

# THE ROLE OF CALCIUM IN SELECTIVE CATION UPTAKE BY PLANT ROOTS

## IV. The Effects of Divalent Cations on Exchangeable and Non-exchangeable Fractions of Monovalent Cations in Plant Roots (1)\*

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Exchangeable and non-exchangeable fractions of inorganic ions in plant roots have been investigated in relation to ion uptake mechanisms by several workers (1-3). As a result, it is generally accepted that ions taken up by metabolic processes are largely non-exchangeable with ions of the same or other species in external solution (1, 3, 4). Accordingly, the exchangeable fraction of ions in plant roots corresponds to ions taken up by passive mechanisms, and the non-exchangeable fraction corresponds to ions taken up by active mechanisms.

In previous papers (5-7), it has been suggested that the calcium effect on the selective monovalent cation uptake was closely related to metabolic processes. Moreover, the second paper of this series showed that the effects of divalent cations on rubidium uptake differed in non-desorption and desorption treatments (6).

In the present paper, the effects of divalent cations on the exchangeable and non-exchangeable fractions of monovalent cations taken up by plant roots are examined in more detail. Further, the procedure of desorption treatment for plant roots is discussed in relation to ion absorption experiments.

### MATERIALS AND METHODS

#### *Preparation of Root Materials*

Excised roots of barley (*Hordeum vulgare* L., variety Akashinriki) were used. Root materials were prepared according to the procedure described previously (5).

#### *Experimental Procedure*

To investigate the effects of divalent cations on the uptake of rubidium and sodium, equimolar mixtures of rubidium chloride and sodium chloride (1.0 mM each) were used as the absorption solution. When calcium chloride or magnesium chloride was added to the absorption

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solutions, its concentration was 0.5 mM. Absorption solutions were labeled with radioactive rubidium ( $^{86}\text{Rb}$ ) or radioactive sodium ( $^{22}\text{Na}$ ). The initial pH of the absorption solutions was about 5.6. In all experiments, 0.5 g of roots were used in 500 ml of absorption solution. The absorption period was 60 minutes, during which the solutions were continuously aerated.

At the end of the absorption period, roots were separated from the solution by means of a nylon mesh filter. To remove any adhering solution, roots were blotted with filter paper, then shaken for 30 seconds in 30 ml of washing solution. This washing operation was repeated once more. Demineralized water was used as the washing solution, except when noted.

Roots of certain plots underwent desorption treatment, i.e. after washing, except for particular cases, roots were shaken repeatedly for 30 minutes in 50 ml of desorption solution, then they were blotted with filter paper. Compositions of desorption solutions are described in the figures of the experimental results. All the desorption solutions were non-radioactive.

Experimental temperatures during absorption, washing, and desorption treatments are also described in the figures.

### *Radioactive Assay*

The procedure for the radioactive assay was identical with that described in previous papers (5-7).

## RESULTS

### *Procedure of Desorption Treatment*

Effects of desorption treatment on the exchangeable and non-exchangeable fractions of rubidium taken up by barley roots were investigated under various conditions of time and shaking. The desorption solution used was an equimolar mixture of rubidium chloride and sodium chloride (1.0 mM each). Results are shown in Fig. 1.

Ions in roots after desorption treatment correspond to the non-exchangeable fraction, and ions in roots from the non-desorption treatment correspond to the exchangeable plus non-exchangeable fractions. Therefore, the difference obtained by subtracting the value of the desorption treatment from the non-desorption treatment corresponds to the exchangeable fraction. Shadowed bars in Fig. 1 show the non-exchangeable fractions, and heights of the dashed lines on the shadowed bars (the differences between the non-desorption and desorption treatments) show the exchangeable fractions. Numbers at the right of the bars are ratios of the exchangeable and non-exchangeable fractions of ions taken up by barley roots. The same system is used in the figures described below.

