

THE ROLE OF CALCIUM IN SELECTIVE CATION UPTAKE BY PLANT ROOTS

V. The Effects of Divalent Cations on Exchangeable and Non-exchangeable Fractions of Monovalent Cations in Plant Roots (2)*

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In the preceding paper (1), it was shown that the exchangeable fraction of rubidium taken up by plant roots decreased in the presence of calcium in every solution used for the absorption, washing, and desorption treatments, while the non-exchangeable fraction increased. Both fractions of sodium were similarly affected by the presence of calcium in the absorption solution.

According to the Donnan equilibrium theory, the adsorption of polyvalent cations by plant roots is larger than that of monovalent cations (2). Some workers (3-5) have reported that the exchange-adsorption of calcium ions by plant roots is larger than that of ammonium or potassium ions. The fact that the exchangeable fraction of rubidium in plant roots was decreased by the presence of calcium in the absorption solution (1) is explainable according to the above theory. On the same basis, however, we cannot explain how the calcium in the washing or desorption solutions produced a similar effect on the exchangeable fraction of rubidium.

The effect of calcium during the desorption treatment must have been caused by interaction between the radioactive rubidium ions in the plant roots and non-radioactive cations in the desorption solution. In this paper, the mechanisms for the decrease of the exchangeable fraction of rubidium, which was desorbed with calcium chloride solution, are examined in comparison to those observed when a mixture of rubidium chloride and sodium chloride was used as the desorption solution.

MATERIALS AND METHODS

Preparation of Root Materials

Excised roots of barley (*Hordeum vulgare* L., cultivar Akashinriki) were used. Root materials were prepared according to a procedure described previously (6). Temperatures during the growing period were 25°C for barley, and 30°C for rice.

In an experiment on the exchange-adsorption of cations by barley

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roots, excised roots prepared by the above procedure were dried at 55°~60°C, then ground in a mortar and pestle, and passed through 60 mesh sieve.

Experimental Procedure

Exchange-adsorption of cations by barley roots (dry powder)

Adsorption of cations by barley roots was determined according to the "percolation method" of Inden et al. (5). Suitable aliquots (0.4~0.8 g) of barley root powder were mixed with 8 g of quartz sand, then the mixtures were packed into columns 10 mm in diameter and 150 mm long. The samples were saturated with hydrogen ion by passing 40 ml of 0.05 N hydrochloric acid and 100 ml of demineralized water through the columns. Each sample was treated with 80 ml of solution which contained equivalent amounts of two cations (0.1 N each, as acetate), and then washed with 100 ml of demineralized water. After this treatment, cations adsorbed by the sample were eluted by passing 50 ml of 0.05 N hydrochloric acid and demineralized water through the column. Flow rates of the solutions used in this experiment were all about 4 ml per minute.

Calcium and magnesium in the eluent were determined by the chelatometric titration with sodium ethylenediamine tetraacetate, and potassium and sodium by flame-photometry.

Effects of calcium on exchange-desorption of monovalent cations from excised plant roots

In experiments of this part, equimolar mixtures of rubidium chloride and sodium chloride (1.0 mM each) were used as the absorption solution, except when noted. Absorption solutions were labeled with radioactive rubidium (⁸⁶Rb) or radioactive sodium (²²Na). The initial pH of the absorption solutions was about 5.7.

Procedures for absorption, washing, and desorption treatments were virtually identical with those described in the preceding paper (1). The procedure for the radioactive assay was identical with that described in previous papers (6-8). The exchangeable and non-exchangeable fractions of the monovalent cations in plant roots were calculated according to the methods given in the preceding paper (1).

All desorption solutions were non-radioactive, and their compositions are given in the figures for the experimental results. Experimental temperatures are also given in the figures.

RESULTS

Exchange-adsorption of Cations by Barley Roots (Dry Powder)

Adsorption of various cations was determined with dry powder of excised barley roots. Results are shown in Table 1. Adsorption of divalent cations, especially calcium, was markedly larger than those of monovalent cations.

