

Selective Inhibition of Absorption and Long Distance Transport in Relation to the Dual Mechanisms of Ion Absorption in Maize Seedlings

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Summary. The influence of several uncouplers of oxidative phosphorylation and inhibitors of terminal electron transport was studied on absorption and long distance transport of both K and Cl at concentrations within each range of the dual isotherm typical of ion uptake by maize roots. At low concentrations in the range of system 1, the system considered to implement ion movement through the plasma membrane, root absorption and long distance transport are equally inhibited by a given inhibitor. In the high range of system 2, the system considered to mediate ion passage through the tonoplast, long distance transport is markedly less sensitive to inhibitors than is absorption. The observations are in accord with the hypothesis that only system 1 is involved in the uptake of ions from the external solution into the symplast, and hence into the xylem. At high concentrations, entrance into the symplasm is deemed to be largely by diffusion and therefore less inhibitor sensitive.

With respect to absorption by the roots, the plasma membrane system is more inhibitor sensitive than is the tonoplast system. It is suggested that the difference in sensitivity is real, and not the consequence of an inequality of inhibitor concentration in the vicinity of the plasma membrane and tonoplast respectively.

When ion absorption by plant roots is examined through a wide concentration range, at least 2 distinct isotherms are noted which differ in their compass by roughly three orders of magnitude (6). The 2 types of isotherms have been taken to reflect separate transport systems or mechanisms (6), and it has been proposed by Torii and Laties (16) that the high affinity system (system 1) operates at the plasma membrane, while the low affinity system (system 2) acts at the tonoplast. Crafts and Broyer (4), Arisz (1), and Lundegardh (11) have long argued that salts move from the root cortex into the stelar cylinder through a cytoplasmic continuum, the symplasm. Pitman (15), on the basis of selectivity and kinetic considerations, has recently made an excellent case for the same view.

In connection with the theory of symplastic transport it follows that passage of ions into the xylem from the external solution involves transport across the plasma membrane of the root cortex cells, but bears no direct relation to ion movement through the tonoplast. In this view it is to be anticipated that system 1, putatively operating at the plasma membrane, controls the kinetic aspects of ion movement into the xylem, and hence the

kinetic aspects of long distance transport to the shoot. In accordance with this contention Lüttge and Laties (10) have established that in the low concentration range the transport of both Cl⁻ and K⁺ to the leaves of young maize seedlings displays precisely the same isotherm as does system 1-mediated absorption in the root. In further affirmation of expectations, transport to the leaves was shown to be linear with concentration in the high concentration range, while root absorption displayed the characteristics of system 2.

It is inherent in the hypothesis of Torii and Laties (16) that at high concentrations ions pass through the plasma membrane diffusively at rates in excess of maximal system 1-mediated transport. It is for this reason that the isotherm for tonoplast transport, implemented by system 2, is perceptible, even though systems 1 and 2 operate in sequence, and not in parallel. It is for this reason also that long distance transport is linear with concentration in the high range. There has been a long standing debate respecting the question of whether salt movement into the xylem represents a metabolically mediated process, or whether a significant passive component is involved as well. We suggest that the argument may be resolved by recognizing that in the low range active transport mediated by system 1 will be primarily responsible for salt entry into the symplasm, and hence into the xylem, while

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at high concentrations diffusion will gain ascendancy in providing ions to the symplasm.

Arisz (2, 3) effectively demonstrated that while salt movement across the plasma membrane and across the tonoplast are both metabolically implemented, the 2 transport systems are markedly different in their susceptibility to metabolic inhibitors, and are hence distinguishable. MacRobbie (12, 13) has further elucidated this distinction. In what follows, we examine the influence of selected inhibitors on absorption and on long-distance transport in both the low and high concentration ranges, with the end in view of establishing the extent of involvement of systems 1 and 2 in the respective processes.

Materials and Methods

Corn seedlings (Oh 43 Rf × C 103 Rf, Illinois Foundation Seeds, Inc.) were grown in 0.2 mM CaSO₄ for 9 to 10 days under artificial light on a 12 hour photoperiod. For each treatment, sets of 5 seedlings, supported on paraffined disks, were floated on 200 ml experimental solution in 250 ml beakers [see (10) for full procedural details]. Chloride solutions were labelled with ³⁶Cl, while ⁸⁶Rb served as a label for K (5, 6). Experiments were of 3 to 4 hours duration, and were carried out at room temperature on the laboratory bench. Although temperature and light, and hence transpiration, varied from day to day, all treatments of a given experiment were carried out at the same time. Hence comparisons of absolute rates, particularly of long distance transport, have meaning within a given experiment but not between experiments. In the figures, absorption and transport of a designated ion at a given concentration represents a single experiment.

