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Correlation between Ion Fluxes and Ion-stimulated Adenosine Triphosphatase Activity of Plant Roots[1]

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ABSTRACT

The energy-dependent influx of Rb^+ into excised roots of corn, wheat, and barley has been determined and compared to the Rb^+-stimulated ATPase activity of membrane fractions obtained from root homogenates of these species. The external Rb^+ concentrations studied were in the range of 1 to 50 mM. The ratio of Rb^+ influx/Rb^+-stimulated ATPase was approximately 0.85 and was nearly constant for all the species and Rb^+ concentrations studied. The correlation coefficient for Rb^+ influx versus Rb^+-activated ATPase was 0.94. The results support the concept that ATP is the energy source for ion transport in roots and that an ATPase participates in the energy transduction process involved in energy-dependent ion transport.

The energy source for ion transport in plants is unknown. Respiratory-inhibitor studies implicate oxidative phosphorylation in the transport process; however, the coupling mechanism is controversial. Atkinson *et al.* (1) and Polya and Atkinson (28), on the basis of changes in ATP levels associated with inhibited rates of ion transport in storage tissues, favor the concept that the electron transfer process itself is somehow involved in energy-linked ion transport. However, on the basis of arsenate (16) and oligomycin inhibition of transport (17, 19, 20) in roots, it would seem that ATP could also serve as the actual energy source for transport. In green algae it has been concluded that anion transport is more closely linked to the photosynthetic electron transport process whereas cation transport is coupled to ATP formation and utilization (25, 30, 31).

In animal tissues it has been shown directly that ATP could provide the necessary energy for Na^+ transport (5, 18). It has also been shown that an ATPase is intimately involved in this ATP-driven reaction (33). The existence of ion-sensitive ATPases in plant tissues has been reported recently for several plant species (2, 4, 6, 8, 11, 13, 15, 21, 26, 32); however, none of these reports has shown the enzyme to have any relationship to ion transport. We (8) have reported on the presence of a membrane-bound, monovalent ion-stimulated ATPase in oat roots which appears to have sufficient activity to account for the observed rates of ion transport. These studies have now been extended to other plant species which possess different rates of ion transport, and the results provide correlative evidence for a role of the ATPase (and therefore ATP) in the energy-dependent transport of Rb^+ and K^+.

MATERIALS AND METHODS

Plant Culture. Roots from 4-day-old, dark-grown wheat (*Triticum vulgare*, 59 × 844), corn (*Zea mays*, WF9 × M14), and barley (*Hordeum vulgare* var. Arivat) were used. The seeds were placed between layers of cheesecloth on stainless steel screens 2 cm above a 0.2 mM $CaSO_4$ solution with gentle aeration. All plants were kept at 25 ± 2 C during the 4-day growing period.

Influx Experiments. Rubidium influx was determined with ^{86}Rb. Roots were excised and washed three times in approximately 250 ml of distilled water. The terminal 6 cm of five roots were cut into 1.5-cm segments and incubated in 50 ml of 0.5 mM $CaSO_4$ plus the desired concentration of RbCl for 30 min. Solutions were maintained at 30 C during the absorption period and gentle aeration was provided. The experiments were terminated by rapid filtration on Buchner funnels. The root segments were rinsed for 30 sec with approximately 10 ml of an ice-cold washing solution (0.5 mM $CaSO_4$–5 mM RbCl) and then placed into ice-cold washing solution for an additional 30 min. The roots were finally rinsed with the wash solution, placed in tared stainless steel planchets and weighed. Root tissue was ashed at 500 C for 1 hr and ash was moistened with 0.25 ml of 1% Photoflo, dried, and counted for radioactivity in a gas-flow counter.

ATPase Experiments. The ATPase experiments were basically as described previously (8). Roots were excised and washed three times in approximately 250 ml of deionized water. The roots were chilled in cold deionized water for 15 min prior to grinding in a mortar and pestle with 100 ml of 0.25 M sucrose, 0.003 M EDTA, 0.01 M tris, pH 7.5. The tissue was ground vigorously for 45 sec, strained through four layers of cheesecloth, and finally diluted with 200 ml of 0.25 M sucrose. In order to evaluate various membrane fractions separately, the brei was subjected to successive centrifugations of 1500*g* for 10 min, 10,000*g* for 15 min, and 80,000*g* for 60 min. The two low speed pellets were resuspended in a wash solution of 0.25 M sucrose, 0.001 M EDTA, 0.01 M tris, pH 7.5, and resedimented. All fractions were finally suspended in fresh wash solution. The soluble fraction was not included in this study since it had previously been shown to possess no monovalent ion activated ATPase (8). ATPase activity of the three fractions was assayed in a 1-ml reaction system consisting of 3 mM ATP-tris, 1.5 mM $MgCl_2$, 20 mM tris, pH 7.2, and various concentrations of RbCl. The reaction was terminated after 30 min at 37 C by addition of 0.8 ml of a solution containing 5N H_2SO_4 and 2.5% ammonium molybdate. The Pi released was determined by the Fiske-Subbarow procedure (9). The ATPase activities reported represent

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just the Rb^+ stimulated component of the enzyme; *i.e.*, the activity in the presence of Mg^{2+} has been subtracted from the activity in the presence of Mg^{2+} + Rb^+.

