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CONTINUOUS LIGHT ALTERS INDOLE-3-ACETIC ACID METABOLISM IN *LEMNA GIBBA*

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Key Word Index—*Lemna*; Lemnaceae; duckweed; auxin half-life; indoleacetic acid turnover; photobiology.

Abstract—Turnover is an important parameter of indole-3-acetic acid (IAA) metabolism, and IAA turnover may also be linked to the modulation of auxin-regulated responses. Previous studies have not, however, addressed whether environmental factors, including light, affect the turnover of IAA. We have used a model system consisting of normal *Lemna gibba* and a line of *L. gibba* that overproduces IAA precursors (MTR1) to measure IAA turnover in intact plants exposed to constant light, constant light followed by 24 h darkness, and a diurnal (12 h light) cycle. Turnover rates were determined by pulse labeling with [$^{13}C_6$]IAA tracer followed by analysis by GC-MS. Free IAA levels and IAA uptake rates were also determined. These studies showed that IAA uptake in both lines was greater in the light in both light conditions (continuous and diurnal) than in the dark. In the dark, IAA turnover time in both lines was less than 1 h and IAA levels were 4–6 ng/g fr. wt. Under constant light, IAA levels were higher in MTR1 and turnover time was much longer in the normal line. These studies suggest—based on the difference in response between the MTR1 and the normal line—that IAA turnover is regulated both by the rate of biosynthesis as well as the rate of catabolism. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Light is one of several important factors in the environment that is utilized by plants to regulate their growth and development [1, 2]. Plants utilize light in two distinct ways: as a source of energy and as an environmental stimulus. The light environment can convey information through variation of at least four attributes: quality, quantity, direction and periodicity. In the natural environment, the information of seasonal change is related to temperature and the duration of the light period, and in many plant species the change between vegetative and reproductive phases is dependent on the changing of the light period [3].

The interactions between light- and auxin-regulated responses in plant growth and development have been studied for many years. The very concept of plant hormones was based, in part, on early careful studies of phototropism [4, 5]. The regulation of plant growth and development by auxin may act through a change in the amount of hormone (either the amount produced or the steady state levels), a change in biological activity, or a change in the sensitivity of the tissue [6]. The turnover rate of a hormone is an important parameter of metabolism, and turnover of IAA has been postulated to be linked to the modulation of auxin-regulated responses [7]. Light is known to interact with several aspects of auxin metabolism, including the regulation of IAA levels and transport [8–10]. However, no information about the effect of environmental changes, including light, on IAA turnover has been published.

In this study, the effects of light on IAA turnover, free IAA levels and IAA uptake were determined using a model system developed to allow rapid rates of turnover to be measured in intact plants. The system is based on two related lines of the duckweed, *Lemna gibba*. One line, 3F7-11, is a product of inbreeding of *L. gibba* G-3, and the other line, MTR1, was selected from a mutagenized population of 3F7-11 based on its resistance to α-methyltryptophan [11]. Because MTR1 is less sensitive to feedback regulation at the