Absorption and transport were calculated on the basis of radioactivity measurements of tissue or tissue extracts, and the experimentally determined specific activity of the external solution. Where

chloride uptake was measured, roots were rinsed in water before being dried and placed directly into fluor for liquid scintillation counting. Shoots were extracted on a steam bath with 20 to 40 % ethanol for several hours, and aliquots of the extract were dried on planchets for counting with a gas flow detector. For the estimation of ⁸⁶Rb, roots were rinsed in unlabeled experimental solution at the end of the experiment, and both roots and shoots were separately extracted for 60 minutes in 20 ml 1:50 nitric acid on a steam bath. Aliquots of the acid extract were plated and counted.

Uptake and transport is expressed per g fresh weight of root, and all results are the average of triplicate determinations involving 5 plants each. Average values and standard deviation of the means are indicated in the figures.

Results

The isotherm for ion absorption in the high range is in fact multiple (5, 7). However, since the distinction of paramount importance is between the low affinity system and the 1 or more high affinity systems, the concept and the presentation are simplified by discussing absorption in the high range simply in terms of system 2. Table I and figure 1 verify that the thrust of the argument is not changed by this simplification. Thus, the influence of *m*-Cl-CCP (8, 9) is markedly greater on absorption than on long-distance transport of K in the high range, whether the external concentration is 8 mM or 40 mM. In both experiments the effect of *m*-Cl-CCP in the low range is equally pronounced on both absorption and long-distance transport, and greater than the effect on absorption in the high range. Figure 2 indicates the same holds true for the absorption and long-distance transport of Cl. A comparison of figure 3 with figure 2 indicates that in the low range the more effective uncoupler, *p*-CF₃O-CCP (8) is also more

Table I. *The Relative Sensitivity of Absorption and Long Distance Transport to Uncouplers and Terminal Oxidase Inhibitors in the Concentration Range of System 1 and System 2*

The medium contained 0.5 mM CaSO₄ in addition to the inhibitors.

Inhibitor	Inhibitor concentration M	Ion	System 1			System 2		
			Concentration mM	% Inhibition Transport Absorption		Concentration mM	% Inhibition Transport Absorption	
<i>m</i> -Cl-CCP	10 ⁻⁶	K/Rb	0.4	95	88	8	56	69
	10 ⁻⁶	K/Rb	0.2	79	81	40	25	59
	10 ⁻⁶	Cl	0.2	87	68	40	34	47
<i>p</i> -CF ₃ O-CCP	10 ⁻⁶	K/Rb	0.2	94	94	40	35	43
KCN	2 × 10 ⁻⁴	Cl	0.2	90	87	40	45	68
NaN ₃	10 ⁻³	Cl	0.2	97	98	40	55	87
	5 × 10 ⁻⁴	Cl	0.2	97	97	40	53	86

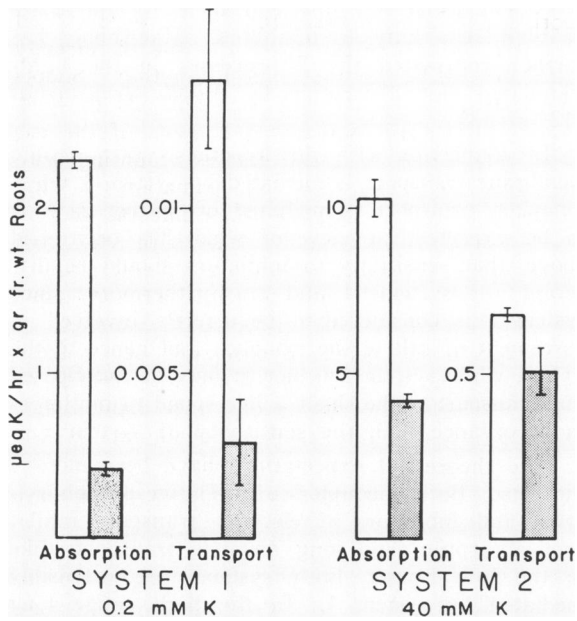


FIG. 1. The effect of *m*-Cl-CCP on K⁺ absorption and transport. Intact seedlings. Inhibitor concentration 10⁻⁶ M, pH 7.0; 0.5 mM CaSO₄. ⁸⁶Rb as K tracer. Inhibitor treated: shaded bars.

effective in suppressing K absorption and long-distance transport. Again, both uptake and transport are inhibited in the low range very much more markedly than in the high range, and root absorption is affected more than transport in the high range.