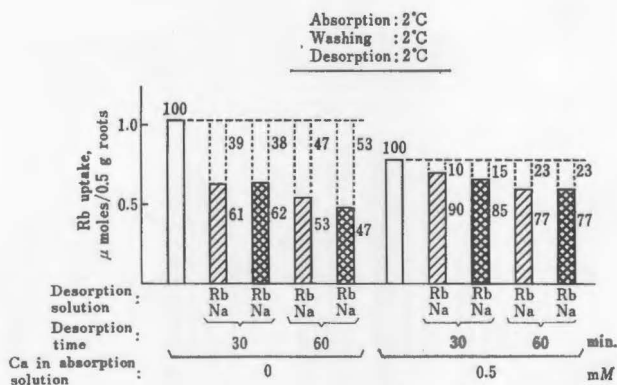


Fig. 1. Effects of desorption treatment on exchangeable and non-exchangeable fractions of rubidium. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride and sodium chloride in the desorption solution: 1.0 mM)

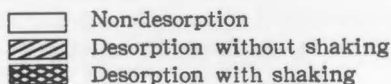


Fig. 1 shows that the non-exchangeable fraction of rubidium was slightly smaller, and the exchangeable fraction was slightly larger at 60 minutes of desorption time than at 30 minutes. But, the difference between values with and without shaking during desorption treatment was very small. Striking effects were observed when calcium was added to the absorption solution. Under the various conditions of time and shaking during desorption treatment, calcium in the absorption solution induced an increase in the non-exchangeable fraction and a decrease in the exchangeable fraction of rubidium.

#### *Effects of Calcium and Magnesium in the Absorption Solution*

Effects of divalent cations in the absorption solution on the exchangeable and non-exchangeable fractions of rubidium and sodium were investigated at different temperatures. The desorption solution used in this experiment was an equimolar mixture of rubidium chloride and sodium chloride (1.0 mM each). Figs. 2 and 3, respectively, show the results on rubidium and sodium uptake.

As shown in Fig. 2, the presence of calcium in the absorption solution decreased the exchangeable fraction and increased the non-exchangeable fraction of rubidium taken up by barley roots at different temperatures. The presence of magnesium was not as effective as that of calcium. Fig. 3 shows that the effects of calcium and magnesium in the absorption solution on the ratios of the exchangeable and non-exchangeable fractions of sodium were similar to the results for rubidium described above.

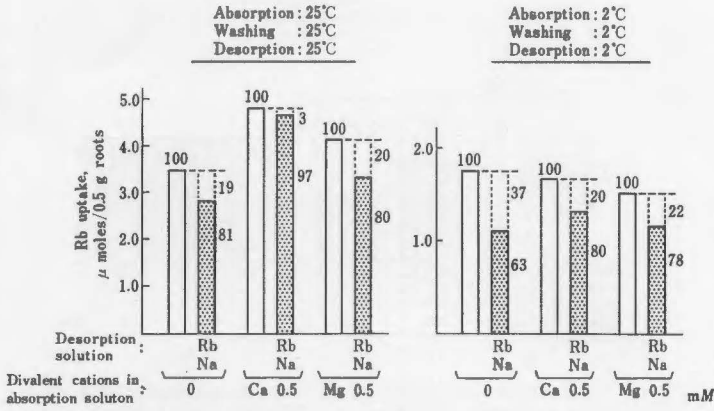


Fig. 2. Effects of divalent cations in the absorption solution on exchangeable and non-exchangeable fractions of rubidium. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride and sodium chloride in the desorption solution: 1.0 mM)

Non-desorption  
 Desorption

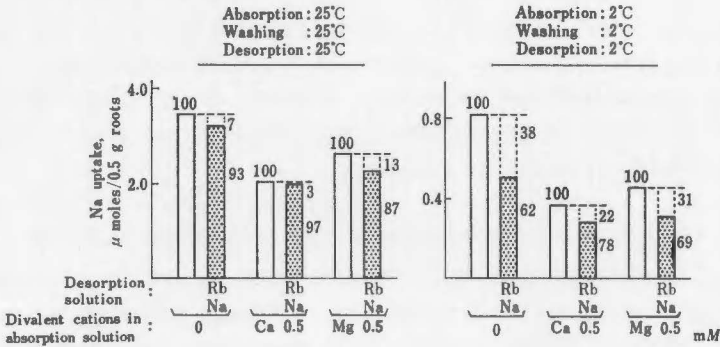


Fig. 3. Effects of divalent cations in the absorption solution on exchangeable and non-exchangeable fractions of sodium. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride and sodium chloride in the desorption solution: 1.0 mM)

