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THE EFFECTS OF CYCLOHEXIMIDE ON THE UPTAKE AND TRANSLOCATION OF AMINO ACIDS IN LEAVES OF SAGITTARIA GRAMINEA MICHX

E. SCHENK

Botanisch laboratorium, Utrecht

SUMMARY

Cycloheximide (CHM) inhibits translocation of ^{14}C-α-amino-iso-butyric acid in excised leaves of *Sagittaria graminea*, but does not affect its uptake. Protein synthesis was also inhibited by CHM.

A possible connection between various processes in which CHM is involved is discussed.

1. INTRODUCTION

The relation between protein synthesis and ion uptake has been studied by several investigators (POLYA 1968, MÜLLER & PASCHINGER 1970, LÄUCHLI et al. 1973). A direct link however, of rates of ion absorption to net protein synthesis as proposed by STEWARD & PRESTON (1941), is difficult to demonstrate, as the inhibitors of protein synthesis used are usually of a non-specific nature. Cycloheximide (CHM) inhibits protein synthesis in 80-S ribosome systems (SIEGEL & SISLER 1963) in a wide variety of eucaryotes including intact plant cells (MACDONALD & ELLIS 1969). Many investigators, however, demonstrated CHM to act on other processes than protein synthesis, e.g. inhibition of DNA synthesis (BROWN et al. 1970), but these effects were generally regarded as secondary effects of the inhibition of protein synthesis. Recently ELLIS & MACDONALD (1970) and MCMAHON (1975) have shown that CHM produced a wide range of independent effects on cellular metabolism, such as a decrease in the size of the ATP pool which may cause an inhibition of ion uptake.

The present work describes investigations which indicate that CHM is able to inhibit long distance translocation of ^{14}C-α-amino-isobutyric acid (AIB) but does not affect its uptake. Concomittantly protein synthesis was inhibited and K^+ efflux was increased. A possible link between these various processes is discussed.

2. MATERIAL AND METHODS

Translocation experiments were carried out as described in previous papers (SCHENK 1972, 1974).

For the measurement of the inhibition of protein synthesis by CHM,

labelled α-amino-iso-butyric acid (AIB) could not be used, owing to its incapability of incorporation into proteins. Therefore ^{14}C-l-leucine was used for that purpose. Its incorporation was measured in excised leaves engaged in translocation as well as in leaf segments incubated in 25 ml distilled water containing labelled leucine (2.5×10^{-4} M), in the presence or absence of CHM (1 μg/ml).

As *Sagittaria* leaves need several hours before translocation can be measured, no incorporation ratios were measured within the 6 hours period following the start of the experiment.

The leaves were rinsed in tap water for 3 minutes and homogenized in 0.05 M borax. After centrifuging ($1000 \times$ g) the supernatant was treated with 5% trichloroacetic acid (TCA) in order to precipitate protein. Samples were counted in a Packard Tricarb Liquid Scintillation Spectrometer.

To test possible K^+ efflux (POLYA 1968), leaves were incubated in 25 ml distilled water with or without CHM (1 or 5 μg/ml). After 2½, 12, 24 and 48 hours, samples were analysed by atomic absorption spectroscopy. Radioactive amino acids were obtained from the Radiochemical Centre at Amersham (U.K.) and CHM from Koch-Light Laboratories (U.K.).

3. RESULTS AND DISCUSSION

In order to study distinct effects on AIB translocation the leaves, of about 15 cm length were divided in three zones (SCHENK 1974): an apical zone I, a middle zone II and a basal zone III.

Fig. 1 shows the results of experiments in which AIB was applied to the apical part of zone I and CHM (1 μg/ml) was applied to either zone I, II or III. *Table 1* shows the relative distribution of AIB after 48 hours of translocation.

When CHM was applied to zone I, uptake of AIB took place at normal (control) ratio but translocation out of this zone did not occur at all.

When zone II was treated with CHM, the absolute amount of AIB which arrived in that zone *(table 1)* was slightly decreased compared to the control. The translocation profile, however, is different and a drastically decreased translocation of AIB out of zone II (from 20%–3%) was found.

The inhibiting effect of CHM on AIB translocation is much less distinct or virtually absent when zone III was treated with the inhibitor.

Regarding these results, CHM seems to have no effect on AIB transport across the plasmalemma (uptake). This is in accordance with results of ELLIS & MACDONALD (1970) and LÜTTGE et al. (1974). The latter did not find an effect on transport across the tonoplast in barley root cells either.

