

Effects of Metabolic Inhibitors on Al-accelerated P and K Uptake in Excised Barley (*Hordeum vulgare* L.) Roots

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Abstract

The energy dependent character of the Al-accelerated P and K absorption in excised roots of barley (*Hordeum vulgare* L. cv. Sanuki-Hadaka) was examined using a multi-compartment transport box technique. Radioisotopes (³²P and ⁸⁶Rb) were used to determine the mobility of P and K in roots. Phosphate and Al were given at concentrations of 0.2 mM, and initial K was 1.2 mM. Two metabolic inhibitors, DNP (2, 4-dinitrophenol) and CCCP (Carbonyl cyanamide-m-chloro phenylhydrazine) were used at concentrations of 1.0, 2.0 and 3.0 μ M for DNP, and 0.2, 0.6 and 1.0 μ M for CCCP. Radioactive tracers were given at two loading sites, root apex and translocation peaks of the respective elements. The Al-accelerated P and K absorption and translocation disappeared in the presence of the metabolic inhibitors. This suggested that the Al-stimulated absorption and translocation of P and K were predominantly energy dependent at the P, K and Al concentrations given. The inhibition was severe in the presence of CCCP, even at the lowest concentration. The tendencies of the inhibitory effects caused by DNP or CCCP was similar at the root apex and the respective

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translocation peaks, though the magnitude of inhibition was higher at the translocation peak loading than at the apex loading. Apparently, the energy dependent character of the acceleration of P and K uptake by Al has no relation with aging of roots, though a wide difference was found between the apex loading and the translocation peak loading in the characteristics of absorption and translocation of inorganic ions.

Introduction

We have observed that in intact plants (Malkanthi *et al.* 1995a) and in excised barley roots (Malkanthi *et al.* 1995b), the absorption of P is accelerated by the presence of Al (0.2 mM) when the pH of the ambient solution is low. In some instances the same phenomenon was observed in regard to K uptake (Malkanthi *et al.* 1995a, b). This property of Al has also been demonstrated for some other species (Cumming *et al.* 1985, Huett and Menary 1980, Konishi and Miyamoto 1984, Konishi *et al.* 1985), but the physiological role of Al in stimulating nutrient ion uptake has not yet been fully understood. Therefore, as an initial step towards investigation of the physiological aspects of Al-stimulation of ion uptake in plants, we examined the possibility of metabolic linkage of P and K absorption in barley roots when Al was added under conditions of low pH. Radioisotopes were used in combination with two metabolic inhibitors, DNP (2, 4-dinitrophenol) and CCCP (Carbonyl cyanamide-m-chloro phenylhydrazine) and the multi-compartment transport box technique (Kawasaki *et al.* 1984). The observations were made at two main loading sites of barley roots, the root apex (0 to 10 mm zone) and the translocation peak (26 to 36 mm zone for P, 39 to 49 mm zone for K). The absorbed solutes were carried upward efficiently at the translocation peaks, though rapid absorption but slow translocation of solutes were found at the root apex (Moritsugu *et al.* 1993, Moritsugu 1995). Both loading sites, the root apex and the translocation peaks, were selected and used for this investigation, because a big difference was found between both loading sites in the characteristics of absorption and translocation of nutrient ions.

Materials and Methods

Excised roots from 5-day old etiolated seedlings of barley (*Hordeum vulgare* L., cv. Sanuki-Hadaka, short-culmed variety, somewhat strong short-culmed nature as compared with "Akashinriki" used by Kawasaki *et al.* 1984) were employed. Seeds of barley were

germinated for 24 h at 25°C in aerated deionized water with ethylene-free atmosphere in the dark. The germinating seeds were spread over a stainless steel screen of 5 mm mesh, and grown on aerated 0.25 mM CaSO₄ for 72 h at 25°C in the dark. Thereafter, a complete nutrient solution (KNO₃ 4 mM, CaCl₂ 3 mM, NH₄H₂PO₄ 1 mM, MgSO₄ 1 mM, Fe 40 μM, B 30 μM, Mn, 5 μM, Zn 0.5 μM, Cu 0.3 μM, Mo 0.01 μM, at pH 5.5) was supplied and the same growth condition was continued for 24 h.

As can be seen from Fig. 1, a root part corresponding to the first compartment (0 to 10 mm from root tip) was used for the loading site of both P and K experiments of the apex loading. A root part corresponding to the third compartment (26 to 36 mm from root tip) was used as the loading site for the translocation peak loading of P experiments. A root part corresponding to the fourth compartment (39 to 49 mm from root tip) was used as the loading site for the translocation peak loading of K experiments.