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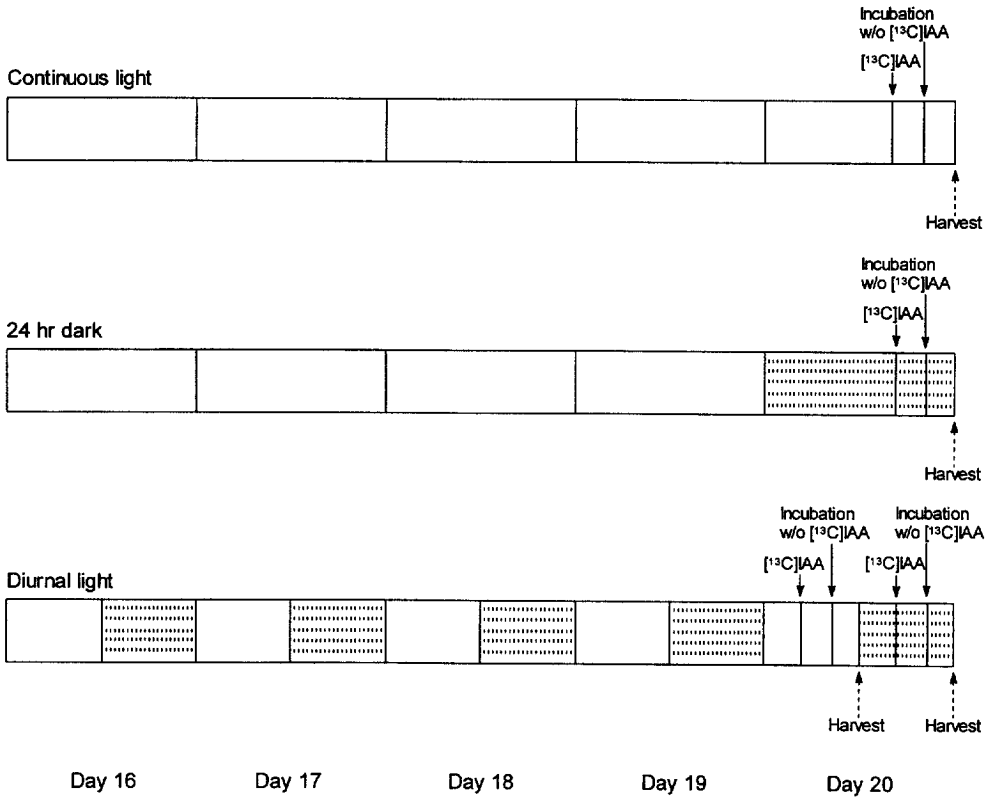

Fig. 1. Experimental protocols for growth of 3F7-11 and MTR1 lines of *Lemna gibba* in various light conditions. The top bar shows the conditions used for the continuous light experiment, the middle bar shows the design for the constant dark experiment. The diurnal photoperiod with two measurements of turnover (at the end of the light period and at the end of the dark period) is illustrated by the bottom bar. All cultures were started from 2 mother/daughter pairs, and for the first 15 days all cultures were maintained under continuous light at 25°.

level of anthranilate synthase [11] and thus has higher levels of indolic precursors, changes in processes involved in IAA biosynthesis should be magnified in MTR1 relative to those in 3F7-11. Thus, comparisons between 3F7-11 and MTR1 should provide information as to whether light-mediated regulatory steps controlling IAA turnover involve either IAA biosynthesis or catabolism, or both.

RESULTS AND DISCUSSION

The effect of diurnal light treatment on growth rate

Light regulates many biochemical reactions such as protein synthesis [12, 13], the activity of specific enzymes [14] and nutrient utilization [15, 16] in higher plants, including the aquatic monocot *Lemna*. In other plants, evidence suggests that light and dark conditions influence the rate of entry of exogenous IAA, the endogenous level of auxins, and processes of conjugation and oxidative breakdown of IAA [17]. Our current studies using the light conditions diagrammed in Fig. 1, show that growth under continuous light also affects IAA turnover, an important aspect of IAA metabolism that has not previously been shown to be altered by environmental changes.

Lemna gibba is a long-day plant. Both normal (3F7-11) and α-methyltryptophan resistant (MTR1) lines grew faster under continuous light than under light/dark conditions (Fig. 2). This increase in the rate of growth may, however, be a reflection only of the total light received during each 24 h period (Fig. 2). This is consistent with our observation (data not shown) that under continuous light of 13 μmol m^{-2} s^{-1}, 3F7-11 and MTR lines grew at about half the rate of plants in continuous light of 25 μmol m^{-2} s^{-1}. Similarly, Landolt and Kandeler [18] found that the growth rate of *Lemna gibba* increased in proportion to the length of day, at least at suboptimal light intensities, and was the highest under continuous light. Based on the fr. wt increase per flask per day, 3F7-11 grew about 20% faster than MTR1 under both diurnal and constant light conditions; however the increase in number of fronds per day was greater in MTR1.

