THE ROLE OF CALCIUM IN SELECTIVE CATION UPTAKE BY PLANT ROOTS

III. The Effect of Calcium on Cation Uptake as Influenced by Metabolic Inhibitors (Cyanide, DNP, and Ouabain)*

Toshio KAWASAKI, Shiro HORI** and Masumi MORITSUGU

In previous papers (1, 2), it has been reported that the stimulating effect of calcium on the rubidium uptake of excised barley roots was closely related to the metabolic process of ion uptake. To investigate in more detail the mechanisms of the calcium effect the present study was done by using some metabolic inhibitors. The metabolic inhibitors used were as follows: (i) cyanide, an inhibitor of metal oxidases in the respiratory chain (3, 4); (ii) 2,4-dinitrophenol (DNP), an uncoupling agent of oxidative phosphorylation (3, 4); (iii) g-strophanthin (ouabain), an inhibitor of adenosine triphosphatase (ATPase) and of the active transport of potassium and sodium in animal cells (5-18).

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 (1, 2).

Experimental Procedure

An equimolar mixture of rubidium chloride and sodium chloride (1.0 m*M* each) was used as the absorption solution in this study, except in the experiment in which cyanide was used. When cyanide was added, the absorption solution was a mixture of rubidium chloride and sodium cyanide (1.0 m*M* each). Absorption solutions were labeled with radioactive rubidium (^{86}Rb) or radioactive sodium (^{22}Na). The initial pH of the absorption solution was adjusted to about 5.4 immediately before an experiment.

Procedures for the absorption experiment, washing operation, and desorption treatment after washing were virtually identical with those described in previous papers (1, 2). In all the experiments, an equimolar mixture of rubidium chloride and sodium chloride (1.0 m*M* each) was used as the desorption solution, which was non-radioactive.

* Data presented in this paper were published in the Journal of the Science of Soil and Manure, Japan, Vol. 41, p. 467-472 (1970), in Japanese.

** Department of Applied Biology, Radiation Center of Osaka Prefecture.

Redioactive Assay

The procedure for the radioactive assay was also identical with that described in previous papers (1, 2).

RESULTS

Influence of Cyanide

The effects of calcium on the uptakes of rubidium and sodium as influenced by cyanide were investigated under various conditions with or without the desorption treatment of roots after an absorption period at 25° and 2°C. Figs. 1 and 2 show the rates of rubidium and sodium

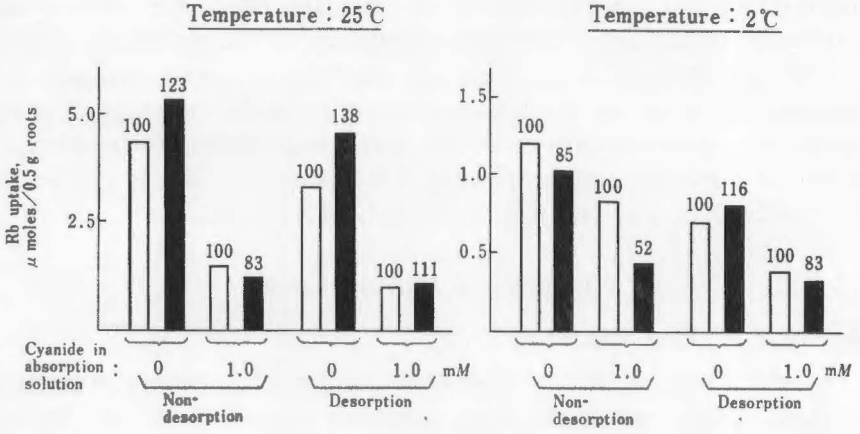

Fig. 1. Effects of calcium and cyanide on the rubidium uptake.
□ $CaCl_2$ in absorption solution: 0 m*M*
■ $CaCl_2$ in absorption solution: 0.5 m*M*