Non-desorption  
 Desorption

*Effects of Calcium in the Desorption Solution*

*a) Species of salt in the desorption solution*

Effects of types of desorption solutions on the exchangeable and non-exchangeable fractions of rubidium were investigated in the absence and presence of calcium in the absorption solution, and at various temperatures. Three types of desorption solutions were used: an equimolar mixture of rubidium chloride and sodium chloride (1.0 mM each), calcium chloride (1.0 mM), and calcium sulfate (1.0 mM). Results are shown in Fig. 4.

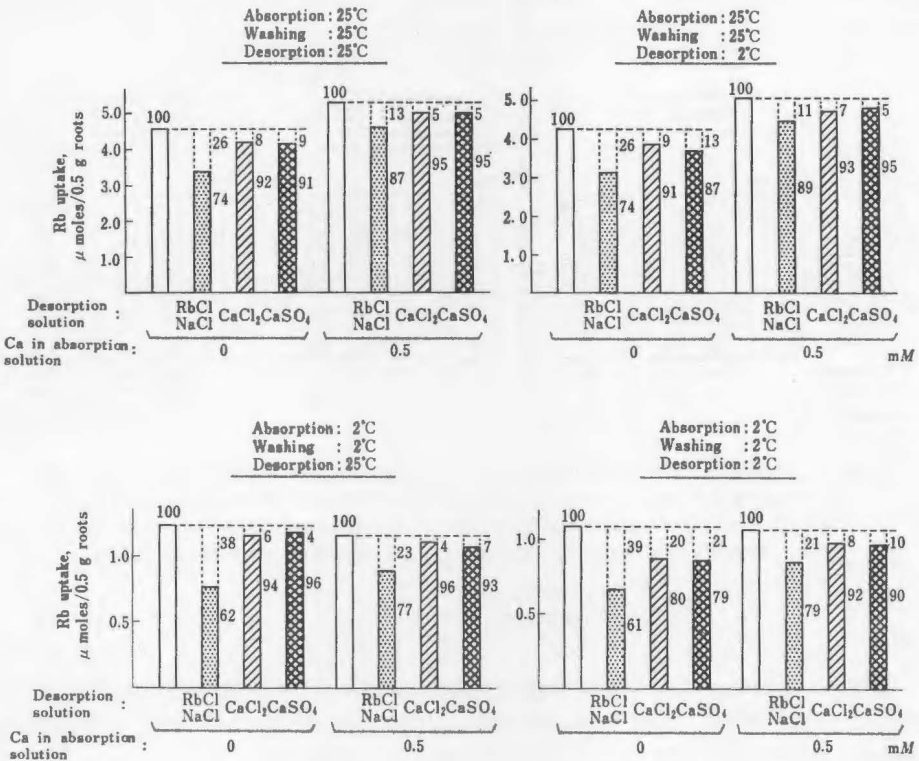
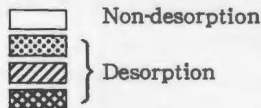


Fig. 4. Effects of desorption treatments with different types of salt solutions on exchangeable and non-exchangeable fractions of rubidium. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride, sodium chloride, calcium chloride, and calcium sulfate in the desorption solution: 1.0 mM)



When calcium chloride or calcium sulfate was used as the desorption solution, the non-exchangeable fraction of rubidium became larger, and

the exchangeable fraction became smaller than when an equimolar mixture of rubidium chloride and sodium chloride was used. The difference between the calcium chloride and calcium sulfate solutions was essentially negligible.

*b) Concentration of calcium in the desorption solution*

Effects of the concentration of calcium in the desorption solution on the exchangeable and non-exchangeable fractions of rubidium were investigated at various temperatures. The desorption solutions used were equimolar mixtures of rubidium chloride and sodium chloride with concentrations of calcium increasing from 0 to 1.0 mM.