TABLE 1
Cation adsorption in barley roots (dry powder)

Treatment*	Cation adsorbed (me/100 g roots**)			
	Ca ⁺⁺	Mg ⁺⁺	K ⁺	Na ⁺
Ca ⁺⁺ +K ⁺	9.80	—	0.15	—
Ca ⁺⁺ +Na ⁺	9.28	—	—	0.07
Mg ⁺⁺ +K ⁺	—	9.19	0.20	—
Mg ⁺⁺ +Na ⁺	—	9.19	—	0.05
Ca ⁺⁺ +Mg ⁺⁺	5.78	3.57	—	—
K ⁺ +Na ⁺	—	—	3.53	3.20

* Cation concentration: 0.1 me/l each (as acetate)

** Dry weight

Effects of Calcium on Exchange-desorption of Monovalent Cations from Excised Plant Roots

(1) *Effects of desorption treatments with different types of salt solutions (Rubidium uptake by excised barley roots)*

Excised barley roots, which had absorbed rubidium from the absorption solution, were subjected to desorption treatments with different types of salt solutions: a mixture of rubidium chloride and sodium chloride (1.0 mM each); calcium chloride solution (1.0 mM); and a mixture of rubidium chloride, sodium chloride and calcium chloride (1.0 mM each). Results are shown in Fig. 1.

As described in the preceding paper (1), ions in roots after the 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 for the desorption treatment from that for the non-desorption treatment corresponds to the exchangeable fraction. Shaded bars in Fig. 1 show the non-exchangeable fractions. Heights of the dashed lines on the shaded bars (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 in this paper, except in Fig. 3.

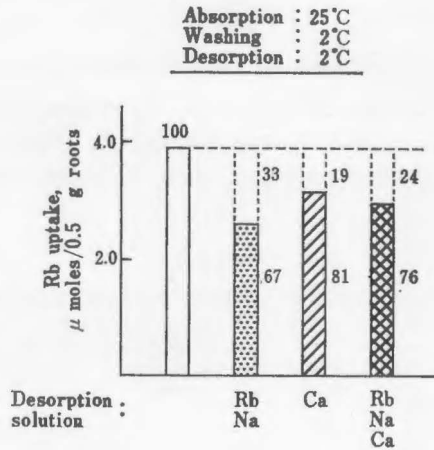


Fig. 1. Effects of desorption treatments with different types of salt solutions on exchangeable and non-exchangeable fractions of rubidium in excised barley roots. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride, sodium chloride and calcium chloride in the desorption solution: 1.0 mM)

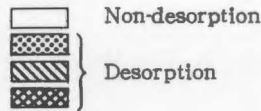


Fig. 1 shows that the calcium in the desorption solution increased the non-exchangeable fraction of rubidium and decreased the exchangeable fraction in agreement with the result of the preceding paper (1). The exchangeable fraction of rubidium was smallest in the desorption solution of only calcium chloride, and became progressively larger in mixtures of rubidium chloride, sodium chloride and calcium chloride; and in rubidium chloride and sodium chloride. The non-exchangeable fraction of rubidium showed the reverse of the above order.

(2) *Effect of 2, 4-dinitrophenol (DNP) in the desorption solution (Rubidium uptake by excised barley roots)*

Effects of calcium on the exchangeable and non-exchangeable fractions of rubidium described above were examined with and without the addition of DNP in the desorption solution. The concentration of DNP used was 0.001 mM, and the temperature was kept at 2°C during the experiment.

Fig. 2 shows that the effects of three types of desorption solutions (a mixture of rubidium chloride and sodium chloride; a mixture of rubidium chloride, sodium chloride and calcium chloride; calcium chloride

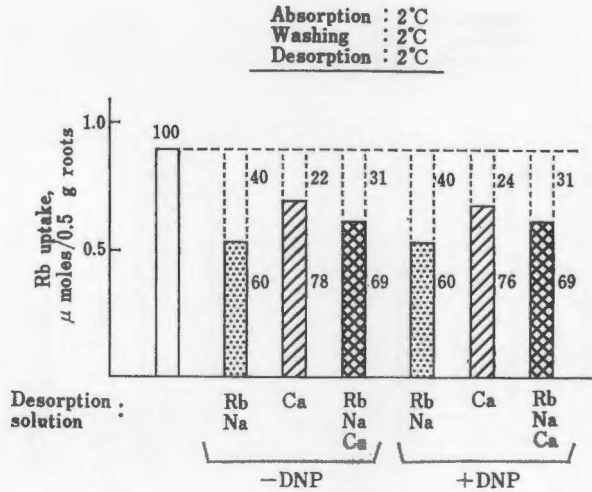
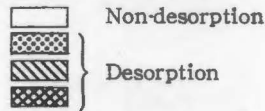


Fig. 2. Effect of DNP in the desorption solution on exchangeable and non-exchangeable fractions of rubidium in excised barley roots. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride, sodium chloride and calcium chloride in the desorption solution: 1.0 mM; DNP in the desorption solution: 0.001 mM)



solution) on the exchangeable and non-exchangeable fractions of rubidium did not change in the presence of DNP during the desorption treatment.