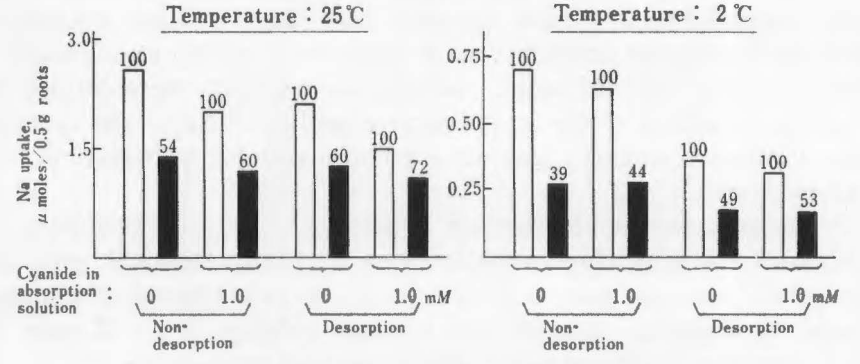

Fig. 2. Effects of calcium and cyanide on the sodium uptake.
□ $CaCl_2$ in absorption solution: 0 m*M*
■ $CaCl_2$ in absorption solution: 0.5 m*M*

uptake, respectively. Numbers on bars indicate the ratio of the results when calcium chloride was added to that when there was no addition. The same system is used in figures presented below.

At 25°C, the rubidium uptake was increased by calcium when no cyanide was added, with or without the desorption treatment, while the calcium effect was not found when cyanide was added. At 2°C, however, the rubidium uptake was decreased by calcium when there was no desorption treatment and it was increased by calcium when there was desorption treatment with no addition of cyanide. The rubidium uptake was, however, decreased by calcium with the addition of cyanide with or without the desorption treatment.

The sodium uptake was decreased by calcium under all the experimental conditions; on the addition or omission of cyanide, with or without the desorption treatment, and at 25° and 2°C.

Influence of DNP

The effects of calcium on the uptakes of rubidium and sodium as influenced by DNP were investigated under various conditions with or without the desorption treatment at 25° and 2°C. Figs. 3 and 4 show the rates of rubidium and sodium uptake, respectively.

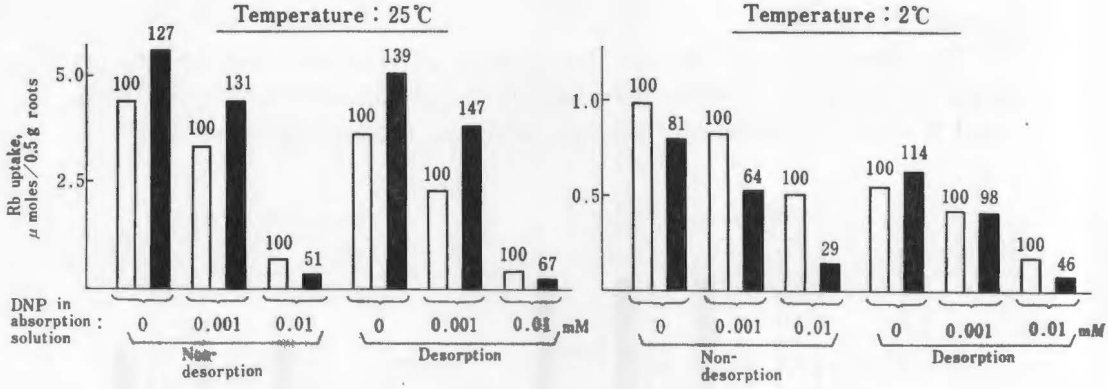

Fig. 3. Effects of calcium and DNP on the rubidium uptake.
□ $CaCl_2$ in absorption solution: 0 m*M*
■ $CaCl_2$ in absorption solution: 0.5 m*M*

At 25°C, the rubidium uptake was increased by calcium when no DNP was added and was decreased by calcium on the addition of 0.01 m*M* of DNP, with or without the desorption treatment. However, in spite of the lowering of the rubidium uptake with the addition of 0.001 m*M* of DNP, the calcium effect was clear. At 2°C, the rubidium uptake decreased on the addition of 0.001 and 0.01 m*M* of DNP, and the effect of