In order to express the ATPase results on a per gram fresh weight basis, so as to be comparable to the influx data, it was necessary to have an estimate of the extent of membrane release by the grinding procedure. This was accomplished as described previously (8) by determining the phospholipid content of the crude extracts and of unhomogenized roots.

Porteins were determined by the procedure of Lowry *et al.* (24).

All experiments were performed in duplicate and the results shown represent the means of three to five experiments.

RESULTS

Rubidium was used in these experiments instead of K^+ because of the greater convenience of determining influx rates with the Rb^+ radioisotope as compared to the K^+ radioisotope. All the results with Rb^+ should be similar to K^+ since Rb^+ transport has been shown to be similar to K^+ transport (23) and Rb^+ activation of the ATPase is nearly identical to the K^+ activation of the enzyme (8).

Table I. *Rb^+-stimulated ATPase Activity of Various Subcellular Fractions of Barley, Wheat, and Corn*

RbCl	ATPase Activity								
	Barley			Wheat			Corn		
	1,500g	10,000g	80,000g	1,500g	10,000g	80,000g	1,500g	10,000g	80,000g
mM	μmoles Pi/g fresh wt·hr								
1	0.68	1.44	3.61	0	1.62	4.42	0.23	1.11	1.79
5	1.21	2.81	7.28	0.65	3.24	7.07	0.33	2.27	1.82
10	1.79	5.48	13.31	0.62	4.57	10.31	0.45	2.85	3.31
25	2.62	7.68	16.84	0.84	6.77	11.49	0.67	3.17	3.57
50	0.95	9.35	22.43	0.72	8.39	13.40	0.83	2.69	4.43

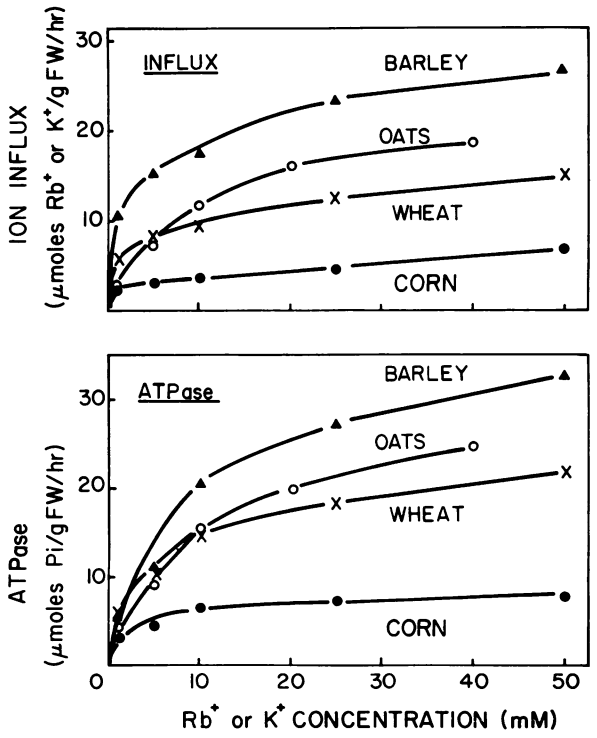

FIG. 1. Rubidium influx and Rb^+-stimulated ATPase activity of corn, wheat, and barley. The data for oats are from a previous paper (8) and pertain to K^+ influx and K^+-stimulated ATPase.

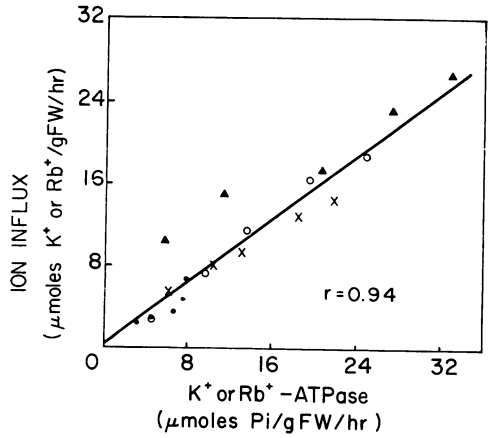

FIG. 2. Correlation between Rb^+ or K^+ and Rb^+ or K^+-stimulated ATPase activity for corn (●), wheat (×), oats (○), and barley (▲).