(3) *Effects of desorption treatments with different types of salt solutions (Rubidium uptake by excised rice roots)*

With excised rice roots, a preliminary experiment on the effect of calcium in the absorption solution was carried out at 25° and 2°C. The desorption solution used in this experiment was a mixture of rubidium chloride and sodium chloride. Results are presented in Fig. 3.

When the temperature of the absorption period was 25°C, the rubidium uptake by excised rice roots was increased by the presence of calcium in the absorption solution, as compared with the uptake in the absence of calcium. These results closely agree with those obtained with excised barley roots (6-8). However, when the temperature of the absorption period was 2°C, the rubidium uptake was decreased by the presence of calcium in the absorption solution. These latter results, under the condition with desorption treatment, were not in accord with those for excised barley roots (7).

Based on the experimental results described above, the effects of the

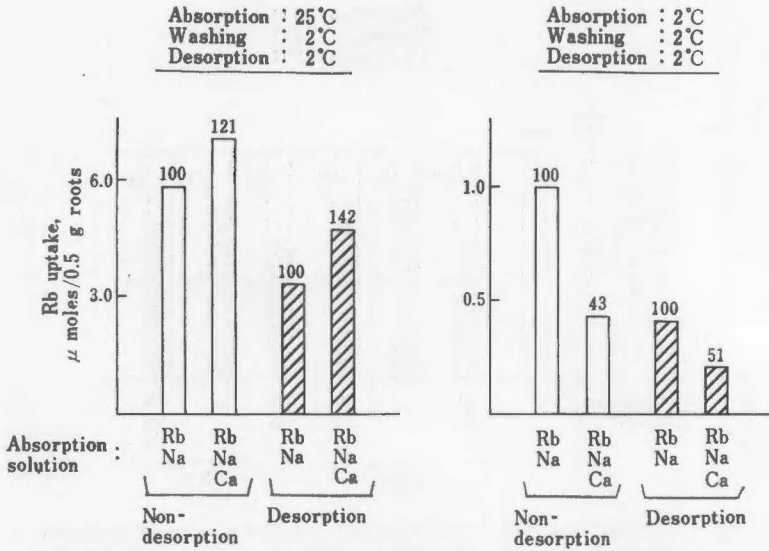


Fig. 3. Effect of calcium in the absorption solution on rubidium uptake by excised rice roots. (Rubidium chloride, sodium chloride and calcium chloride in the absorption solution: 1.0, 1.0 and 0.5 mM each; Rubidium chloride and sodium chloride in the desorption solution: 1.0 mM)

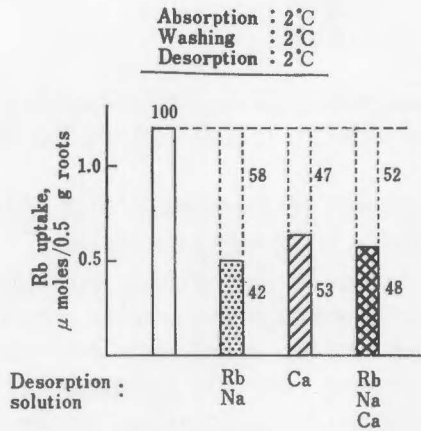
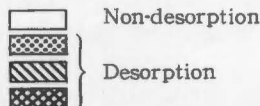


Fig. 4. Effects of desorption treatments with different types of salt solutions on exchangeable and non-exchangeable fractions of rubidium in excised rice roots. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride, sodium chloride and calcium chloride in the desorption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



three types of desorption solutions on the exchangeable and non-exchangeable fractions of rubidium were examined with excised rice roots at a low temperature (2°C). Results are shown in Fig. 4.