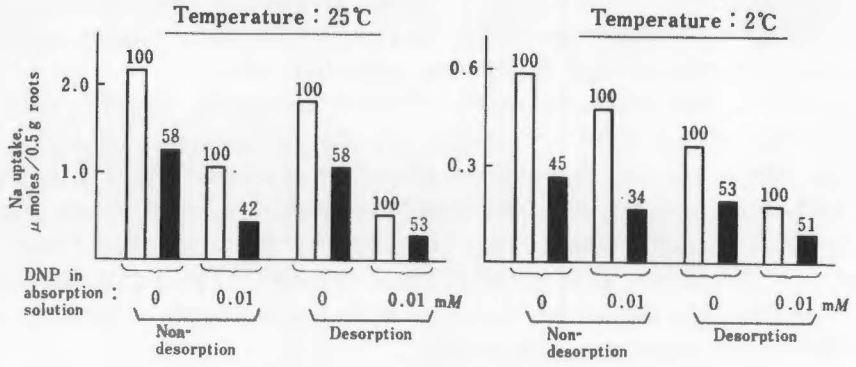

Fig. 4. Effects of calcium and DNP on the sodium uptake.
□ $CaCl_2$ in absorption solution: 0 m*M*
■ $CaCl_2$ in absorption solution: 0.5 m*M*

calcium on the rubidium uptake became more inhibitory with increasing concentrations of DNP.

The sodium uptake was decreased by calcium under all the experimental conditions; on the addition and omission of 0.01 m*M* of DNP, with or without the desorption treatment, and at 25° and 2°C.

Influence of Ouabain

The effects of calcium on the uptakes of rubidium and sodium as influenced by ouabain were investigated under various conditions. Figs. 5 and 6 show the rates of rubidium and sodium uptake, respectively.

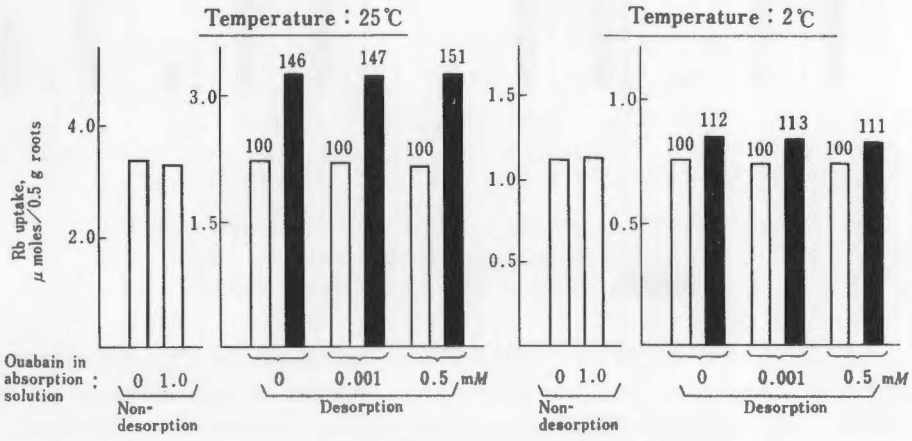

Fig. 5. Effects of calcium and ouabain on the rubidium uptake.
□ $CaCl_2$ in absorption solution: 0 m*M*
■ $CaCl_2$ in absorption solution: 0.5 m*M*

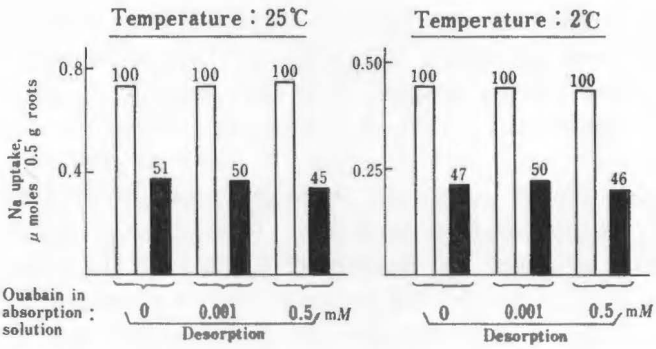

Fig. 6. Effects of calcium and ouabain on the sodium uptake.
□ $CaCl_2$ in absorption solution: 0 m*M*
■ $CaCl_2$ in absorption solution: 0.5 m*M*

The rubidium uptake was increased by calcium on desorption treatment at both 25° and 2°C, but ouabain up to 1.0 m*M* did not affect the rubidium uptake in either the presence or absence of calcium. The sodium uptake was decreased by calcium, but ouabain up to 0.5 m*M* did not affect the sodium uptake.

