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GLYCINE METABOLISM IN ETIOLATED BARLEY LEAVES ON EXPOSURE TO LIGHT

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; glycine; turnover; light; porphyrins

Abstract—Of a large number of amino acids examined, changes in glycine were the only ones which were correlated with the ability of dark-grown barley leaves to synthesise protochlorophyllide, δ-aminolaevulinic acid and chlorophyll on exposure to light. A rapid depletion was found in endogenous glycine in barley leaves after day 7. Illumination of the leaves increased the rate of glycine depletion. Glycine concentrations were high throughout the young leaf. The top and middle leaf sections however, which had maximal chlorophyll synthesising potential exhibited the most pronounced decrease in glycine as the leaf aged. Using glycine-[^{14}C] pulse techniques the half life of glycine in 7 and 14-day-old dark-grown leaves was 3.5 and 4.4 min respectively. Light treatment lengthened the half life to 6.9 and 12.1 min in 7 day and 14-day-old-leaves. Sustained illumination continued to decrease glycine turnover.

INTRODUCTION

Protochlorophyllide (P650) in etiolated barley leaves decreases between days 7 and 10 of dark-growth [1]. This correlates with the ability of the tissue to produce chlorophyll on exposure to light [1] and in the rate of endogenous δ-aminolaevulinic acid (ALA) synthesis [2]. The identity of the direct precursors of ALA in higher plants is still uncertain with some controversy as to whether the classical animal and bacterial system (i.e. glycine + succinyl-CoA → ALA [3]) is operative [4]. Several reports show that glycine-[^{14}C] is a poor precursor of ALA accumulated in the presence of laevulinic acid [5–7] which is a competitive inhibitor of ALA-dehydratase, and also that glycine-[^{14}C] is a rather poor precursor of porphyrins in barley leaves [8]. Because of the poor recovery of radioactive ALA after glycine-[^{14}C] feeding in barley leaves [9] an attempt was made to measure glycine turnover and to correlate endogenous glycine concentrations with chlorophyll synthesis.

RESULTS

Of all the amino examined in 6 to 10-day-old dark-grown leaves [10] only changes in glycine correspond to profiles obtained for protochlorophyllide (P650) and chlorophyll [1]. The results for glycine are given in Fig. 1. Six-day-old dark-grown leaves had a glycine concentration of *ca* 1 μmol/gfr. wt, this slightly increased by day 7, after which it rapidly decreased to below 0.2 μmol/g fr. wt at day 10. Illumination of 6-day-old leaves induced a rapid decrease in glycine, depleting the pool(s) by *ca* 42% in 24 hr.

In order to localise the changes in glycine, leaf blades of dark-grown and illuminated material were cut into 3 equal lengths with the coleoptile-enclosed stem forming a 4th section. The glycine concentrations in each section are given in Fig. 2. In all parts of the shoot, glycine was at its maximum in 6- and 7-day-old dark-grown tissue. The characteristic decrease in glycine between day 7 and 10 took place throughout the shoot. The decreases amounted to 1.38, 1.30 and 1.18 μmol/g fr. wt in the top, middle and bottom third of the blade respectively.

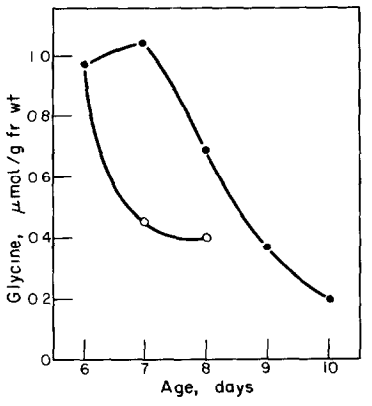

Fig. 1. Glycine in ageing etiolated leaves of barley. Seedlings were grown in the dark, at 25°, for up to 10 days. Six-day-old leaves were also illuminated for 24 and 48 hr. The primary leaf was extracted in hot (70°) 80% EtOH and glycine isolated by high voltage electrophoresis before estimation. ●—● dark-grown leaves; ○—○ light treatment.