The experimental setup of an excised root in a multi-compartment transport box and the

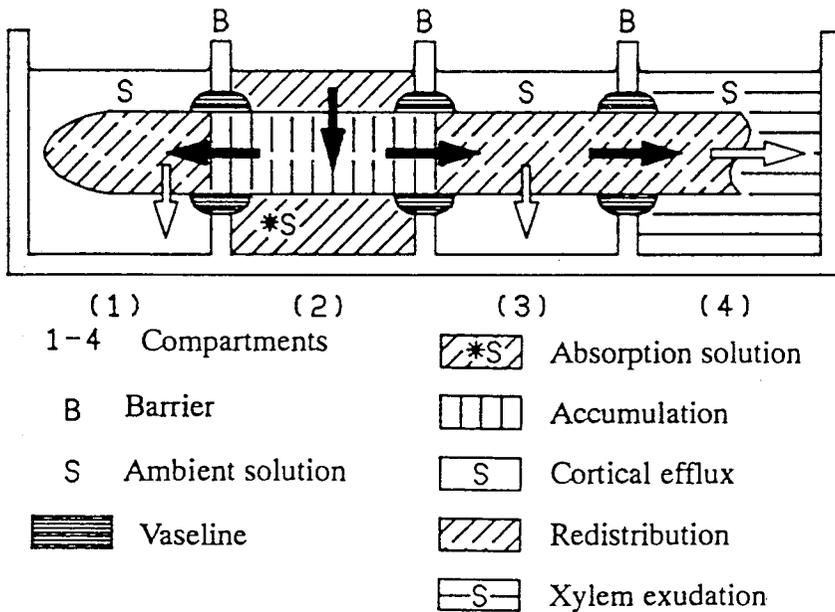


Fig. 1. Multi-compartment transport box with excised root and fractionation of absorbed solutes. The apparatus is made of plexiglass with several compartments. Each compartment is 10mm long, 50mm wide, 15mm deep, with plexiglass barriers (3mm wide) between compartments.

fractionation of absorbed solutes are illustrated in Fig. 1. All of the non-radioactive compartments (except the second compartment in the example of Fig. 1) were filled with 4 ml of non-radioactive ambient solution which is the same chemical composition to the

radioactive absorption solution except radioactivity. The ambient solutions contained 0.2 mM KH_2PO_4 or 1.0 mM KCl in the P or the K experiments. In addition 0.2 mM $\text{KAl}(\text{SO}_4)_2$ was given in the ambient solution of +Al treatment. Therefore, in the K experiments, total K^+ concentration was increased to 1.2 mM. To maintain the same K^+ concentration throughout K experiments, 0.2 mM KCl was given to Al-free treatments (-Al in Fig. 4 and 5). To all the experiment solution, 0.5 mM CaCl_2 was given. The pH of all the ambient solution was maintained at 3.8 using 0.1 mM MOPS (3-morpholinopropanesulfonic acid) buffer, which suffered to maintain the initial pH throughout the course of an experiment. Metabolic inhibitors were used at the concentrations of 1.0, 2.0 and 3.0 μM for DNP and 0.2, 0.6 and 1.0 μM for CCCP, both from Sigma Chemicals, St. Louis, USA. In addition, these inhibitors were given to all the compartments except the control treatments (-Al and +Al in each Fig.) independently of radioactivity.

The second compartment was radioactivated in the example of Fig. 1. Though this compartment was not used as the loading compartment in the present investigation, Fig. 1 was enough to explain the experimental conditions keeping down occupying area. The radioisotopes ^{32}P and ^{86}Rb were used as tracers for P and K. The radioactive tracer (about 25 kBq, a definite amount for each course of experiment, 1.000 ml with a Gilson micropipette) was added in 3 ml of the original ambient solution at the loading compartment, and mixed well with four strokes of the micropipette to get homogeneity. Each end time of the mixing of the radioactive compartment was recorded respectively as the start of each experiment.

The absorption experiments worked for about 20 h at 25°C in the dark. To depress loss of water by evaporation (concentration effect), the multi-compartment transport box in a course of an absorption experiment was put under a clear plastic cover which was attached to the inside of a wide aluminium plate, and the inside air of the cover was isolated by shallow water placed in the aluminium plate. At the end of the absorption period, the ambient solutions except where radioactivity was given were collected separately as a sample for the cortical efflux or the xylem exudation, and the end times of the absorption experiments were recorded respectively. The radioactive absorption solution used was collected with rinsing water to treat legally as radioactive wastes.