DISCUSSION

A great number of experiments on ion uptake have been performed with metabolic inhibitors, especially respiratory inhibitors, and ion uptake has been found to be largely dependent upon the aerobic respiration in plant roots. In this study, the relation between the calcium effect in ion uptake and a series of metabolic processes made up of aerobic respiration, the formation of ATP, and the hydrolysis of ATP, was investigated. Three metabolic inhibitors were used: cyanide, a known inhibitor of metal oxidases in the respiratory chain; DNP, a known uncoupling agent in oxidative phosphorylation; and ouabain, a known inhibitor of ATPase in animal cells.

Waisel (19) reported that calcium decreased the rubidium uptake in a supply of nitrogen gas or DNP. In the present experiments also, the stimulating effect of calcium on rubidium uptake was reduced or abolished on the addition of cyanide (Fig. 1). A similar trend was found on the addition of DNP. The rubidium uptake decreased with increasing concentrations of DNP, and the effect of calcium on this uptake was clear when the rubidium uptake was slightly reduced. However, when the rubidium uptake was markedly reduced, the calcium effect became inhibitory.

It has been reported that the oxygen uptake in barley roots was not reduced by about 0.1~0.001 m*M* of DNP (20, 21); thus, the abolition

of the calcium effect on the addition of cyanide may be due to the formation of ATP being decreased indirectly by the inhibition of aerobic respiration. These results suggest that the stimulating effect of calcium on rubidium uptake is related to the supply of energy.

On the other hand, animal cells (i.e., erythrocytes and nerve cells etc.) have been found to contain ATPases which were activated in the presence of sodium and/or potassium (6-8, 12-17, 22-25), and these ATPases were inhibited by ouabain (6-8, 12-17). In addition, ouabain has been reported to inhibit the active transport of sodium and potassium (5, 7, 9-11). Thus, a close linkage has been established between active ion transport and ATPase activity in animal cells (6, 7, 12, 26).

Recently, cation-activated ATPase has been found in plant cells (27-36), and a possible relationship between ion uptake and ATPase activity was suggested (31, 35). Although it has been reported that ouabain inhibited ion transport in algal cells (37, 38) and in higher plant cells (39), many experiments have shown that ouabain did not inhibit ATPase activity (28, 29) or ion transport (40-43) in higher plants. In the present investigation, ouabain at concentrations up to about 1.0 m*M* did not affect the rubidium and sodium uptakes, though a concentration of ouabain for half maximal inhibition was about $10^{-4}\sim10^{-3}$ m*M* on both the systems of ATPase and ion transport in animal cells (7, 8, 13, 18). Also, the calcium effect on monovalent cation uptake was not affected by ouabain. Since the influence of ouabain in plant cells is obscure at the present time, it is necessary to account these discrepancies.

SUMMARY

The mechanism of the calcium effect on selective cation uptake by excised barley roots was investigated with three metabolic inhibitors. The inhibitors used in this study were cyanide, DNP and ouabain.

1) The rates of rubidium and sodium uptake decreased in the presence of 1.0 m*M* of cyanide. Calcium chloride increased the rubidium uptake in the absence of cyanide, but this stimulating effect of calcium on rubidium uptake was not observed in the presence of 1.0 m*M* of cyanide.

2) The rate of rubidium uptake decreased with an increasing concentration of DNP. When the rubidium uptake was slightly reduced, the stimulating effect of calcium on rubidium uptake was clear, whereas the calcium effect was abolished when the rubidium uptake was markedly depressed. The sodium uptake also decreased in the presence of DNP.

The results suggest that the calcium effect on selective monovalent cation uptake is closely related to the energy supply.

3) The rates of rubidium and sodium uptake were not influenced significantly by the addition of ouabain up to 1.0 m*M*; the stimulating effect of calcium on the rubidium uptake did not change regardless of the addition of ouabain.