It was described above that the effect of calcium in the absorption solution on the exchangeable and non-exchangeable fractions of rubidium differed markedly between excised barley roots and excised rice roots for desorption treatment at a low temperature. As shown in Fig. 4, however, the effects of the three types of desorption solutions on the exchangeable and non-exchangeable fractions of rubidium in excised rice roots were similar to those for the excised barley roots of Fig. 1.

(4) *Effects of desorption treatments with different types of salt solutions (Sodium uptake by excised barley roots)*

It was previously found that the sodium uptake by excised roots decreased in the presence of calcium in the absorption solution under various conditions (7). The effects of three types of desorption solutions on the exchangeable and non-exchangeable fractions of sodium were examined with excised barley roots at a low temperature (2°C).

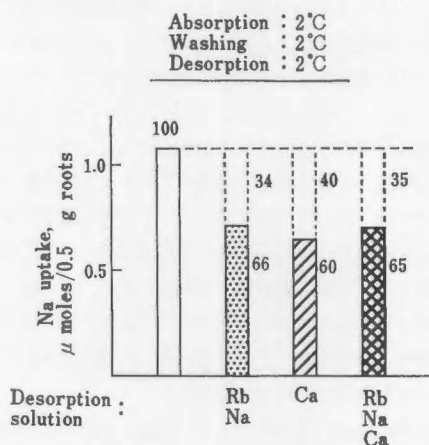
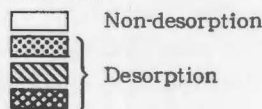


Fig. 5. Effects of desorption treatments with different types of salt solutions on exchangeable and non-exchangeable fractions of sodium in excised barley roots. (Rubidium chloride and sodium chloride in the absorption solution: 1.0 mM; Rubidium chloride, sodium chloride and calcium chloride in the desorption solution: 1.0 mM)



As shown in Fig. 5, the non-exchangeable fraction of sodium was slightly decreased by the use of calcium chloride solution, but differences were very small among the three types of desorption solutions.

DISCUSSION

It was previously found that the exchangeable fraction of rubidium was decreased by the presence of calcium in the absorption, washing and desorption solutions, while the non-exchangeable fraction was increased. To investigate the causes of this results, four mechanisms are proposed and examined below.

If the desorption solutions used in experiments were (a) a mixture of rubidium chloride and sodium chloride and (b) calcium chloride solution, the mechanisms to be examined are as follows:

(i) When a mixture of rubidium chloride and sodium chloride was used as the desorption solution, an isotopic-exchange between the radioactive rubidium in the plant roots and non-radioactive rubidium in the desorption solution might have occurred in addition to the exchange-desorption of radioactive rubidium. Consequently, the exchangeable fraction of rubidium which was desorbed with a mixture of rubidium chloride and sodium chloride should be larger than that with calcium chloride solution.

(ii) When a mixture of rubidium chloride and sodium chloride is used as the desorption solution, the specific activity (total radioactivity of ^{86}Rb per gram of rubidium) of the desorption solution should be very small in comparison with that when calcium chloride solution was used. If plant roots reabsorbed the same amount of rubidium from the desorption solution, the amount of reabsorbed ^{86}Rb should be smaller in the desorption treatment with a mixture of rubidium chloride and sodium chloride than with calcium chloride solution. Therefore, the apparent exchangeable fraction of rubidium might be larger when a mixture of rubidium chloride and sodium chloride was used as the desorption solution, i. e. the apparent exchangeable fraction of rubidium might be smaller in a desorption solution of calcium chloride.

(iii) In previous papers (1, 7, 8), it was found that rubidium uptake was increased by the presence of calcium in the absorption solution. When calcium chloride solution was used in the desorption treatment, the decrease in the exchangeable fraction of rubidium might be due to the acceleration of reabsorption of rubidium during desorption.

(iv) If the three mechanisms described above cannot account for the facts that the exchangeable fraction of rubidium was decreased, and the non-exchangeable fraction was increased by the presence of calcium under various conditions, then the only mechanism which would account for the results would be that calcium inhibited, perhaps metabolically, the rubidium efflux from the plant roots.

A preliminary experiment showed that the adsorption of divalent cation by excised barley roots was markedly larger than that of mono-

valent cation (Table 1), in good agreement with the results for various species of plant roots (3-5). As stated previously, this result may explain why the exchangeable fraction of rubidium was decreased by the presence of calcium during absorption, but it does not explain the effects of calcium in the washing solution and the desorption solution in the preceding paper (1).