In contrast to its uptake, translocation of AIB in *Sagittaria* leaves is strongly inhibited, particularly when CHM was applied to zone I. The question arises why CHM has a 100%-inhibiting effect when applied near the site of uptake (zone I), but when applied to zone II or III this inhibition is much less.

As *table 1* demonstrates, this phenomenon cannot be explained in terms of differential sensitivity of the leaf zones to CHM treatment. In this experi-

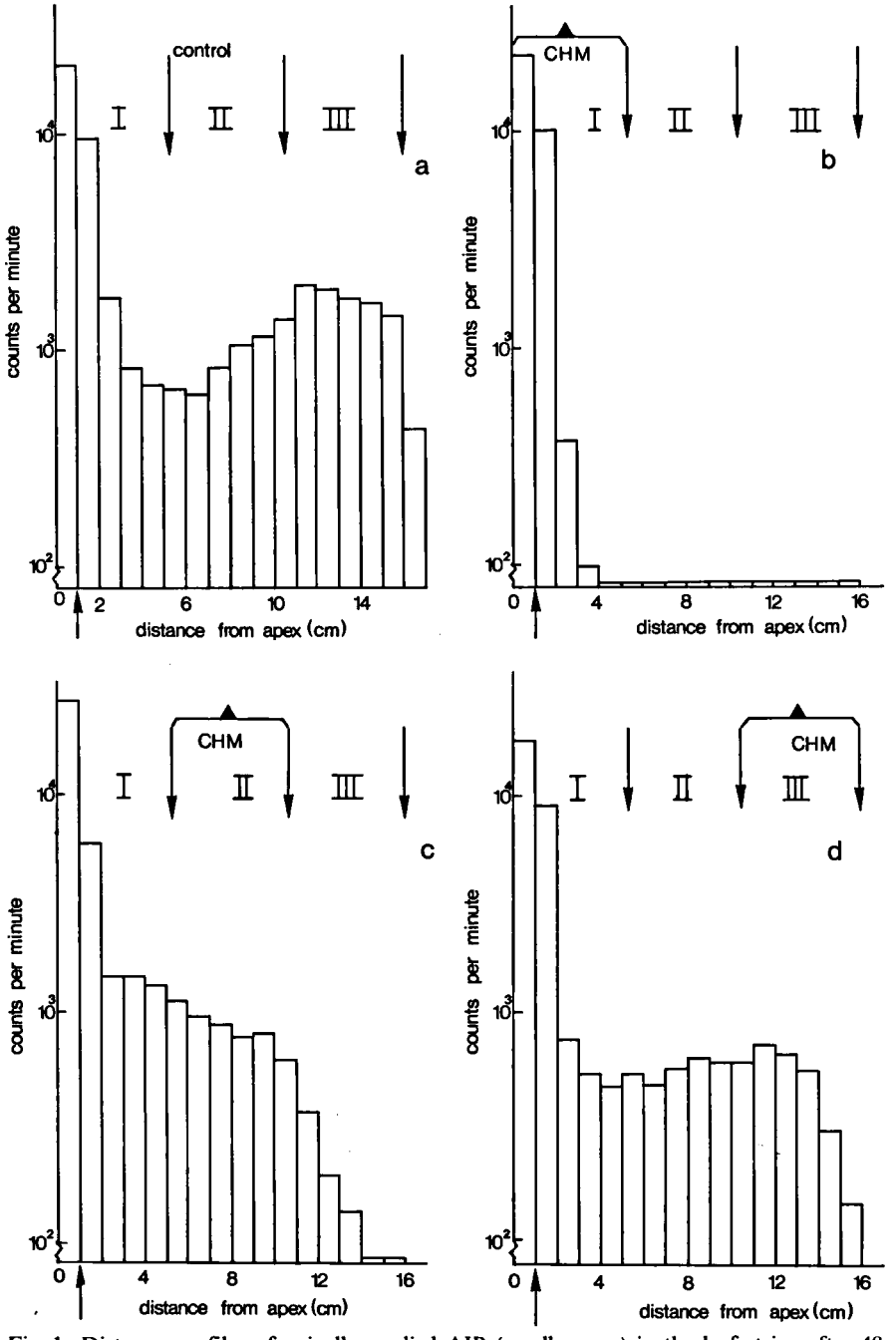

Fig. 1. Distance profiles of apically applied AIB (small arrow) in the leaf strips after 48 hours of translocation. (a) control. (b) CHM 1 μg/ml in zone I. (c) CHM 1 μg/ml in zone II. (d) CHM 1 μg/ml in zone III.