Effect of light on IAA uptake

In *Lemna*, the uptake of potassium and nitrate is light-dependent [19, 20] and we have observed that

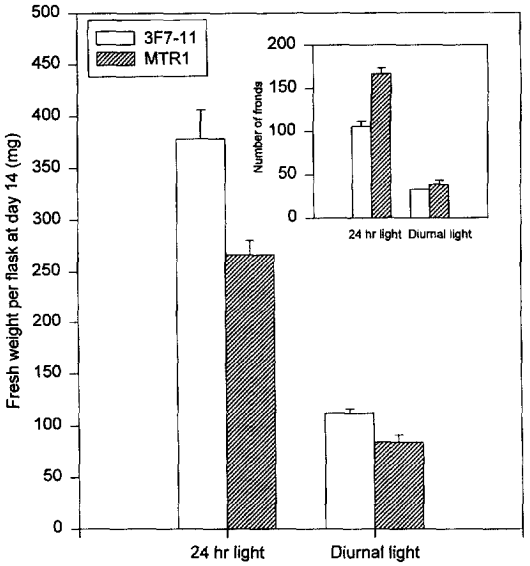

Fig. 2. The effect of diurnal light (D/L = 12:12) on growth rate of normal (3F7-11) and α-MT resistant (MTR1) lines of *Lemna gibba*. Growth rates were based on the increase in fr. wt (mg) and number of fronds per flask per day. Plants grown in continuous light at half intensity grew at rates similar to those grown in diurnal conditions (data not shown). The experiment was started with two mother/daughter two-frond pairs of 3F7-11 or MTR1 per flask and cultures were grown for 14 days. For each treatment, $n = 4$ and error bars indicate standard error.

some component of the IAA uptake process may also be light dependent, in both the inbred and MTR1 lines (Fig. 3). This result is consistent with previous work on other plants where IAA transport, which presumably involves both export and uptake, can be influenced by light [9, 21–23]. IAA uptake under continuous light and during the light period of the diurnal cycle (D/L-light), was around 40% higher than either uptake in plants kept for 24 h in complete darkness, or uptake during the dark period of the diurnal cycle (D/L-dark).

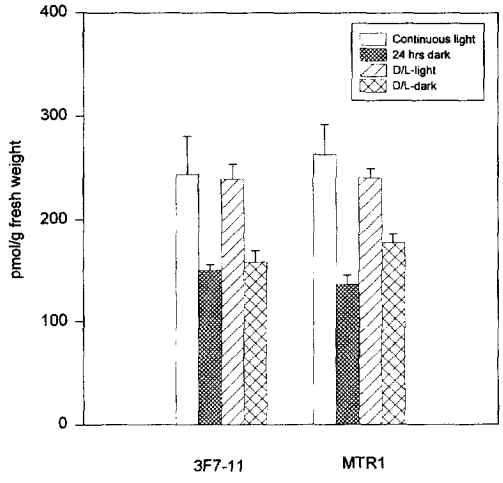

Fig. 3. Uptake of indole-3-acetic acid during a 1 h incubation by both normal (3F7-11) and resistant (MTR1) lines is shown for plants grown in different light conditions. For each treatment, $n = 4$ and error bars indicate standard error.

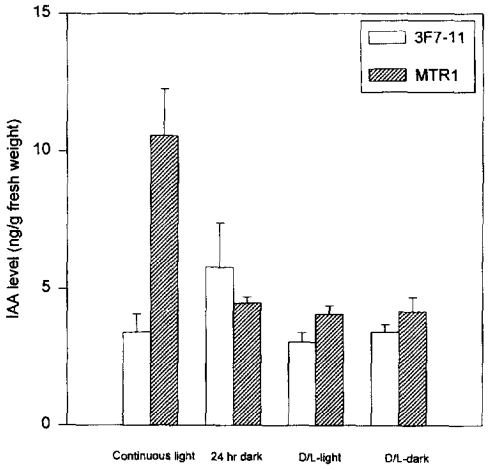

Fig. 4. The effect of light on free indole-3-acetic acid levels was determined for both normal (3F7-11) and resistant (MTR1) lines. Free IAA levels were determined by stable isotope technique and detected by GC-MS. For each treatment, $n = 4$ to 7 and error bars indicate standard error.