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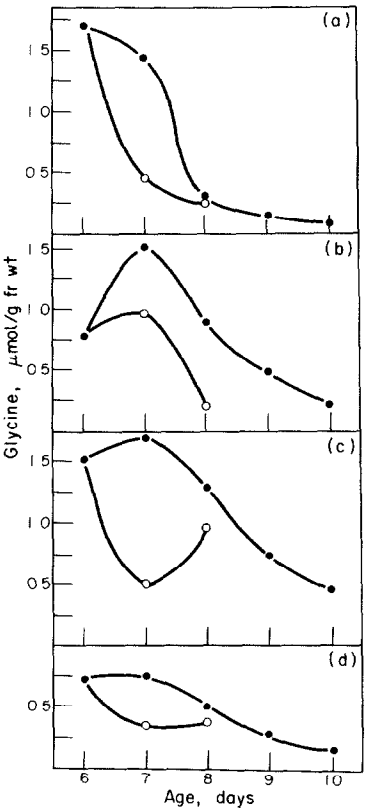

Fig. 2. The distribution of glycine in ageing leaves of barley Seedlings were grown in the dark, at 25°, for up to 10 days Six-day-old leaves were also illuminated for 24 and 48 hr. The primary leaves were cut into four sections. the blade (expanded lamina) was divided into three equal lengths, the stem region, enclosed by the coleoptile, formed the fourth section. Leaf sections were extracted in 80% EtOH at 70° and glycine isolated by high voltage electrophoresis before estimation. (a) top (b) middle (c) bottom and (d) coleoptile region. ●—● dark grown leaves; ○—○ light treatment.

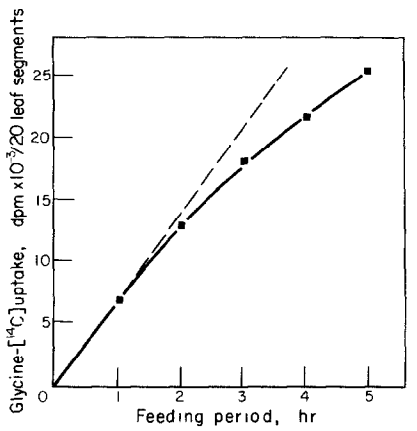

Fig. 3. Time course of glycine-[^{14}C] uptake in etiolated leaf segments. Ten-day dark-grown barley leaves were cut 4 cm below the tip, the top 2 cm were discarded and the remaining portion incubated in 2.5 μCi glycine-[U^{14}C] in K–Pi buffer (10 mM, pH 7.2) for up to 5 hr in the dark. At intervals, leaf samples were removed, washed, and extracted in hot (70°) 80% EtOH The extracts, after partitioning against Et_2O, were assayed for radioactivity. A deviation from the linear rate of uptake was observed after 75 min feeding.

Glycine turnover

Trial experiments showed that 1 cm barley segments, from 10-day-old dark-grown leaves, absorbed glycine-[^{14}C] at a linear rate for 60 to 75 min, thereafter the rate gradually declined (Fig. 3). This suggests that during the first 75 min of feeding, glycine-[^{14}C] is being incorporated into both 'active' and 'inactive vacuolar' pools. When the active pools are saturated the rate of uptake declines. To ensure complete saturation of the active pools, glycine-[^{14}C] feeding has to be maintained for at least 75 min. A two-hour feeding period was, therefore, adopted in all experiments measuring glycine turnover. Turnover was measured in segments from 7 and 14-day dark-grown leaves kept in the dark or after illumination. Gycine-[^{14}C] turnover calculations were made from the relationship of glycine-[^{14}C] present in the tissue against time of incubation after adjusting for the amount leached into the incubation medium. A typical curve is given in Fig. 4. In dark-grown leaves, the mean half-life of glycine-[^{14}C] was 3.5 min in 7-day-old dark-grown leaves and 4.4 min in 14-day-old material (Table 1.) Two separate determinations, each with replicates, were made and on this small statistical population, the difference between 3.5 and 4.4 minutes is not significant. When leaves of these ages were illuminated for 4 hr the half-life lengthened to 6.9 min in 7-day-old leaves, twice as long as in the dark treatment, and 12.1 min in 14 day-old tissue, three times longer than in the dark. These results indicate that light suppresses glycine turnover. As the non-radioactive glycine pools are in a steady state over 3 hr in 7 and 14-day-old cut leaves, light appears to inhibit both the synthesis and degradation of glycine. In 6-day-old dark-grown tissue, after 28 hr illumination, the half-life was 8.5 min nearly 2.5 times longer in the 7-day-old dark control.

