Ion Uptake and Transport through Barley Seedlings: Differential Effect of Cycloheximide

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Cycloheximide inhibits transport of K through barley roots without affecting K uptake and accumulation in the root cells. The inhibitor acts upon protein synthesis but does not appear to uncouple respiration. Requirement of protein synthesis for ion transport through roots is possibly due to involvement of symplasmic transport or vesicular secretion into the xylem.

Excised roots have the ability to take up ions from solution (in particular K, Cl) and in certain conditions can also secrete them from the cut end of the xylem. In the intact plant this appears to be the normal process for supply of K to the shoot from the root *via* the transpiration stream. For barley and maize the amount transported through excised roots is the same as that transported to the shoot in intact plants [1—3].

Ion secretion from the root can be studied by collecting and analysing the solution exuding from the basal end of excised roots in microcapillary tubes [4, 5], or by measuring amounts of ions transported to the surrounding solution [2, 6, 7].

Experiments of this kind have shown that both uptake and secretion of K and Cl are inhibited by the uncoupler CCCP [5, 8]. Apart from uptake to the root other processes requiring metabolic energy appear to be involved in transport through the root. Their nature is uncertain but seems to be of a different kind to the uptake process, since uptake and transport are affected differently by various compounds. Abscisic acid can inhibit transport through the root without affecting uptake [9]. Cytokinins have little effect on accumulation, but inhibit transport by at least 50% [10—12]. This paper shows that the inhibitor of protein synthesis, cycloheximide (CHM), also inhibits transport of K through the roots of barley seedlings without affecting K uptake and accumulation in cells of the roots.

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Abbreviations: CCCP, Carbonylcyanide *m*-chlorophenylhydrazone; CHM, cycloheximide; PCMB, *p*-chloromercuribenzoate.

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Materials and Methods

Transport of ions through the roots of high-salt seedlings can be measured within minutes after excising, whereas roots of low-salt seedlings need several hours for transport to develop [2, 5]. For this reason roots of high-salt barley seedlings were used for measurement of ^{86}Rb transport using a technique described elsewhere [2]. Briefly, excised roots were set up in a series of chambers so that the apical 5 – 7 cm was in radioactive solution and about 0.5 cm at the cut end in non-radioactive solution of the same concentration. The solutions contained 5 mM KCl + 0.1 mM $CaSO_4$ with or without ^{86}Rb as a tracer for K and were aerated. This solution was changed at intervals and tracer content measured to estimate transport through the root. For the conclusions drawn in this paper it is not relevant that ^{86}Rb labelling might not give exact quantitative estimates of K^+ uptake and transport (*cf.* ref. [13—15]). For our purposes it is even sufficient that ^{86}Rb behaves in high-salt barley seedlings qualitatively like K.

Accumulation [2] was estimated from the amount of ^{86}Rb in the roots at the end of the transport experiment. The control rate was used to estimate the content of ^{86}Rb at the time of addition of CHM.

Uptake of ^{86}Rb was measured with excised roots from barley seedlings grown for 6 days on complete nutrient solution [16]. The aerated solutions contained 5 mM KCl + 0.1 mM $CaSO_4$ with or without ^{86}Rb as a tracer and were kept at 25 °C. The experiments were done essentially as described elsewhere [13].

Guttation experiments were done with 7-day-old barley seedlings grown on complete nutrient solution in the light. Guttation fluid was collected in microcapillary tubes from plants in aerated solution containing 5 mM KCl + 0.1 mM $CaSO_4$.

For uptake of ^{14}C-leucine and incorporation into protein, 5 mm segments of barley roots were put for

2 hours in aerated solutions containing 20 μM L-leucine (specific activity 6 μCi $^{14}C/\mu$mole leucine) + 5 mM KCl + 0.1 mM $CaSO_4$ with or without CHM at 25 °C. The roots were then rinsed for 2 min with unlabelled solution at 0 °C and immediately frozen with liquid N_2. The frozen roots were homogenised in 0.1 N NaOH, centrifuged, and the supernatant treated with 10% trichloroacetic acid to precipitate protein. The precipitate was washed and counted. Total uptake of ^{14}C-leucine in roots was also measured.