Effect of light on free IAA levels

Free IAA levels in 3F7-11 and MTR1 lines grown in the dark or in a diurnal light cycle followed by light or dark periods were all similar (Fig. 4). In continuous light, the free IAA level in 3F7-11 remained at the same low level as that found in the diurnal light treatments. However, MTR1 plants grown in continuous light had about twice the free IAA level as normal or MTR1 plants grown with any other light condition. This result may be related to hormone turnover rates, as described below.

Turnover of IAA in relation to light

In the MTR1 line, turnover of IAA was always relatively rapid, with a turnover time of less than 1 h under all four light conditions (Fig. 5). Normal (3F7-11) plants had about the same IAA turnover rate as the MTR1 line under all light treatments studied except under continuous light, where it had an 8× slower turnover rate of around 7.5 h. This result is consistent with the dark period being a determining factor in establishing the more rapid IAA turnover rate in the normal line.

The effect of light on IAA turnover in *Lemna* may be most closely related to an adaptation to continuous light, in that the dramatic change in $t_{1/2}$ for IAA in 3F7-11 was not seen in the diurnal experiments, but appeared only when plants were grown in continuous light. As noted above, growth under continuous light influences IAA turnover in 3F7-11, but does not

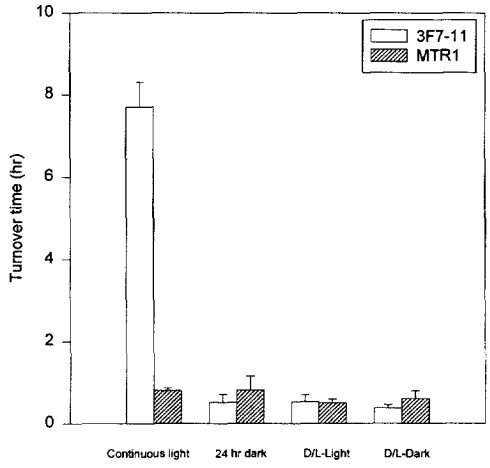

Fig. 5. Light affects turnover of IAA in normal (3F7-11) but not in resistant (MTR1) lines of *Lemna gibba*. For each treatment, $n = 8$ or 9 and error bars indicate standard error.

influence IAA turnover in MTR1 (Fig. 5). The apparent inability of MTR1 to down-regulate its rate of turnover under any light condition suggests that the elevated level of IAA precursors in these plants are "pushing" IAA turnover at constitutively high rates.

With 24 h dark treatment, free IAA in MTR1 is the same as in 3F7-11; however both normal and resistant lines had a high rate of IAA turnover (Figs 4 and 5). Higher levels of free IAA in continuous light grown MTR1 relative to levels in the 24 h dark treatment (Fig. 4), even when the $t_{1/2}$ for IAA remains constant, suggests that continuous light may decrease IAA degradation. A possible hypothesis that relates the differences in IAA levels to the changes in turnover rates can thus be proposed. If it is assumed that compared with 3F7-11, MTR1 cannot easily control IAA biosynthesis due to an excess level of precursors [11], then a dual effect of adaptation to continuous light on both synthesis and catabolism would have different metabolic consequences in the two lines. In the normal line, 3F7-11, both processes would be impeded in continuous light, thus decreasing the rate of turnover and causing the level of IAA in the tissues to be unaffected. However, in MTR1, where growth in continuous light may not be able to reduce the rate of synthesis while catabolism is reduced, the levels of IAA would increase.

While this hypothesis explains the data presented, additional studies on the effect of the light environment will be necessary to fully test this hypothesis. An understanding of the biochemical basis for these changes can only be obtained once the pathways for IAA metabolism are known and specific steps can be studied individually. Nevertheless, our study illustrates the importance of turnover measurements in understanding environmental effects on IAA metabolism and, as with many current studies [7, 24], illustrates the usefulness of appropriate genetic materials for metabolic studies. If our hypothesis is correct, turnover is the parameter of IAA metabolism that responds to continuous light since a different IAA turnover rate was shown in the normal line with different light conditions.

