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EFFECT OF CYCLOHEXIMIDE ON THE CATABOLISM OF LEVULINIC ACID TO CO_2 BY ETIOLATED LEAVES OF *HORDEUM VULGARE*

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; biochemistry; levulinic acid; cycloheximide; chloramphenicol.

Abstract—When etiolated barley (*Hordeum vulgare* L. var. Larker) shoots are incubated with [4-^{14}C]levulinic acid, they evolve $^{14}CO_2$. Cycloheximide inhibits this catabolism, and the effect is distinct from any effect this antimetabolite has on fatty acid oxidation or respiration. We suggest that a protein which is synthesized on 80 *S* ribosomes and which has a short half-life is necessary for levulinic acid catabolism to CO_2.

INTRODUCTION

Levulinic acid (LA), an inhibitor of δ-aminolevulinic acid (ALA)-dehydratase (EC 4.2.1.24), has been employed in a number of studies on the biochemistry of the greening process [1]. However, a potential problem in using LA in this capacity is that it is not metabolically inert; bacteria and yeast [2] as well as barley shoots [3] can metabolize this compound. In an accompanying paper [4], we report on the metabolism of [4-^{14}C]LA in etiolated and greening barley shoots. The present study examines the effect of cycloheximide and chloramphenicol on the metabolism of [4-^{14}C]LA to $^{14}CO_2$ by etiolated barley shoots. We suggest that this catabolism is dependent on the synthesis of proteins on cytoplasmic ribosomes. A preliminary report on this work has appeared [5].

RESULTS AND DISCUSSION

The effect of cycloheximide (CHI) and chloramphenicol (CAM) on [4-^{14}C]LA catabolism to $^{14}CO_2$ is shown in Table 1. Neither compound at the concentrations employed substantially influenced the uptake of [^{14}C]LA. CHI, but not CAM, inhibited $^{14}CO_2$ evolution from [4-^{14}C]LA.

The specificity of the CHI effect was examined by incubating etiolated barley leaf segments with [1-^{14}C]*n*-caproic acid (Cap) or [1,4-^{14}C]succinic acid (Suc) in the presence or absence of the inhibitor. In both cases CHI reduced $^{14}CO_2$ production by 12% (Table 1). Assuming that this represents the extent of respiratory inhibition by CHI, then the specific inhibition of this compound on LA catabolism to CO_2 was estimated to be about 47% (59% − 12%). Maximum inhibition was not observed until 3 hr after the addition of label despite the fact that the leaves were preincubated for 1 hr with the inhibitor (Fig. 1a). When CHI was administered 2 hr after [^{14}C]LA, it began to exert an effect after the third hour (Fig. 1b). Inhibition became maximal by the fifth hour, i.e. 3 hr after addition of CHI, and reached about 24% (after correcting for the effect of CHI on respiration, 36% − 12%). The decline in the rate of $^{14}CO_2$ evolution in the presence of CHI obeys first-order kinetics (Fig. 1, inserts).

These results suggest that a short-lived protein (half-life *ca* 4 hr) is necessary for CO_2 production from LA. This

Table 1. The effect of CAM and CHI on the metabolism of LA, caproic and succinic acids

	Label taken up		$^{14}CO_2$ evolved	
	DPM × 10^{-6}	% of control	label taken up	% of control
[4-^{14}C]LA	3.586 ± 0.107*	100	0.0120 ± 0.0009	100
[4-^{14}C]LA + CAM	3.496 ± 0.017	97.5	0.0114 ± 0.0010	95
[4-^{14}C]LA + CHI	3.424 ± 0.045	95.5	0.00487 ± 0.0002	40.6
[1-^{14}C]Cap	7.800 ± 0.083	100	0.314 ± 0.011	100
[1-^{14}C]Cap + CHI	7.924 ± 0.035	101.6	0.278 ± 0.008	88.5
[1,4-^{14}C]Suc	4.255 ± 0.069	100	0.558 ± 0.001	100
[1,4-^{14}C]Suc + CHI	3.954 ± 0.037	92.9	0.490 ± 0.009	87.8

*S.e. for two to three determinations.