To test possible effects of CHM on respiration, O_2 uptake by roots was measured. Roots were pretreated for 2 hours with aerated solutions containing 5 mM KCl + 0.1 mM $CaSO_4$ with or without CHM at 25 °C. Oxygen uptake was determined with the Rank O_2 electrode over a period of 10 min.

Results and Discussion

Fig. 1 gives results for transport of ^{86}Rb from roots of plants grown under two different conditions (see legend). In both cases the rate of transport rose rapidly in the first hour as the specific activity increased in the transport system. Transport then rose slowly for plants grown in $CaSO_4$/KCl (10% per hour), but fell by about 20% per hour for the plants grown in the complete nutrient solution. The two different types of plants were used because we were concerned to show that any effect of CHM was not unique to plants growing in one set of conditions. Other experiments have shown a consistent difference in transport between plants growing on $CaSO_4$/KCl and on complete nutrient solution of the kind demonstrated in Fig. 1 (*cf.* ref. [17]).

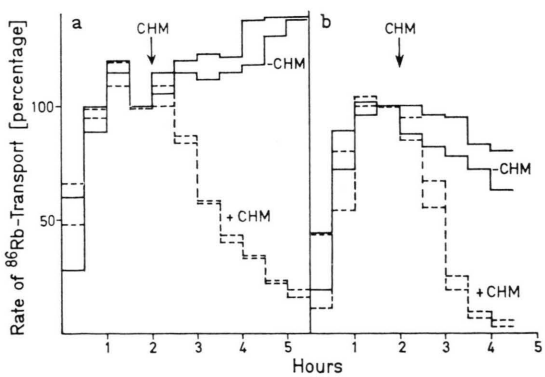

Fig. 1. Transport of ^{86}Rb through roots of barley seedlings grown in the dark under two different nutrient conditions: a. grown for 5 days on 0.1 mM $CaSO_4$, then on 10 mM KCl + 0.1 mM $CaSO_4$ for 24 hours ($CaSO_4$/KCl); b. grown for 6 days on complete nutrient solution [16] containing 10 mM KnO_3. At the time indicated stock CHM in water was added to give 10 μg/ml CHM in the labelled solution. Each bar represents one set of about 150 mg of roots. Roots were excised about 30 min before the time zero. Rates of transport are plotted as a percentage of that in period 4, immediately before addition of CHM. Values in this period were: Fig. 1 a: Control = 0.40; + CHM = 0.50; Fig. 1 b: Control = 1.7; + CHM = 1.6 μmole $g \cdot h^{-1}$.

After 2 hours in radioactive solution CHM was added to the labelled solution. Fig. 1 shows that 10 μg/ml CHM strongly inhibited ^{86}Rb transport in both kinds of roots. In several experiments, transport was reduced to nearly 10% of the controls within $2^1/_2$ hours. Addition of 1 μg/ml CHM had less effect, but still reduced transport to about 60% of the controls. For example in $CaSO_4$/KCl roots, transport was 55% of controls after $3^1/_2$ hours and in roots from nutrient solution it was 65% after $2^1/_2$ hours.

Inhibition of transport in intact plants by CHM was demonstrated by measuring its effect on guttation. At 10 μg/ml CHM guttation was stopped completely within an hour.

Though transport (and guttation) was inhibited (Fig. 1), CHM did not affect the accumulation of ^{86}Rb in root cells (Table I). Uptake of ^{86}Rb by roots was also insensitive to CHM; the amount of

Table I. Effect of CHM on accumulation of ^{86}Rb in barley roots.