The roots were cut beside the barriers, but at countersides of the radioactivated compartment as shown in Fig. 1 to prevent radioactive contamination. Namely, the root segments of the radioactive compartment were sampled with both non-radioactive barrier zones as shown in Fig. 1, or with a single barrier zone in the case of the apex loading. Radioactive root segments were sampled after about 10 min of desorption treatment with a non-radioactive solution of the same chemical composition with the radioactive absorption

solution. After a simple blotting with water sprayed (softend) filter papers, root segments including the desorbed ones were rapidly weighed by an electronic balance with a precision of 0.1 mg. Radioactivities of respective root segments and ambient solutions including ^{32}P or ^{86}Rb were measured using a liquid scintillation counter (Aloka) by the Cerenkov luminescence method (Haviland and Bieber 1970).

Rates of absorption and translocation were calculated for each fraction, using the radioactivities of respective root segments and ambient solutions, respective fresh weights of root segments, and the duration of each absorption experiment and concentration of P and K in the absorption solution. Fractions of absorbed and translocated solutes were classified as shown in Fig. 1. (a) The fraction found in the root segment at the radioactive tracer was given, was termed "accumulation". (b) The fraction that leaked into the medium through the cortex during translocation was termed "cortical efflux" (the activity discharged from the cortex into the solution in the cut-end compartment was ignored). (c) The fraction translocated into the root segments in the non-radioactive compartments but not yet exuded through xylem into the cut end compartment was termed "redistribution". (d) The fraction translocated and exuded through the cut end to the ambient solution was termed "xylem exudation". (e) The sum of all the above fractions was termed "total absorption". In addition, all fractions except accumulation were translocated fractions.

In the present work, the absorption experiments were carried out using two different loading sites. When the radioactive tracer for P (^{32}P) or K (^{86}Rb) was applied to the root apices (the first compartment, 0~10 mm), the method was designated as apex loading, and when the tracer was placed into each translocation peak zone, the third compartment, 26~36 mm for P, or the fourth compartment, 39~49 mm for K (Malkanthi *et al.* 1995b, Moritsugu *et al.* 1993, Moritsugu 1995), that was termed translocation peak loading. Simultaneously, two control treatments were used to evaluate Al and inhibitor effects respectively. Those were treatment with non-Al medium (Al control, -Al) and treatment with Al-containing medium without inhibitor (inhibitor control, +Al). The difference between the inhibitor control (+Al, without inhibitor) and the treatments with both Al and inhibitor (inhibitor treatments) was used to evaluate the effects of metabolic inhibitors on P and K absorption under the given experimental conditions.

Abbreviations : CCCP (Carbonyl cyanamide-m-chloro phenylhydrazone, concentrations: 0.2, 0.6 and 1.0 μM in Figs. 3 and 5). DNP (2, 4-dinitrophenol, concentrations: 1.0, 2.0 and 3.0 μM in Figs. 2 and 4). MOPS (3-morpholinopropanesulfonic acid).

Results and Discussion

Figure 2 shows the effects of Al and 3 different levels of DNP on the absorption and translocation of P loaded at the root apex (top cut in Fig. 2 A) and at the translocation peak zone (bottom cut Fig. 2 B) of P in excised barley roots. Compared with the Al-free control (-Al in Fig. 2) and the control to inhibitor treatment (+Al in Fig.2), the addition of Al remarkably increased the total absorption (left hand cut of Fig. 2) and the translocation (right hand cut) of P. This tendency is found commonly throughout the other figures (Fig. 3 to 5). Therefore, the present work adds general support to previous findings regarding to Al-accelerated P absorption and translocation in intact plants (Malkanthi *et al.* 1995a) and in excised barley roots (Malkanthi *et al.* 1995b).

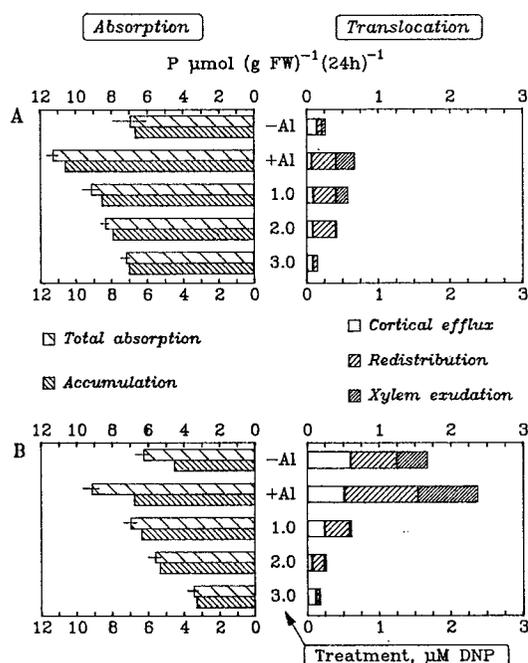


Fig.2. Effect of DNP on Al-accelerated P absorption and translocation in excised barley roots.

