for Na, and is

inhibited when SO₄ is the counterion instead of Cl (5). The present investigation deals with the high affinity mechanism 1 only. Competitive effects of H ions on absorption by the low affinity mechanism 2 are not likely to be very pronounced because of the wide concentration ratios of substrate cations to H ions at the high concentrations of substrate cations at which the second mechanism operates. Progressive injury, on the other hand, might involve the second mechanism as well as the first.

Competition. The experiment shown in figure 2 shows that H ions competitively interfere with the absorption of Rb. This finding is considered evidence that the carrier sites implicated in the absorption of Rb (and K) have appreciable affinity for H ions. On the basis of the K_M and K_I values for Rb and H ions, respectively, the affinity of these sites for H ions is about one third their affinity for Rb ions.

One interpretation of the effect of Ca in minimizing the interference by H ions with the absorption of monovalent cations has been that Ca blocks the access of H ions to the absorption sites (9). However, the results of our experiments done in the presence of Ca indicate that under these conditions, H ions do have access to the Rb carrying sites since H ions competitively interfere with Rb absorption (fig 2). The present results are in keeping with other evidence leading to the conclusion that cations are absorbed in exchange with H ions which are released in the process (2, 8, 10).

Injury. The conclusion that H ions reduce the rate of absorption of monovalent cations through competition for identical carrier sites is in accord with much earlier work. In several of these investigations Ca was found to mitigate or reverse the inhibiting effect of H ions (2). The present results show that one function of Ca is to prevent or minimize injury to the selective transport mechanism caused by H ions. At pH 3.9 the rate of Rb absorption progressively declined in the absence of Ca, and was nil after about 1 hour (fig 3, 4). In the presence of Ca, this progressive impairment was prevented. Since in the absence of Ca, the rate of Rb absorption is not constant, the effect of Ca is much more pronounced in long than in short experiments, as may be seen by a comparison of the pH 4.1 curves in figures 6 and 7.

The Effects of Ca. Viets (11) and others (2) have shown that Ca accelerates the absorption of various ions by plant tissues. The Ca effects described here are not believed to be accelerations of ion absorption in the sense discussed by Viets, but rather to represent reversal of inhibitory H ion effects. At pH 5.7, the effects of Ca at concentrations up to several mmole per liter were slight or absent (fig 5, 7). Nevertheless, at higher concentrations of Rb and of Ca, positive effects of Ca can be demonstrated even at pH 5.7. These findings suggest, though they do not prove, that the Viets effect involves not the high affinity mechanism 1 discussed here, but the low affinity mechanism 2 which comes into play at higher concentrations of Rb or K (5).

The effects elicited by H ions parallel in large measure those produced by Na ions (1, 5). Both of these ionic species competitively inhibit K-Rb absorption, and both tend to cause a progressive and eventually irreversible impairment of the K-Rb transport mechanism. Ca serves as a protective agent against such damage from Na and H ions. On the other hand, Ca does not prevent these ions from competitively inhibiting K-Rb absorption. The present results thus extend the previous conclusions (1) concerning the essential role of Ca ions in selective ion transport.

duce progressive damage to the absorption mechanism.

The findings reported here on absorption of Rb are believed to apply equally to the absorption of K.

Summary

Experiments are described in which excised barley roots absorbed Rb from solutions of RbCl ranging in concentration from 0.002 to 0.20 mM, in the absence and presence of $CaCl_2$, both at low pH values (3.9–4.1) and at pH 5.7. The temperature was 30°.

In the absence of Ca, the rate of Rb absorption from solutions at pH 3.9 declined with time, and fell to nil in about 1 hour. In the presence of 0.50 mM Ca the rate of Rb absorption remained constant for at least 2 hours. One effect of Ca therefore is to protect the absorption mechanism from progressive impairment by H ions at low external pH.

Over the range of Rb concentrations from 0.002 to 0.20 mM, the rate of Rb absorption was a function of the external Rb concentration according to the Michaelis-Menten relation. Comparison of absorption isotherms at pH 4.1 and 5.7 showed H ions to inhibit competitively the absorption of Rb.

It is concluded that H ions have 2 distinct effects on the absorption mechanism. In the absence of Ca, H ions quickly bring about nonphysiological conditions and the absorption mechanism is progressively impaired. In the presence of Ca, H ions inhibit Rb absorption by competing with Rb for the Rb carrying transport sites. This competition need not in-

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