Table II. *Ratio of Rb^+ Influx/Rb^+-stimulated ATPase of Barley, Wheat, and Corn*

RbCl	Rb^+ Influx/Rb^+-ATPase		
	Barley	Wheat	Corn
mM	μmoles Rb^+ per g fresh wt per hr/μmoles Pi per g fresh wt per hr		
1	1.86	0.93	0.72
5	1.35	0.78	0.64
10	0.87	0.60	0.57
25	0.86	0.65	0.64
50	0.81	0.66	0.83

Table I shows the Rb^+-stimulated ATPase activity for three membrane fractions at five Rb^+ concentrations for corn, wheat, and barley roots. All membrane fractions contained Rb^+-activated ATPase but most of the activity, especially in barley, was present in the microsomal fraction. Corn possesses the least Rb^+-activated ATPase followed in order by wheat and barley.

Figure 1 shows the Rb^+ influx on a per gram fresh weight per hour basis and the entire (sum of the three fractions, Table I) Rb^+-stimulated ATPase on a per gram fresh weight per hour basis for the various plant species. The data for oats were presented in an earlier report (8) and are included here for comparative purposes. This comparison shows quite clearly that different ion transport capacities are associated with different rates of ion-stimulated ATPase. These data are shown as a correlation in Figure 2 for the four species and the five external concentrations examined. The least squares line of best fit is shown and the correlation coefficient is 0.94.

The ratio of K^+ influx/K^+-stimulated ATPase for oats was previously shown to be about 0.76 (8). Table II shows the ratio of Rb^+ influx/Rb^+-stimulated ATPase (based on data of Fig. 1) for barley, wheat, and corn. The ratio is fairly constant for the three species studied and for the various Rb^+ concentrations employed. It appears, however, that the ratio is slightly higher at low Rb^+ concentrations, especially for barley.

DISCUSSION

The correlation between ion influx and ion-activated ATPase reported here strongly supports the concept that ATP serves as the energy source for ion transport in plant roots and that an

ATPase functions in the energy transduction process. Of course, the high correlation observed could be fortuitous, but considering the fairly large range covered for Rb^+ influx (5.6–26.7 μmoles of Rb^+/g fresh wt·hr) and the Rb^+-activated ATPase (3.13–32.9 μmoles of Pi/g fresh wt·hr) this does not seem likely. Furthermore, the close approximation of zero flux with zero ion-activated ATPase (Fig. 2) appears to implicate the enzyme in ion transport.

A possible relationship of an ATPase to ion transport is also suggested by the work of Sexton and Sutcliffe (32). They report a distribution of ATPase activity along the root axis of peas which strongly resembles the pattern of ion transport along the root axis of corn (3, 14).

The ratio of Rb^+ influx/Rb^+-stimulated ATPase was nearly constant for all the Rb^+ concentrations and species examined (Table II). The reason for the high ratios for barley at the two lowest Rb^+ concentrations is unknown. The average ratio for the four species and five concentrations was 0.85. Similar ratios for animal tissues range from about 1 to 5 (34) with values of 2 to 3 being most common (27). The actual values reported here, however, should be treated with caution from a quantitative standpoint. They could undoubtedly be altered slightly by varying the experimental conditions of either the influx or ATPase experiments or in the techniques employed in the ATPase isolation procedure. Variations of this type, however, should have only a slight effect on the over-all correlation of the two processes.

By inspection of Figure 2 it is apparent that the kinetics of the influx and ATPase for the various species are quite similar. However, no quantitative treatment of the data has been made since only the system II concentration range (7) was examined, and the contribution of system I to the results is unknown. Attempts are currently being made to determine the kinetics for both concentration ranges for both the influx and the ion-stimulated ATPase.

The membrane system possessing the monovalent ion-activated ATPase is of considerable interest. Since high external ion concentrations were employed in these experiments, either the plasmalemma (36, 37) or tonoplast (22) would be strong candidates for the enzyme location, if indeed the ATPase is related to transport. Hall (12) has reported the existence of an ATPase on the plasmalemma of corn roots, and Poux (29) has shown that the tonoplast of small vacuoles and either the plasmalemma or cell walls of cucumber root cells contain ATPase activity. In these studies, however, the Wachstein-Meisel (35) cytochemical procedure was employed and this technique has been criticized recently because of the use of high lead concentrations. Thus, the membrane localization of the monovalent ion-activated ATPase reported here is unknown.

In summary, the ATPase described here and previously (8) possesses some properties which suggest it may have a role in ion transport. The enzyme is associated with some type of membrane, and it possesses a specificity toward monovalent ions (8) which appears to be similar to the specificity of ion transport (10). In addition, the ion-stimulated ATPase activity varies from species to species in accordance with a variation in ion transport rates (Fig. 1 and 2). These similarities and correlations of ion influx and ion-stimulated ATPase activities obviously need much further supplementation, but they are quite suggestive that the ATPase plays a role in the ion transport process.

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