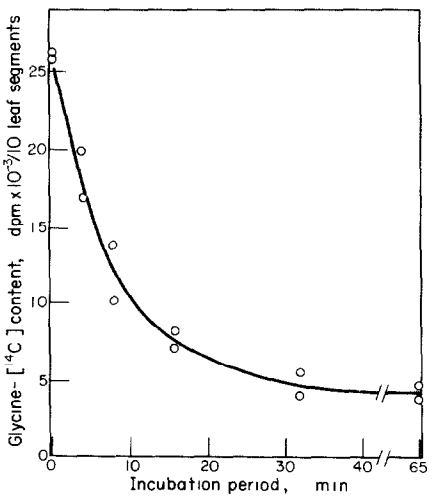

Fig. 4. Typical glycine-[^{14}C] turnover curve. Leaf segments (2 cm), incubated in 2.5 μCi glycine-[U^{14}C] in K–Pi buffer (50 mM, pH 7.2) were thoroughly washed and then incubated in buffer for up to 1 hr. At intervals, leaf samples were removed, rapidly killed in hot EtOH and glycine extracted and purified by high voltage zone electrophoresis before counting. The results are the mean of two replicates using 7-day-old dark-grown leaves illuminated for 2 hr before feeding.

Table 1. The half-line of glycine-[^{14}C] in etiolated and greening leaves of barley

Dark age (days)	Light (hr)	Glycine-[^{14}C] half life (min)	
		Range	Mean
6	28	8.5–8.5	8.5
7	0	2.6–4.4	3.5
7	4	5.3–8.5	6.9
14	4	12.0–12.2	12.1

Half-lives were calculated from glycine-[^{14}C] depletion curves in leaf segments from 7 and 14-day-old dark-grown leaves or after 4 hr illumination (including 1 hr as intact seedlings). Six-day-old dark-grown leaves were illuminated for 28 hr (25 hr as intact seedlings). The results show the range of calculated half-lives from three experiments, each experiment with duplicated samples.

DISCUSSION

The young barley shoot is rich in glycine where it can form over 5% of the total amino acids at day 7. By day 10 however, glycine constitutes only 0.9% of the total amino acids. Depletion in glycine between day 7 and 10 indicates that most of the glycine pool(s), in young leaves, is capable of being rapidly utilised in cell metabolism and that any 'inactive' vacuolar pool is negligible. Glycine turnover calculations confirm this. The half-life of glycine-[^{14}C] is less than 5 min in 7 and 14-day-old dark-grown material However light supresses glycine turnover and diminishes the endgenous glycine pool size(s). It appears that the synthesis of glycine is inhibited on illumination. Glycine-[^{14}C] metabolism is rapid in coffee seedlings [11], grape leaves [12], wheat leaves [13], spinach leaves [14] and tobacco plants [15]. While these reports do not give turnover rates, Wang and Burris [16, 17] using young green wheat leaves, found that after 45 min feeding with glycine-[^{14}C] that only 3% of the total label taken up was present as glycine in dark-treatments while 10% remained as glycine in the light. It appears that in barley as in wheat leaves, glycine-[^{14}C] turnover is substantially faster in the dark than in the light. Glycine turnover is so rapid in higher plant leaves that experiments comparing its relative incorporation with the view to precursor identification in studies on porphyrin synthesis should be approached with some caution.