Table 1. Relative distribution in percentages of the total amount of AIB in the leaf strips after 48 hours of translocation. The influence of CHM (1 μg/ml) on AIB uptake and translocation.

All percentages are averages of the amounts of AIB in the respective zones in three series of three leaf strips.

	Zone I	Zone II	Zone III
Control: AIB apically applied	68	12	20
AIB and CHM in zone I	100	–	–
Idem, but CHM 18 hrs after AIB	71	11	18
AIB in zone I and CHM in zone II	87	10	3
AIB in zone I and CHM in zone III	83	10	7
Idem, but CHM 18 hrs after AIB	74	11	15
Control; AIB basically applied	1	4	95
AIB and CHM in zone III	–	–	100
Control; AIB applied in the middle	11	73	16
AIB and CHM in zone II	1	96	3

ment, the AIB was applied together with CHM to either zone II or III and here too, translocation out of this zone was inhibited. The possibility for a time-dependent, gradual breakdown of CHM can be rejected, as experiments in which CHM was applied 18 hours after the start of the experiment show that the inhibition of AIB translocation is less than when CHM is added together with AIB *(table 1)*.

Since carbonyl cyanide m-chlorophenylhydrazone (CCCP), an uncoupler of respiration, did stimulate the accumulation of AIB in *Sagittaria* leaves rather than inhibiting its translocation (SCHENK 1974), the observed translocation-inhibiting action of CHM cannot be attributed to an uncoupling of respiration.

The results may point to an involvement of protein synthesis, in particular of proteins with relatively long turn-over times, involved in translocation.

Results of leucine incorporation in TCA insoluble fractions *(table 2)* show that CHM, in the concentrations used, indeed acts as a protein synthesis inhibitor in leaves of *Sagittaria*.

This interpretation is in agreement with conclusions of VAN STEVENINCK & VAN STEVENINCK (1972), based on experiments with beetroot slices. These authors showed that the inhibitory effect of CHM when added after the start of the experiment, did not become apparent until after a certain time interval, which was thought to be correlated with the development of a specific and relatively stable ion transport mechanism.

It could, however, also be possible that CHM inhibits the passage of AIB out of a cytoplasmatic compartment in which the amino acid arrives after its uptake, into a special cytoplasmatic compartment involved in intracellular and intercellular translocation. If arrived in this compartment, CHM cannot influence the amino acid translocation.

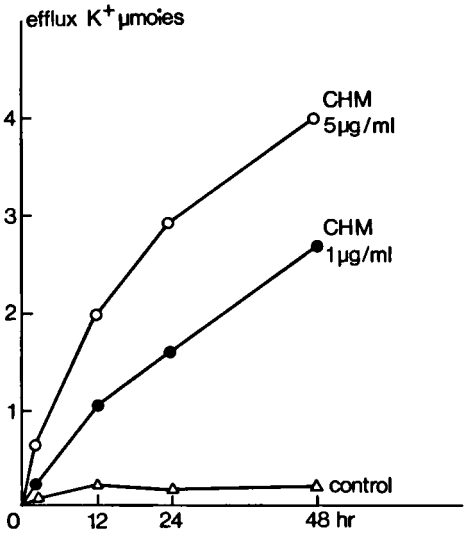

Fig. 2. Time course of K^+ efflux in medium, in the absence of CHM (△), in the presence of 1 µg/ml CHM (●) and in the presence of 5 µg/ml CHM (○).

Table 2. Effect of CHM (1 µg/ml) on ^{14}C-l-leucine incorporation.
A: leaf segments incubated in labelled leucine (2.5×10^{-6} M)
B: excised leaves engaged in translocation. Leucine (2.5×10^{-6} M) was applied by means of a small agar block containing the radioactive amino acid.

	Leucine uptake (%)	Incorporation of leucine (%)	Inhibition (%)
A			
− CHM	48.5	42.6	
+ CHM	50.0	15.2	64.3
B			
− CHM	40.3	34.6	
+ CHM	31.4	19.0	45.0

The observation of an increased K^+ efflux *(fig. 2)* is comparable with results of POLYA (1968) with experiments on CHM-inhibition of Tris-induced cation uptake in beet disks. He explained the observed extrusion of K^+ by an increased permeability of the plasmalemma. This leads to the suggestion of an involvement of membrane systems in symplasmatic translocation, although a satisfactory explanation for the nature of the translocation mechanism in *Sagittaria* leaves cannot be presented yet.

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