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CHLOROPHYLL FORMATION AND GLYCINE METABOLISM IN LAEVULINIC ACID TREATED BARLEY LEAVES

ALLAN K. STORBART† and GEORGE A. F. HENDRY‡

† Cell Metabolism Laboratory, Department of Botany, The University, Bristol BS8 1UG, U.K.; ‡ Department of Biochemistry, The Medical School, The University, Bristol BS8 1TP, U.K.

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Key Word Index—*Hordeum vulgare*; Gramineae; leaves; chlorophyll; δ-aminolaevulinic acid; glycine turnover; total amino acid, laevulinic acid.

Abstract—Laevulinic acid (LA) inhibited chlorophyll formation and δ-aminolaevulinic acid (ALA) accumulation in dark-grown barley leaves. Mole ratios (ALA: chlorophyll × 8) indicate that LA decreased ALA production by about 30%. The turnover of glycine-[^{14}C] in 7-day-old leaves treated with LA was 70% slower than in control tissue and this resulted in an increase in endogenous glycine. Total amino acid also increased in LA treated leaves. The data indicate that any contribution made by glycine to ALA synthesis in LA-treated barley leaves would be significantly restricted.

INTRODUCTION

Laevulinic acid (LA) is a competitive inhibitor of δ-aminolaevulinic (ALA) dehydratase [1]. The treatment of higher plants with LA brings about an accumulation of ALA and an inhibition of chlorophyll synthesis. Theoretically, if LA has no effect on any other aspect of metabolism, there should be a 1:1 mole ratio of chlorophyll inhibition and the ALA accumulated (1 mole chlorophyll = 8 moles ALA). Claims have been made for such ratios in *Chlorella* [2], maize leaves [3] and cucumber cotyledons [4]. Barley leaves [4, 5], however, give ratios which suggest that LA may have effects other than on ALA-dehydratase and LA also appears to effect the ultrastructure of mitochondria as well as plastids in maize and bean leaves [6]. Gassman and Castelfranco [7] report that the $^{14}CO_2$ released from ALA-[^{14}C] in greening barley leaves was partially inhibited by LA. Protochlorophyllide turnover was also slower in etiolated barley leaves treated with LA [8].

Laevulinic acid is used extensively in attempts to elucidate the direct precursors of ALA and porphyrins in plants [9]. Its effect, therefore, on other aspects of plant metabolism is of some importance. This report deals with the effect of LA on glycine and amino acid metabolism in barley leaves.

RESULTS

Laevulinic acid (5×10^{-2} M, pH 7.0) was fed to 8 cm tops of 8-day-old dark-grown leaves for 6 hr in the light. Chlorophyll and 'accumulated' ALA were estimated in the LA treated leaves and in water controls. Mole ratios of 'accumulated' ALA and the difference in chlorophyll of the LA and control leaves (1mole chlorophyll = 8 moles ALA) were consistently low with typical values of 1:1.5. The discrepancy between the observed ratio and the calculated ratio, based on the assumption that LA has no effect other than on ALA-dehydratase, was some 50% and indicates that the accumulation of ALA in LA treated barley leaves was about 30% less than expected.

Glycine-[^{14}C] turnover in LA treated leaves

Segments from 7- and 14-day-old dark-grown leaves were fed LA (10^{-2} M) in the presence of glycine-[^{14}C] for 4 hr in the light. The uptake of glycine-[^{14}C] was linear over this period. After 4 hr the leaf segments were transferred to buffer. At regular intervals segments were removed, washed and glycine extracted, and after purification assayed for radioactivity. The half-life of glycine was calculated from the turnover of glycine-[^{14}C] in the leaf tissue. Typical results (Table 1) indicate that LA treatment lengthens the half-life of glycine-[^{14}C] by 73 and 24% in tissue from 7- and 14-day-old leaves respectively.