The greatest change in glycine occurred in the regions with the highest potential for chlorophyll production on illumination [1, 9]. Since the regulation of porphyrin synthesis appears to be exerted through ALA synthesis [3] it may be that ALA formation is also linked to the rate of glycine metabolism. Certainly, changes in glycine concentration and not glutamate [9, 10] have a close relationship to the synthesis of ALA and porphyrin levels [1] in developing barley leaves.

EXPERIMENTAL

Barley seeds (*Hordeum vulgare*, cv Proctor) were obtained from K. Wilson Ltd., Wellingborough, Northants and stored at room temp. in bins. Seeds were soaked for 16 hr in H_2O, planted in trays of vermiculite and germinated at 25° in the dark. Seedling illumination was from banks of Atlas fluorescent super-white 65/80 W lamps with an illuminance, at seedling level, of 3500–4000 lx. Glycine-[U-^{14}C] (112 mCi/mmol) was purchased from the Radiochemical Centre, Amersham and repurified before use.

Glycine extraction and determination. Amino acids were extracted in simmering (70°) 80% aq. EtOH. After reduction in vol., lipids were removed by partition with Et_2O, and glycine purified from the aq. phase by high voltage zone electrophoresis (HCOOH–EtOAc electrolyte, pH 1.95 at 85V/cm, for 28 min). After elution glycine was determined with ninhydrin [18].

Glycine turnover. In dark experiments the primary leaves of 7 and 14-day-old barley seedlings were cut 4 cm below the tip 1 hr before feeding and the top 2 cm discarded. The retained 2 cm segments were floated in cold (7°) K–Pi buffer, pH 7.2, and tied with nylon thread into groups of ten. Dark-grown leaves used for light treatment experiments were illuminated as intact seedlings for 1 or 25 hr before cutting and tying. It was found that such treatment allowed the non-radioactive glycine pools to be maintained at a more or less steady level, at least during the 2 hr of feeding, and 1 hr incubation. The feeding medium comprised 1 ml glycine-[U-^{14}C] (5–10 μCi, sp. act. 112 mCi/mmol), in 8.5 ml K–Pi buffer (50 mM,pH 7.2) containing 1 drop 0.1% Triton X-100 as a leaf wetting agent and 0.5 ml $CaSO_4$ (final concn 10 μM). Feeding was continued for 120 min in the dark or, where indicated, the light (900–1000 lx at flask level), the flasks being held in a shaking water bath (100 strokes/min, 24°). After incubation the tissue was removed from the flasks and the 'bundled' segments washed in glycine (1 mM) for 45 sec, H_2O for 30 sec. and K–Pi buffer (50 mM, pH 7.2) for 30 sec. At precisely 2 min after the end of feeding the first samples were dropped into simmering (70°) 80% aq. EtOH. The remaining tissue was placed in flasks containing buffer only. At intervals of 4, 8, 16, 30, 45 and 60 min, tissue was removed and killed. After immersion in hot 80% EtOH the nylon threads were cut and discarded. The extracts were reduced on a hot plate (70°) to one-fifth vol., decanted and fr. 80% hot EtOH added to the tissue. Extraction was repeated 4× until the plant material was colourless. The decanted extracts were bulked and reduced to 1 ml and partitioned 3 times against Et_2O. Glycine was isolated by high voltage zone electrophoresis. After elution radioactivity was assayed with a liquid scintillation counter

The half-life [19,20] in the steady state, was calculated as that time where $P_o - P_s/2$, P_o is glycine-[^{14}C] present in the tissue at the start of incubation, and P_s is glycine-[^{14}C] present at the end of the exponential decline in glycine-[^{14}C] levels. P_s was usually found within 45 min after the start of incubation and appears to represent the amount of glycine present in the 'inactive vacuolar' pools.

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