The effect of the terminal oxidase inhibitors, cyanide and azide on chloride absorption and long-distance transport is depicted in figures 4 and 5. With both inhibitors, absorption and transport are almost equally affected in the low range, and ab-

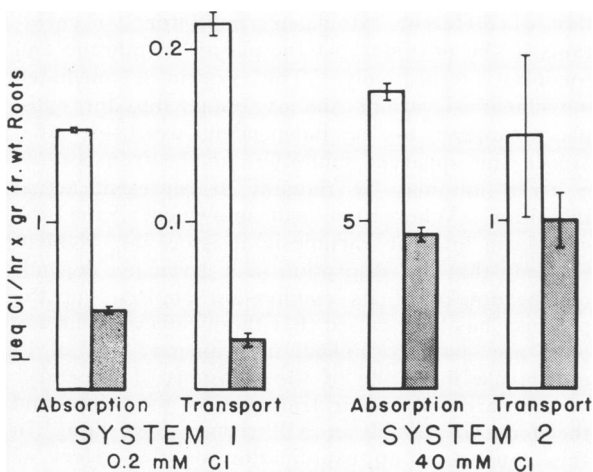


FIG. 2. The effect of *m*-Cl-CCP on Cl⁻ absorption and transport. Intact seedlings. Inhibitor concentration 10⁻⁶ M, pH 7.0; 0.5 mM CaSO₄.

sorption is more markedly inhibited than transport in the high range. The concentrations of both uncouplers and oxidase inhibitors were chosen on the basis of their maximum influence, without irreversible injury, on oxidative phosphorylation and respiration respectively.

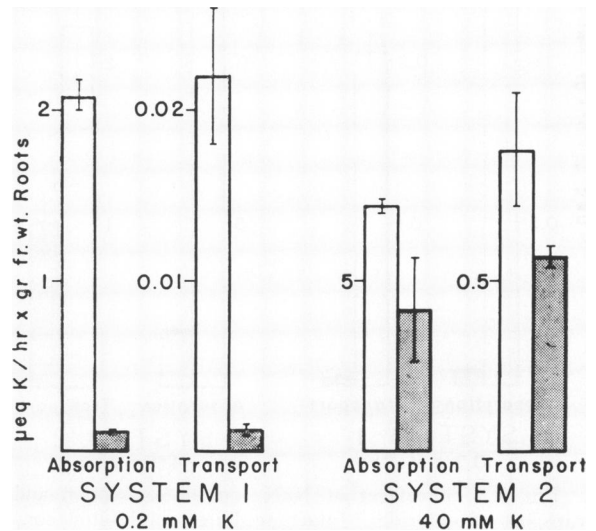


FIG. 3. The effect of *p*-CF₃O-CCP on K⁺ absorption and transport. Intact seedlings. Inhibitor concentration, 10⁻⁶ M, pH 7.0; 0.5 mM CaSO₄. ⁸⁶Rb as K tracer.

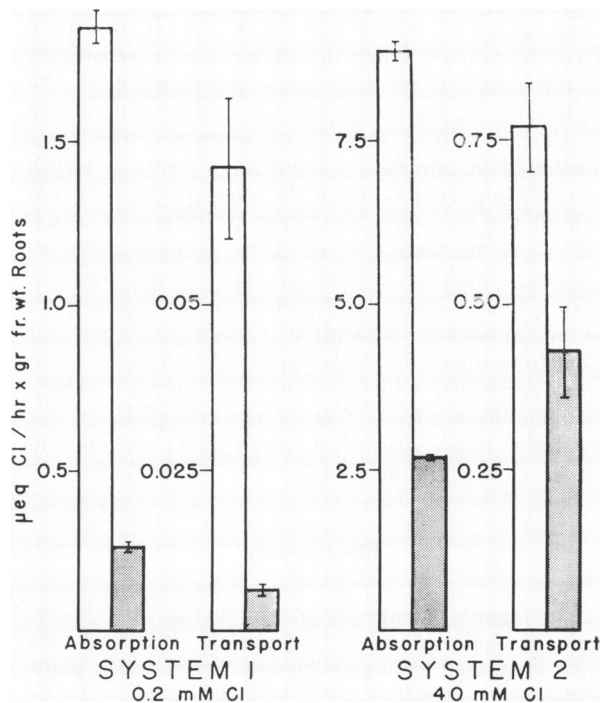


FIG. 4. The effect of KCN on the absorption and transport of chloride. Intact seedlings. Inhibitor concentration, 0.2 mM, pH 7.0. 0.5 mM CaSO₄.