The effect of LA on glycine and total amino acid

Seven-day-old dark-grown leaves were trimmed to 4 cm and stood in a LA solution (pH 7) for 1 hr in the dark

Table 1. The effect of laevulinic acid on the turnover of glycine-[^{14}C]

Dark age (days)	Light treatment (hr)	Glycine-[^{14}C] half-life (min)		
		Control (−LA)	LA treated	% increase
7	4	8.3	14.4	73.5
14	4	12.4	15.4	24.2

Segments from 7- and 14-day dark-grown leaves were incubated in buffer containing glycine-[^{14}C] (2.5 μCi) and LA (10^{-2} M) in the light for 4 hr. The tissue was then transferred to buffer only and samples taken at regular intervals. Glycine was extracted and purified by high voltage electrophoresis before radioassay. Half-life values were calculated from turnover curves

followed by 4 hr in the light. The glycine and total amino acid content of the leaves were then determined. The results (Fig. 1) show that LA at all concentrations (10^{-1} M to 10^{-4} M) induced an increase in glycine. Laevulinate at 10^{-1} M brought about a 30% increase in 'total amino acid'. At 10^{-2} M, LA had only a slight effect on total amino acid (*ca* 12%) whereas glycine increased by 60%.

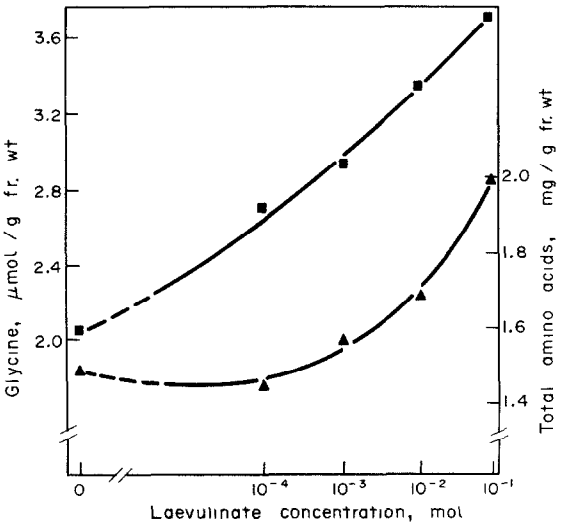

Fig. 1. The effect of laevulinic acid on glycine and total amino acid content. 4 cm tops from 7-day-old dark-grown leaves were fed LA (pH 5) for 1 hr in the dark and 4 hr in the light. Glycine and total amino acid were then determined. ■—■; glycine. ▲—▲; total amino acid.

DISCUSSION

Laevulinic acid had a considerable effect on glycine metabolism in barley leaves. It was found to slow down glycine turnover and to increase the endogenous glycine content. Comparisons of glycine and 'total amino acid' content in leaves treated with high concentrations of LA indicated that LA may also modify the 'turnover' of other amino acids. Since the turnover of glycine in barley leaves is a rapid process [10] and LA slows this down one might expect that the incorporation of glycine-[^{14}C] into ALA in LA fed leaves would be low relative to other precursors, in a system where possibly more than one pathway exists for the synthesis of ALA [9]. Treatment with LA, certainly in barley, would decrease any contribution made by glycine to ALA through the classical ALA-synthetase system. The observed mole ratios of 'accumulated' ALA to the difference in chlorophyll between the LA and control leaves suggest that a 20 to 30% decrease in ALA accumulation has taken place. While it is possible that an ALA 'degrading' system is operative it is of interest that in leaves where glycine synthesis is diminished by α-hydroxymethanesulphonic acid treatment [11] chlorophyll production is also down by *ca* 20%.

EXPERIMENTAL

Barley seeds (*Hordeum vulgare* cv Proctor) were soaked in H_2O for 16 hr, sown in moist vermiculite, and grown in the dark at 26°. Light treatment was from Atlas Fluorescent super white tubes (3000 lux at seedling level). Glycine-U-[^{14}C] (112 mCi/mM), obtained from the Radiochemical Centre, Amersham, was purified by high voltage paper electrophoresis (HVE) before use. Laevulinate solutions were freshly prepared from the fully dehydrated chemical. Chlorophyll was extracted and determined in 80% Me_2CO [12]. δ-Aminolaevulinic acid was extracted in hot aq. EtOH and purified by HVE (formate–acetate electrolyte, pH 1.97 at 85 V/cm for 28 min. mobility relative to glycine, 1.15). After elution ALA was determined spectrophotometrically as the Ehrlich product [13]. Amino acids were extracted in hot aq. EtOH. Glycine (after purification by HVE) and 'total amino acid' were determined with ninhydrin [14]. Radioactivity measurements were made using liquid scintillation counting. Experimental procedures for the determination of glycine turnover and half-life calculations have been given previously [10].

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