From a comparison of the presence and absence of calcium in desorption solutions containing the same amount of monovalent cations (Fig. 1), it was concluded that the decrease in the exchangeable fraction of rubidium in the presence of calcium was not caused by a difference in isotopic-exchange. [Results (Fig. 1) also showed that the effect of calcium in the desorption solution was not due to a difference in specific activities for the desorption solutions.

To examine the effect of calcium on the reabsorption of rubidium during desorption, experiments were carried out under conditions in which the rubidium uptake is inhibited by calcium. In the previous paper (Part III) of this series (7), when DNP (0.001 mM) was added to the absorption solution, the rubidium uptake was decreased by the presence of calcium at 2°C. In Fig. 2, however, the exchangeable fraction of rubidium, after treatment with three desorption solutions, showed no difference in order between the conditions for the addition and non-addition of 0.001 mM DNP at 2°C, i.e. the exchangeable fraction of rubidium after desorption treatment with a mixture of rubidium chloride, sodium chloride and calcium chloride was smaller than that with a mixture of rubidium chloride and sodium chloride. Accordingly, the decrease in the exchangeable fraction of rubidium in the presence of calcium could not be due to acceleration of the reabsorption of rubidium in the desorption solution.

Moreover, rubidium uptake by excised rice roots decreased in the presence of calcium at 2°C during the absorption period, in contrast to results at 25°C (Fig. 3). However, the exchangeable fractions of rubidium, after treatment with desorption solutions containing calcium, were smaller than with a desorption solution containing no calcium (Fig. 4). Although it has been reported that the low potassium release from excised plant roots is due to the reabsorption of effluxed potassium (9), the above results also suggest that the decreasing effect of calcium on the exchangeable fraction of rubidium was not due to stimulation of the reabsorption of rubidium.

The three mechanisms proposed on (i) to (iii) cannot explain the facts that the exchangeable fraction of rubidium was decreased by the presence of calcium in the absorption, washing and desorption solutions, and the non-exchangeable fraction of rubidium was increased. Therefore, it was concluded that calcium inhibited rubidium efflux from plant

roots, but that the inhibition of rubidium efflux was not due to the apparent decrease in the exchangeable fraction of rubidium.

As shown in previous papers (6-8), stimulation of the rubidium uptake by calcium is related to the metabolic processes of ion absorption. In addition, it has been shown in this paper that calcium inhibited the efflux of rubidium from plant roots, in agreement with Hooymans' report (10).

On the other hand, calcium has been reported to inhibit the efflux of sodium (11). However, in this experiment, the calcium in the desorption solution scarcely affected the exchangeable fraction of sodium (Fig. 5), while the calcium in the absorption solution decreased it (8).

SUMMARY

In the preceding paper (8), it was reported that the exchangeable fraction of rubidium in plant roots was decreased by the presence of calcium in every solution used in the absorption, washing and desorption treatments, while the non-exchangeable fraction was increased. Both fractions of sodium were similarly affected by the presence of calcium in the absorption solution. This paper reports further investigations in relation to the results reported previously.

1) The exchange-adsorption of divalent cations, especially calcium, by barley roots (dry powder) was larger than that of monovalent cations. This suggests that the character of the exchange-adsorption of plant roots may induce a decrease in the exchangeable fraction of monovalent cations due to the presence of calcium in the absorption solution. However, the effects of calcium in the washing or the desorption solution cannot be similarly explained.

2) It is unlikely that the decrease in the exchangeable fraction of rubidium due to the presence of calcium in the desorption solution can be ascribed to differences in the isotopic-exchange between plant roots and various desorbing solutions or to differences in the specific activities of the effluxed radioactive rubidium in the desorption solution. Further, the decrease in the exchangeable fraction of rubidium by calcium was not due to the stimulating effect of calcium on the reabsorption of effluxed rubidium.

3) The exchangeable fraction of sodium in plant roots was hardly affected by the presence of calcium in the desorption solutions.

4) Based on the above results, it is reasonable to assume that the decrease in the exchangeable fraction of rubidium due to the presence of calcium was caused by the inhibitory effect of calcium on the exchange-desorption of rubidium from plant roots.

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