A : Apex loading
B : Translocation peak loading

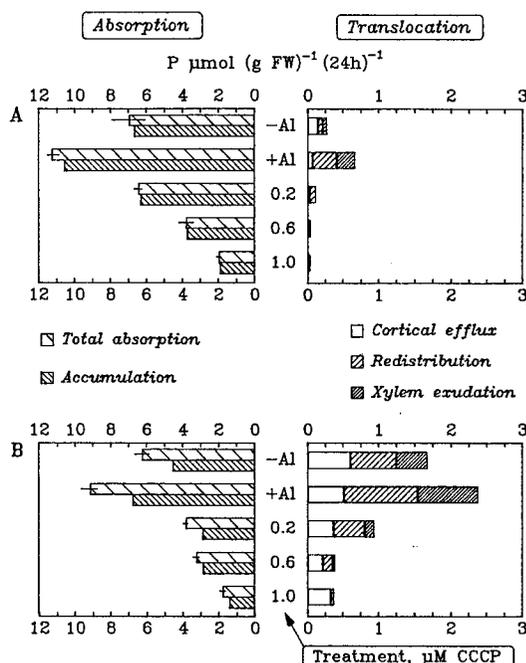


Fig.3. Effect of CCCP on Al-accelerated P absorption and translocation in excised barley roots.

A : Apex loading
B : Translocation peak loading

Common legends for Figs. 2 & 3: Lines on bar ends show the standard deviation of 4 replicated experiments in -Al and +Al, and of 2 replicated experiments in the remaining inhibitor treatments. Media are composed of 0.2 mM KH_2PO_4 , 0.5 mM CaCl_2 , 100 mM MOPS at pH 3.8, and 0.2 mM $\text{KAl}(\text{SO}_4)_2$ except the -Al control.

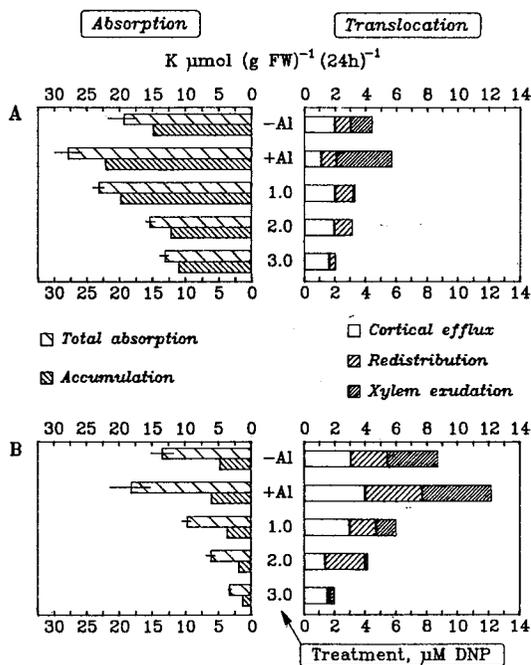


Fig. 4. Effect of DNP on Al-accelerated K absorption and translocation in excised barley roots.

A : Apex loading

B : Translocation peak loading

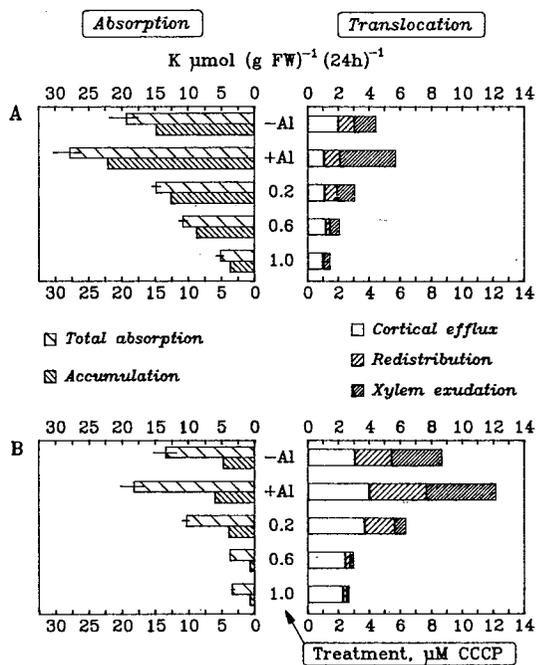


Fig. 5. Effect of CCCP on Al-accelerated K absorption and translocation in excised barley roots.