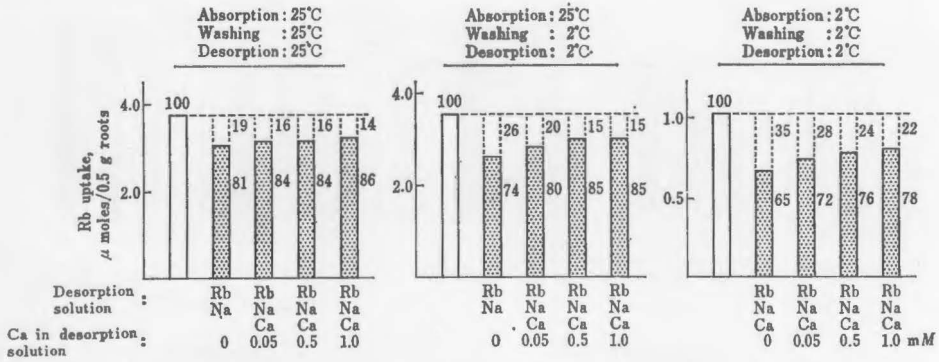


Fig. 5. Effects of the concentration of calcium in the desorption solution on exchangeable and non-exchangeable fractions of rubidium. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride and sodium chloride in the desorption solution: 1.0 mM)

□ Non-desorption  
 ▨ Desorption

Fig. 5 shows that the non-exchangeable fraction of rubidium increased, and that the exchangeable fraction decreased with increasing concentrations of calcium in the desorption solution.

*Effects of Calcium in the Washing Solution*

Effects of calcium in the washing solution on the exchangeable and non-exchangeable fractions of rubidium were investigated at various temperatures. Demineralized water and two concentrations of calcium chloride solutions (0.05 and 0.5 mM) were used as the washing solution. After the absorption treatment, roots were shaken for 30 seconds in 30 ml of washing solution. This washing operation was repeated twice. An equimolar mixture of rubidium chloride and sodium chloride (1.0 mM each) was used as the desorption solution in all treatments. Results are presented in Fig. 6.

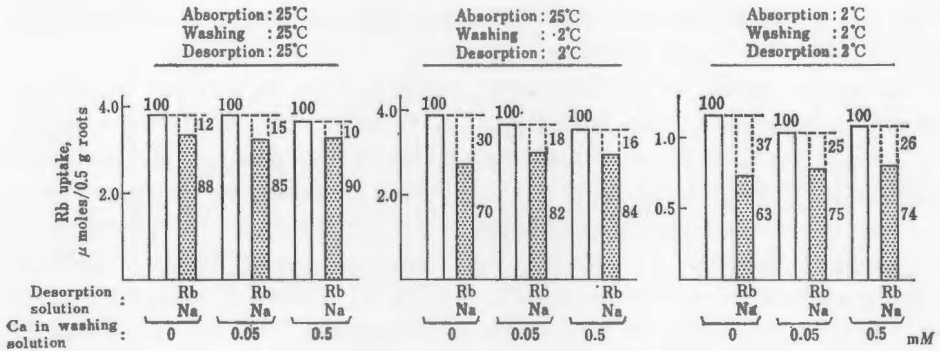


Fig. 6. Effects of the concentration of calcium in the washing solution on exchangeable and non-exchangeable fractions of rubidium. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride and sodium chloride in the desorption solution: 1.0 mM)

Non-desorption  
 Desorption

Although the results were not so clear at a temperature of 25°C, the presence of calcium in the washing solution changed the ratios of the exchangeable and non-exchangeable fractions of rubidium in barley roots after desorption treatments. When calcium chloride solutions were used as the washing solution, the non-exchangeable fraction of rubidium became larger, and the exchangeable fraction became smaller than that when demineralized water was used.

DISCUSSION

*Effects of Calcium on the Exchangeable and Non-exchangeable Fractions of Monovalent Cations in Plant Roots*

It has been suggested that calcium plays an important role in the permeability of plant cells (8-14), and that calcium decreases the loss of sodium, rubidium, and potassium from plant roots (12, 15-18).