Nutrient conditions	Rate of ^{86} Rb accumulation [μmole $g \cdot h^{-1}$]		
	Control	+ 1 μg/ml CHM	+ 10 μg/ml CHM
$CaSO_4$/KCl	0.80	0.65	1.20
complete nutrient solution [16]	1.25	1.25	1.30
complete nutrient solution [16]	1.25	1.30	1.15
complete nutrient solution [16]	1.55	1.60	1.25
mean percentage of control	100	98	105

Table II. Effect of CHM on uptake of ^{14}C-leucine and incorporation into protein by barley roots. Nutrient condition: Complete nutrient solution [16].

	Total leucine uptake [a]		Incorporation into protein [a]	
	[nmole $g \cdot h^{-1}$]	[%]	[nmole $g \cdot h^{-1}$]	[%]
control	200	100	66	100
1 μg/ml CHM	200	100	40	61
10 μg/ml CHM	226	113	25	38

[a] Data calculated per g fresh weight of roots.

^{86}Rb taken up after 60 and 120 min at 10 μg/ml CHM was 99 and 112% of the controls respectively.

Cycloheximide is generally used as an inhibitor of cytoplasmic protein synthesis, but Ellis and Mac Donald [18] showed that other physiological processes in higher plants may also be affected and in particular that CHM may act as an uncoupler of respiration. Protein synthesis in high-salt barley roots was certainly inhibited by CHM both at 1 and 10 μg/ml (Table II). At both 1 and 10 μg/ml CHM the total uptake of ^{14}C-leucine was the same as for the controls. The rate of incorporation into the trichloroacetic acid-insoluble fraction (protein), however, was steady over the two hours measured and reduced to 61 and 38% of the controls at 1 and 10 μg/ml CHM respectively.

In contrast, CHM did not appear to affect respiration (Table III). Oxygen uptake was not stimulated, as one might expect if CHM would act as an uncoupler. The uncoupler CCCP, however, increased O_2 uptake by almost 100% (Table III). In addition, CCCP strongly reduces ion uptake and accumulation

Table III. Effect of CHM and CCCP on oxygen uptake by barley roots. Nutrient condition: Complete nutrient solution [16].

	Rate of O_2 uptake [μmole g·h^{-1}]	[%]
control	12.9 ± 1.0	100
1 μg/ml CHM	13.4 ± 1.5	104
10 μg/ml CHM	12.2 ± 0.8	95
control	12.7 ± 1.5	100
2 μM CCCP [a]	23.4 ± 1.3	184

[a] CCCP was added directly to the incubation vessel of the O_2 electrode and O_2 uptake measured over a period of 10 min.

in high-salt barley roots [8]. Since CHM did not stimulate respiration and in particular had no effect on K uptake and accumulation, it seems unlikely that it could have been acting simply as uncoupler of respiration. The action of CHM as an inhibitor of protein synthesis is in line with the conclusion of Van Steveninck and Van Steveninck [19] that the ion transport mechanisms in cells of beet-root slices depend on the synthesis and decay of specific proteins.

These results provide further evidence that transport of K through the root differs from uptake and accumulation of K in cells of the cortex. There is also the implication that transport involves protein synthesis. Inhibition of transport with CHM is thus consistent with Baker's observation that PCMB inhibited exudation from *Ricinus* seedlings, though in this case only exudation of fluid was measured and no data on ion uptake and transport through the root were reported [20]. *

In studying transport of ions across the root it is possible that we are looking at the overall effect of one or more processes, for example, at symplasmic transport and also at secretion of ions into the xylem by a separate process. For giant algal cells (*Chara, Nitella*) it appears that in addition to active transport across the plasmalemma, there may also be a vesicular mechanism of transport through the cytoplasm [21]. Secretion into the xylem could also be by such a mechanism, differing in kind from the mechanism of uptake into a cortical cell, which appears to be due to a membrane-bound enzyme system [22].

One can readily accept that both symplasmic transport within a membrane system (endoplasmic reticulum?), and secretion by vesicles would require some degree of membrane turnover and therefore of protein synthesis. Involvement of protein synthesis in the transport process would also explain the action of abscisic acid and cytokinins on transport (*cf.* ref. [17]), since both these substances have selective action on enzyme synthesis [23].