A : Apex loading

B : Translocation peak loading

Common legends for Figs. 4 & 5: Lines on bar ends show the same conditions as Fig. 2. Media are composed of 1.0 mM KCl, 0.5 mM CaCl₂, 100mM MOPS at pH 3.8, and 0.2 mM KAl(SO₄)₂ (except -Al) or additional 0.2mM KCl (only -Al).

When the roots were exposed to DNP, the total P absorption and the translocation of P decreased (Fig. 2). This kind of depression by inhibitors was found in the other figures presented here (Fig. 3 to 5), and several authors have reported the stimulative effects of Al on K uptake in potatoes (Lee 1971), wheat (Mugwira *et al.* 1980), and tea (Konishi *et al.* 1985), respectively.

The process of rise and fall of root absorption ability accompanied by root aging was observed on several ions separately (Moritsugu *et al.* 1993, Moritsugu 1995). It was caused by characteristic differences accompanied by root age, namely, young roots fixed easily several inorganic ions, then the fixing ability of ions decreased, and the ion transferring ability increased to maximal range but finally the ability decreased gradually. In the present study, the root apex that has strong ability of fixation of each ion and the translocation peak of P where the uptake and translocation ability of P are noticeable, and the translocation peak zone of K characterized by the rapid K intake and succeeding quick

translocation toward plant top were selected as loading sites.

It is clear from the present work that Al influences the metabolic pathways in the absorption of P and K, because Al-accelerated P and K absorption are predominantly metabolic process under experimental conditions given commonly 25°C in the dark, pH 3.8 (MOPS buffer), CaCl₂ 0.5 mM, individually K⁺ 1.2 mM, P or Al 0.2 mM.

The comparison between experimental results obtained by the apex loading and by the translocation peak loading, revealed that the energy dependent character in relation to Al-acceleration of P or K absorption was common for the two loading sites, though the magnitude of the inhibition was not the same. This suggests that the existence of an energy requiring pathway in relation to Al-acceleration of P and K absorption in barley roots is common beyond the root age, even though the absorption characteristics of several ions are widely different by root age (Moritsugu *et al.* 1993, Moritsugu 1995).

The translocation of P were more rapid in the case of translocation peak loading than in the case of apex loading (comparison between right hand two cuts in Figs. 2 and 3). This is a clear characteristic of the translocation peak loading (Moritsugu *et al.* 1993, Moritsugu 1995), however, this is not a complete explanation, because the rate of P accumulation is remarkable (*ca.* 70%) to the total absorption rate, and the rate of K accumulation is apparently slow (*ca.* 30%) to the total absorption rate of K in the case of the translocation peak loading (Fig.4 and 5-B). There is another reason that P looks like to be immovable and fixed easily within the loading sites as compared with the case of K.

The rate of total K absorption (Fig. 4 and 5, left cuts) as well as translocation (right cuts) was more rapid than those of P (Fig. 2 and 3). This may be caused by the difference of nutrient concentration of the ambient solution, 0.2 mM for P and 1.2 mM for K, respectively.

Although the accumulation contributes to a great extent to the total absorption especially in the case of the apex loading (top cuts, A in each Fig.), the Al-acceleration on P and K movements was somewhat marked in the case of the translocation peak loading (bottom cuts, B in each Fig). This phenomenon was clearer in the net translocated fraction (xylem exudation and the redistribution) than in the cortical efflux (loss during movement of P and K). On uncertainty of the cortical efflux, there were some reasons as follows: physical leakage caused by a small pin point hole or split connecting the radioactive compartment and the next compartments, physiological wrinkles of root surface or wound of root tissues caused by considerable acidity of ambient solution (pH 3.8). In the case of the translocation peak loading, the above contaminating conditions became twice as compared with that of the apex loading. Therefore, to consider in detail according to the rate of the cortical efflux was not recommendable. The rate of translocation except the cortical efflux decreased steadily by

the addition of inhibitors and the Al-acceleration was lost. This phenomenon was found even in the lowest concentration except in an example (apex loading of P in DNP experiment).

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