In the present investigation, it was observed that the exchangeable fraction of rubidium taken up by plant roots decreased in the presence of calcium in every solution for absorption, washing, and desorption treatments, while the non-exchangeable fraction increased (Figs. 1, 2, 4-6). Both fractions of sodium were affected in a similar manner by the presence of calcium in the absorption solution (Fig. 3). The above results do not suggest that the effects of calcium in the absorption solution on the ratios of the exchangeable and non-exchangeable fractions of monovalent cations induced the selective uptake of monovalent cations described in previous papers (5-7), because the effects of calcium on the



ratios of the exchangeable and non-exchangeable fractions of rubidium were similar to that of sodium.

However, even when the total cations were equivalent in all the desorption solutions, the exchangeable fraction of rubidium was decreased by the use of calcium salt solutions in the desorption treatment, whereas the non-exchangeable fraction was increased, as compared with the use of a mixture of monovalent cations (Fig. 4). The effects of calcium salt described above did not differ in extent between calcium chloride and calcium sulfate solutions (Fig. 4). Thus, it was considered that the effects of calcium salt on the exchangeable and non-exchangeable fractions of monovalent cations were due to calcium ion, but not to accompanying anions. Further, these calcium effects were observed when a total time of treatment was as short as 1 minute, too (Fig. 6).

The stimulating effect of calcium on rubidium or on potassium uptake by plant roots is thought to be dependent on the metabolic processes of ion absorption (11, 15; 19-24). However, it has been suggested that this effect of calcium is caused by an inhibition of the efflux of potassium from plant roots (25). In this paper, it was clearly shown that calcium represses the exchange-desorption of monovalent cations, especially rubidium. Further studies on the effects of calcium discussed above will be presented in a following paper.

#### *Desorption Treatment for Plant Roots in the Ion Absorption Experiment*

In spite of the importance of the washing treatment in ion absorption experiments with plant roots, there are only a few reports on the washing technique of plant roots after absorption treatment (1, 26). Washing methods for plant roots also differ in many papers on ion absorption by plants. Previously, we examined on the washing operation with water for plant roots in ion absorption experiments (27). In the present paper, the results of investigations on desorption treatment with salt solutions were presented.

The non-exchangeable fraction of rubidium was slightly smaller, and the exchangeable fraction was slightly larger at 60 minutes of desorption time than at 30 minutes (Fig. 1). Effects of shaking during desorption treatment on the exchangeable and non-exchangeable fractions were very small (Fig. 1). Accordingly, in the experiment of monovalent cation absorption, 30 to 60 minutes of desorption time with occasional shaking suffice for the desorption treatment of plant roots.

The exchangeable fraction of rubidium was larger at a lower temperature for desorption treatment than that at a higher temperature (Figs 4-6). These results may be due to high rates of re-absorption of cations which have been eluted during desorption treatment at a higher



temperature. Thus, the washing and desorption treatments should be carried out at low temperature.

The most striking effects on the exchangeable and non-exchangeable fractions of monovalent cations was observed in the presence of calcium in the absorption, washing, and desorption solutions (Figs. 1-6). Desorption solutions must be used after careful consideration, and the composition should be described clearly in all papers.

#### SUMMARY

The effects of divalent cations, especially calcium ion, on the exchangeable and non-exchangeable fractions of monovalent cations were investigated in relation to the processes of cation absorption by excised barley roots.

1) The exchangeable fraction of rubidium in plant roots was decreased by the presence of calcium salt in every solution for absorption, washing, and desorption treatments, while the non-exchangeable fraction was increased. Both fractions of sodium were also affected in a similar manner by the presence of calcium salt in the absorption solution. The presence of magnesium salt was not as effective as that of calcium salt.

The effects of calcium salt described above did not differ in extent between the calcium chloride and calcium sulfate solutions in the desorption treatment. Accordingly, it was concluded that the effects of calcium salt on the exchangeable and non-exchangeable fractions of monovalent cations were due to calcium ion, but not to accompanying anions.

2) It was not considered that the effects of calcium in the absorption solution on the ratios of the exchangeable and non-exchangeable fractions induced the selective uptake of monovalent cations by plant roots, because the effects of calcium on rubidium uptake were similar to those of sodium uptake.

3) In connection with the experimental method of cation absorption by plant roots, the effects of time, temperature, and salt composition during desorption treatment on the exchangeable and non-exchangeable fractions were discussed.

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