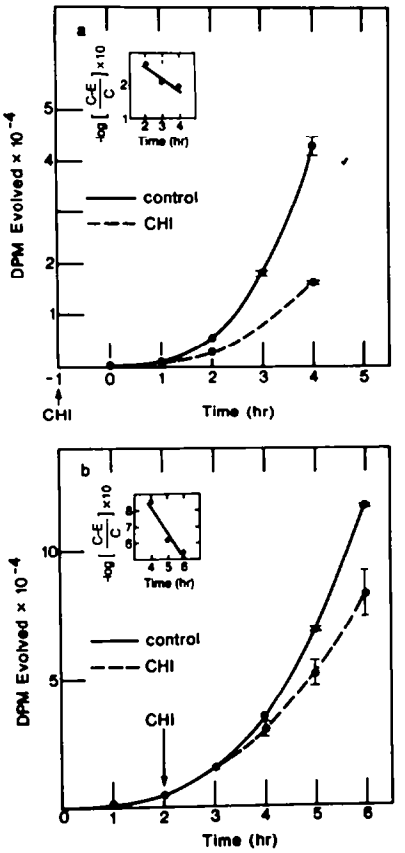

Fig. 1. The effect of CHI on the metabolism of [4-^{14}C]LA to $^{14}CO_2$ by etiolated barley shoot segments incubated in the dark. (a) Tissue was incubated for 1 hr with CHI prior to the addition of [4-^{14}C]LA. (b) Tissue was incubated for 2 hr with [4-^{14}C]LA prior to the addition of CHI. Inserts: the decline in the rate of CO_2 production after addition of CHI. The data are plotted as: the negative log of the amount of $^{14}CO_2$ produced in the absence of CHI (C) minus that in the presence of CHI (E) divided by C vs. time, beginning with the second hour after the addition of [^{14}C]LA in (a) or CHI in (b). Values represent the mean of two determinations; s.e. are indicated with bars.

protein is apparently synthesized on cytoplasmic ribosomes.

EXPERIMENTAL

Growth and preparation of plant tissue. Seeds of *Hordeum vulgare* L. var. 'larker' (Field Seed Farm; Byron, MN) were germinated and grown in darkness for 7 days and then the apical 5 cm of the shoots was harvested according to the method of Duggan *et al.* [3]. One gram samples of tissue were placed into 125-ml Erlenmeyer flasks containing 0.1 M Pi buffer (pH 3.0) and the flasks stoppered. All manipulations involving living material were performed under a dim green safelight.

Incubation with chloramphenicol (CAM) and cycloheximide (CHI). In one experiment the tissue was preincubated for 1 hr in darkness in the presence of either 450 μg/ml CAM or 50 μg/ml CHI. The radioisotope, either [4-^{14}C]LA, [1-^{14}C]Cap or [1,4-^{14}C]Suc, was then added to the incubation medium, bringing the final vol. to 1.0 ml. Respired $^{14}CO_2$ was measured at the designated times as previously described [3].

In another experiment, tissue was incubated with [4-^{14}C]LA for 2 hr prior to the addition of CHI. The incubation was then continued for an additional 4 hr. Respired $^{14}CO_2$ was monitored throughout the incubation.

Biochemicals. [4-^{14}C]LA (20 mCi/mMol) was purchased from Amersham Corp., Arlington Hts., IL and purified by TLC [4]. [1-^{14}C]Cap (19 mCi/mMol) and [1,4-^{14}C]Suc (51 mCi/mMol) were obtained from Research Products International Corp., Mt. Prospect, IL. CHI (Acti-dione) was a gift from the Upjohn Pharmaceutical Co., Kalamazoo, MI. CAM, sodium succinate salt (Chloromycetin), was a gift from Parke-Davis & Co., Detroit, MI.

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