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* *Note added in proof.* Measurements of the effect of 0.25 mM PCMB on barley roots showed that K transport through roots from 5 mM KCl + 0.1 mM $CaSO_4$ was inhibited by 90%, but it was found that uptake to root cells was inhibited too by 75%.

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Ketocarotinoide in den Früchten von *Lonicera webbiana* und *Lonicera ruprechtiana*

Ketocarotinoids of the Berries from *Lonicera webbiana* and *Lonicera ruprechtiana*

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Ketocarotinoids, pigments of caprifoliaceae, *Lonicera*

The main pigments of red Lonicera berries are Rhodoxanthin and two hitherto unknown ketocarotinoids named Loniceraxanthin and Webbiaxanthin. The structural formula for Loniceraxanthin is established (**2**) and for Webbiaxanthin a structural formula is discussed (**3**).

Rote Lonicerafrüchte können klar durchscheinend rot oder opalisierend erscheinen: Im ersteren Falle wird die Färbung durch Anthocyane, im zweiten durch Carotinoide hervorgerufen. Bis jetzt sind nur orange gefärbte Früchte von *Lonicera*-Arten untersucht worden, die Zeaxanthin als Hauptpigment enthalten [1].

Wir haben nun die Ketocarotinoide der rot gefärbten Früchte von *Lonicera webbiana* und *Lonicera ruprechtiana* isoliert; beide Arten stimmen weitgehend überein *.

Experimentelles

Im Verteilungs-Dünnschichtchromatogramm [2] auf Zellulosedünnschichten, die mit Triglyceriden (Öl) imprägniert sind, und mit dem Fließmittel MeOH : Aceton : Wasser = 10 : 2 : 1 erkennt man neben unübersichtlichen Spuren von Begleitpigmenten drei Hauptzonen I – III, die violettrot (I), R_f 0,35, orangerot (II), R_f 0,45, und gelb (III), R_f 0,75, erscheinen und in mehr oder weniger vergleichbaren Mengen vorliegen, wobei I für die Farbe der Beeden ausschlaggebend sein dürfte.

Trägt man nicht den Urextrakt, sondern eine verseifte Probe auf, so ist II restlos verschwunden. Bei der Aufarbeitung ist daher Vorsicht geboten und Alkali auszuschließen.

Trennung der Pigmente: Mit Rücksicht auf II, das gegen Kieselgel, MgO, $ZnCO_3$ u. ä. nicht stabil ist, wurde über Zellulosepulversäule (MN 300) mit PÄ-CCl_4-Gemischen getrennt. Man erhält neben zahlreichen Mischfraktionen reines I in großer und II in kleiner Menge. III liegt noch unrein vor und kann über eine $ZnCO_3$-Säule vollends rein erhalten werden. Die zahlreichen Nebenpigmente werden verworfen.

Ergebnisse

Pigment **1** (Rhodoxanthin): Das violettrote Pigment hat das Spektrum des Rhodoxanthins. Es stimmt im Chromatogramm mit Rhodoxanthin aus Eibenbeeren überein und liegt wie dieses als Gemisch dreier Isomeren (wohl *cis-trans*) vor, die auf Zellulose und Polyamid knapp voneinander getrennt werden und etwas farbverschieden sind. Mit $NaBH_4$ läßt es sich reduzieren, wobei man ein Zwischen- und Endprodukt erhält.

Das erste ist mit Eschscholtzxanthon identisch, das zweite mit Eschscholtzxanthin. Beweisend ist vollends die Überführung in Zeaxanthin über das Dihydrorhodoxanthin, das durch Reduktion mit Zinkstaub in Pyridin und Eisessig zugänglich ist [3] (siehe Reaktionsschema).

Pigment **2** (Loniceraxanthin): Dieser Farbstoff ist im verseiften Extrakt nicht mehr enthalten. Wir

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