EXPERIMENTAL

Plant materials

A *Lemna gibba* inbred line, 3F7-11, and an α-methyltryptophan resistant line, MTR1, were used [11]. All cultures were started with two single mother and daughter frond pairs and were grown aseptically on liquid E medium [25] at 25° under continuous light which was provided by a mixture of cool-white fluorescent and incandescent lamps at 25 μmol m^{-2} s^{-1} [26].

For growth rate measurements, cultures were started with two mother and daughter frond pairs of 3F7-11 or MTR1 and were grown in 125 ml flasks in continuous light or under a 12 h light and 12 h dark photoperiod. Fr. wts and frond numbers were determined after 14 days.

Measurement of free IAA levels

About 0.5 g fr. wt of plants from cultures treated with each of the different light conditions was used for determination of free IAA levels. For 3F7-11, 0.5 g is about 120 fronds and for MTR1 it is about 140 fronds. [$^{13}C_6$]IAA was used as internal standard and [^{3}H]IAA as tracer for GC-MS analysis, as previously described [11].

IAA uptake and turnover rate

Lemna were grown as described above for determination of free IAA levels. *Lemna* fronds (1.5–3.0 g/dish) were incubated for 1 h with 20 ml of E medium supplemented with 60 kBq of [^{3}H]IAA (Amersham, 851 GBq/mmol) and 363 ng of [$^{13}C_6$]IAA in a plastic Petri dish (100 × 60 mm). Five 0.1-ml samples were taken from the isotope containing medium before incubation and four 0.1-ml samples were taken after incubation. The IAA uptake was determined from the decrease in radioactivity in the medium. After taking samples from the feeding soln for IAA uptake determinations, fronds were washed with sterile distilled H_2O to remove exogenous IAA, and about half of the fronds in each dish were harvested (t_0) by gently blotting the fronds dry, weighing and freezing in liquid N_2. The remaining fronds in each dish were transferred to fresh E medium without labeled IAA for an additional 1 h incubation, for determination of IAA turnover rate. After the second incubation period, samples were washed with sterile distilled H_2O and harvested (t_1) as above.

The turnover rate was determined by measuring the isotopic enrichment in the free IAA pool using GC-MS analysis [11]. The turnover time ($t_{1/2}$) was calculated based on the equation for a first-order reaction:

$$\ln C_0/C_t = k(t_1 - t_0)$$

$$t_{1/2} = \ln 2/k$$

where t is, in this experiment, 1 h, and C_0 and C_t are the enrichments in [$^{13}C_6$]IAA in the isolated IAA pool at 0 and 1 h time points, respectively.

24 h dark treatment

Cultures were grown in E medium at 25° under continuous light for 19 days, then placed in complete darkness for 24 h before feeding labeled IAA, as shown in Fig. 1. Plants were kept in the dark except during processing and handling, which was done with the aid of a dim, phototropically inactive, green safelight.

Diurnal light experiment

Cultures were grown in E medium at 25° under continuous light for 15 days, then transferred to a growth chamber (Environmental Growth Chamber Model Q6521) with a 12 h dark/light cycle at 25° for 5 days. Determination of IAA uptake rate and turnover time was started 3 h before the change in the light condition (i.e. 3 h before the end of the light period or 3 h before the end of the dark period). Processing and handling of samples from the dark period was done as for the 24 h dark treatment. Thus, for both continuous light and diurnal light treatments, plants were grown for a total of 20 days. Plants grown with diurnal light were handled differently for the growth rate experiments than for IAA uptake and IAA turnover experiments. In the growth rate experiments, cultures were started from two mother/daughter pairs at day 1, and the growth rate was expressed as the average increase of fr. wt and numbers of fronds per day after a 14 day diurnal light treatment.

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