REFERENCES

1. Kawasaki, T. and Hori, S. 1968. The role of calcium in the selective cation uptake by plant roots. I. Effect of cation concentrations in absorption solution. Ber. Ohara Inst. Landw. Biol. Okayama Univ. 14: 123-132.
2. Kawasaki, T. and Hori, S. 1973. The role of calcium in the selective cation uptake by plant roots. II. Effects of temperature, desorption treatment and sodium salt on rubidium uptake. Ber. Ohara Inst. Landw. Biol. Okayama Univ. 15: 19-28.
3. James, W. O. 1953. The use of respiratory inhibitors. Ann. Rev. Plant Physiol. 4: 59-90.
4. Sakamura, T. 1958. Shokubutsu Seirigaku. I. p. 377-384. Shôkabô. Tokyo.
5. Schatzmann, H. J. 1953. Herzglykoside als Hemmstoffe für den aktiven Kalium- und Natriumtransport durch die Erythrocytenmembran. Helv. Physiol. Acta 11: 346-354.
6. Skou, J. C. 1960. Further investigations on a $Mg^{++}+Na^{+}$-activated adenosintriphosphatase, possibly related to the active, linked transport of Na^{+} and K^{+} across the nerve membrane. Biochim. Biophys. Acta 42: 6-23.
7. Post. R. L., Merritt, C. R., Kinsolving, C. R. and Albright, C. D. 1960. Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. J. Biol. Chem. 235: 1796-1802.
8. Bontings, S. L., Simon, K. A. and Hawkins, N. M. 1961. Studies on sodium-potassium-activated adenosine triphosphatase. I. Quantitative distribution in several tissues of the cat. Arch. Biochem. Biophys. 95: 416-423.
9. Kinoshita, J. H., Kern, H. L. and Merola, L. O. 1961. Factors affecting the cation transport of calf lens. Biochim. Biophys. Acta 47: 458-466.
10. Yoshida, H., Nukada, T. and Fujisawa, H. 1961. Effect of ouabain on ion transport and metabolic turnover of phospholipid of brain slices. Biochim. Biophys. Acta 48: 614-615.
11. Kean, E. L., Adams, P. H., Winters, R. W. and Davies, R. E. 1961. Accumulation of potassium anaerobically by renal medullary slices. Science 134: 1622-1623.
12. Skou, J. C. 1962. Preparation from mammallian brain and kidney of the enzyme system involved in active transport of Na^{+} and K^{+}. Biochim. Biophys. Acta 58: 314-325.
13. Bonting, S. L., Caravaggio, L. L. and Hawkins, N. M. 1962. Studies on sodium-potassium-activated adenosine-triphosphatase. IV. Correlation with cation transport sensitive to cardiac glycosides. Arch. Biochem. Biophys. 98: 413-419.
14. Whittam, R. 1962. The asymmetrical stimulation of a membrane adenosine triphosphatase in relation to active cation transport. Biochem. J. 48: 110-118.
15. Wheeler, K. P. and Whittam, R. 1962. Some properties of a kidney adenosine triphosphatase relevant to active cation transport. Biochem. J. 85: 495-507.
16. Yoshida, H., Fujisawa, H. and Ohi, Y. 1964. Influence of protamine on Na^{+}, K^{+}-dependent ATPase solubilized from brain microsome. Biochim. Biophys. Acta 88: 455-458.
17. Nakao, T., Tashima, Y., Nagano, K. and Nakao, M. 1965. Highly specific sodium-potassium-activated adenosine triphosphatase from various tissues of rabbit. Bichem. Biophys. Res. Commun. 19: 755-758.