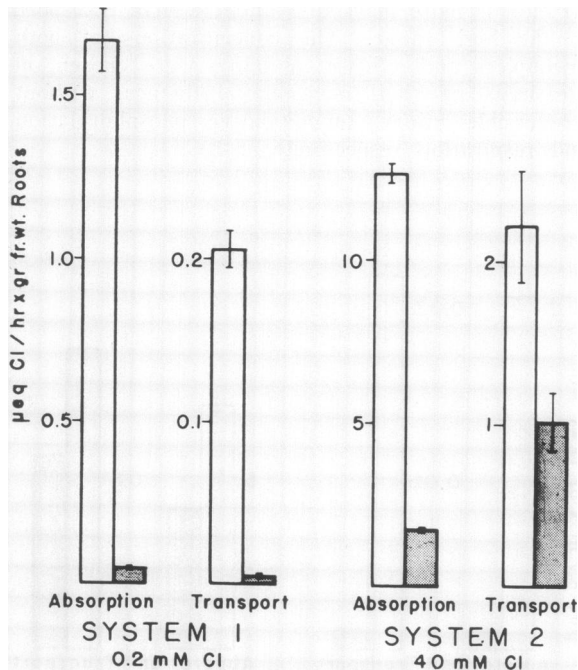


FIG. 5. The effect of NaN_3 on the absorption and transport of chloride. Intact seedlings. Inhibitor concentration, 0.5 mM, pH 5.8; 0.5 mM CaSO_4 .

System 1 manifests a uniformly greater sensitivity to the inhibitors studied than does system 2 (table I). Specifically, absorption is more effectively inhibited in the low range than in the high. To determine whether the difference in sensitivity is intrinsic, or an artifactual consequence of the difference in time required for inhibitor to arrive at the susceptible locus, root segments were preincubated in inhibitor. Segments 20 mm in length (ca 200 mg fr wt) from the region 20 to 60 mm behind the tip were incubated in 10^{-6}M *m*-Cl-CCP ($5 \times 10^{-4}\text{M}$ CaSO_4) for 2 to 4 hours. Subsequently, inhibitor sensitivity was determined as before in a 1 hour absorption period. Aging leads to an enhanced absorption capacity. Hence for each time interval samples preincubated in inhibitor were compared with preincubated controls. There was no discernible effect of preincubation on the inhibitor sensitivity of K absorption by roots in either concentration range.

Discussion

In ingenious experiments involving the absorption and long distance transport of chloride in the thin, elongated leaves of the submerged water plant, *Vallisneria spiralis*, Arisz (2) has firmly established that ion passage into the cytoplasm across the plasma membrane, and ion passage into the vacuole across the tonoplast are both metabolically

dependent processes, distinguishable on the basis of their sensitivity to inhibitors. MacRobbie has further contrasted the 2 processes in the light-stimulated salt absorption by *Nitella translucens* (12, cf 13).

In connection with the hypothesis that plasma membrane transport is mediated by system 1 while tonoplast transport is mediated by system 2, it is to be expected, in view of what has been said above, that sensitivity to inhibitors should be different for systems 1 and 2. Furthermore, since system 1 is considered to be directly involved in movement of salt to the symplast and hence to the xylem, while system 2 is not, it may be anticipated that transport to the shoot will respond to inhibitors in accordance with the sensitivity of system 1.

The theoretical expectations have been met by the data. Both uncouplers of oxidative phosphorylation and inhibitors of electron transport inhibit root absorption and long distance transport alike in the low range. Both systems are ostensibly mediated by system 1. In the high range long distance transport is markedly less inhibitor sensitive than root absorption. The latter observation is in accord with the presumption that in the root, vacuolar uptake is carrier-mediated in the high range, while penetration into the symplast is largely diffusive. Movement within the symplast is much more rapid than can be expected from simple diffusion (3, 15), but the absorption isotherms do not bear upon this question. The vacuoles represent a diversionary sump so far as transport to the shoot is concerned, and the data reaffirm the independence of the root accumulation and long distance transport processes in the high range.

If with respect to inhibitor absorption a pseudo steady-state is reached in the cytoplasm in a relatively short time, as with salt absorption (12, 14), then the difference in sensitivity of systems 1 and 2 to the chosen inhibitors is real, and not a reflection of different cytoplasmic inhibitor concentrations in the proximity of the plasma membrane and tonoplast respectively. The system 1 mediated movement of cations and of anions into the cytoplasm appear to be independent processes (6). Hence the apparent inhibition of the transport of a given ion may be thought to represent actual inhibition. Where net salt movement from the cytoplasm into the vacuole is at issue, it is evident not only that the absorption of a given ion depends on the uptake of its counter-ion (6, 16), but the prospect has been raised that the members of an ion pair are actually linked in transport (12). In either case inhibition of the transport of 1 member of the pair will impair transport of the other, and the focus of inhibition will remain uncertain. In this connection an examination of the effect of inhibitors on the individual fluxes at the plasma membrane and tonoplast respectively (12, 14) may provide the means of resolving the problem.

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