18. Frank, R. N. and Goldsmith, T. H. 1967. Effects of cardiac glycosides of electrical activity in the isolated retina of the frog. J. Gen. Physiol. 50: 1585–1606.
19. Waisel, Y. 1962. The effect of Ca on the uptake of monovalent ions by excised barley roots. Physiol. Plantarum 15: 709–724.
20. Mitsui, S. and Kumazawa, K. 1955. Dynamic studies on nutrients uptake by crop plants (Part 9). Uptake of K, PO_4 and NH_4 by barley seedlings as influenced by 2,4-dinitrophenol. J. Sci. Soil Manure, Japan 26: 241–244.
21. Dokiya, Y., Kumazawa, K. and Mitsui, S. 1964. The comparative physiological study on the uptake of iron, manganese and copper by plants (Part 1). The uptake of ^{59}Fe, ^{54}Mn and ^{64}Cu by rice and barley seedlings as influenced by metabolic inhibitors (Dynamic studies on the nutrient uptake by corp plants, Part 44). J. Sci. Soil Manure, Japan 35: 367–372.
22. Skou, J. C. 1957. The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochim. Biophys. Acta 23: 394-401.
23. Järnefelt, J. 1961. Sodium-stimulated adenosine-triphosphatase in microsomes from rat brain. Biochim. Biophys. Acta 48:104–110.
24. Järnefelt, J. 1961. Mechanism of sodium transport in cellular membranes. Nature 190: 694–697.
25. Emmelot, P. and Bos, C. J. 1962. Adenosine triphosphatase in the cell-membrane fraction from rat liver. Biochim. Biophys. Acta 58: 374–375.
26. Archdeacon, J. W., Rohrs, H. C. and Marta, H. 1964. Sodium and potassium contents of rabbit and cat marrow cells and sodium- and potassium-stimulated ATPase. Biochim. Biophys. Acta 82: 647–649.
27. Gruener, N. and Neumann, J. 1966. An ion-stimulated adenosine triphosphatase from bean roots. Physiol. Plantarum 19: 678–682.
28. Dodds, J. J. A. and Ellis, R. J. 1966. Cation stimulated adenosine triphosphatase activity in plant cell wall. Biochem. J. 101 (Suppl.): 31.
29. Atkinson, M. R. and Polya, G. M. 1967. Salt-stimulated adenosine triphosphatases from carrot, beet, and *Chara australis*. Austral. J. Biol. Sci. 20: 1069-1086.
30. McClurkin, I. T. and McClurkin, D. C. 1967. Cytochemical demonstration of a sodium-activated and a potassium-activated adenosine triphosphatase in loblolly pine seeding root tips. Plant Physiol. 42: 1103-1110.
31. Fisher, J. and Hodges, T. K. 1969. Monovalent ion stimulated adenosine triphosphatase from oat roots. Plant Physiol. 44: 385–395.
32. Hall, J. L. and Butt, V. S. 1969. Adenosine triphosphatase activity in cell-wall preparations and excised roots of barley. J. Exp. Botany 20: 751–762.
33. Sexton, R. and Sutcliffe, J. F. 1969. Some observations on the characteristics and distribution of adenosine triphosphatases in young roots of *Pisum sativum*, cultivar Alaska. Ann. Botany 33: 683–694.
34. Kylin, A. and Gee, R. 1970. Adenosine triphosphatase activities in leaves of the mangrove *Avicennia nitida* Jacq. Influence of sodium to potassium ratios and salt concentrations. Plant Physiol. 45: 169–172.
35. Fisher, J. D., Hansen, D. and Hodges, T. K. 1970. Correlation between ion fluxes and ion-stimulated adenosine triphosphatase activity of plant roots. Plant Physiol. 46: 812–814.
36. Hall, J. L. 1971. Further properties of adenosine triphosphatase and β-glycerophosphatase from barley roots. J. Exp. Botany 22: 800-808.
37. MacRobbie, E. A. C. 1962. Ionic relations of *Nitella translucens*. J. Gen. Physiol. 45: 861-878.
38. Raven, J. A. 1967. Ion transport in *Hydrodictyon africanum*. J. Gen. Physiol. 50: 1607–1625.
39. Brown, H. D., Jackson, R. T. and Dupuy, H. J. 1964. Transport of sugar in *Allium*: Effects of inhibitors and ethylene. Nature 202: 722–723.

40. Mengel, K. 1963. Der Einfluss von ATP-Zugaben und weiterer Stoffwechselagenzien auf die Rb-Aufnahme abgeschnittener Gerstenwurzeln. Physiol. Plantarum 16: 767-776.
41. Hodges, T. K. 1966. Oligomycin inhibition of ion transport in plant roots. Nature 209 : 425-426.
42. Pitman, M. G. and Saddler, H. D. W. 1967. Active sodium and potassium transport in cells of barley roots. Proc. Nat. Acad. Sci. USA 57 : 44-49.
43. Cram, W. J. 1968. The effects of ouabain on sodium and potassium fluxes in excised root tissue of carrot. J. Exp. Botany 19 : 611-616.