

Light Mediates Accumulation of Lichen Phenolics in the *Evernia prunastri* Thallus

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Abstract

Lichen phenolics accumulate in the thalli of *Evernia prunastri* floated on acetate in the dark following a bimaximal temporal pattern. Red light pulses strongly increase the first accumulation of both atranorin and chloroatranorin but repress the second one. Far-red following red light depresses the amount of both phenolics as well as that of everninic and usnic acid even below the values found in thalli incubated in the dark. However, the second maximum, produced after 7–8 hr incubation, slightly diminishes after far-red light treatment. Accumulation of everninic acid seems to be more complicatedly regulated, also depending on the size of the pool of depside precursors, orsellinic and everninic acids.

Keywords: Lichen, lichen phenolics

Abbreviations: HIR — high irradiance reaction, LIR — low irradiance reaction,
P_{fr} — far-red absorbing form of phytochrome

1. Introduction

Phytochrome regulates the synthesis of many secondary compounds, including phenols as well as flavonoids (McClure, 1975). Other phenolics, such as those that form lignin, are also under phytochrome control (Camm and Towers, 1977). However, no attempt has been made to explain the action of light on the biosynthesis and accumulation of lichen phenolics on the basis of a photomorphogenetic process. In a general way, it has been supposed that accumulation of lichen phenolics in the light is related to the availability of acetyl-CoA derived from the fungal oxidation of photosynthates (Scott, 1971;

Mosbach, 1973). As a response to light, phenols can be secreted to the cortex, where they build a screen which absorbs the short wavelength radiation (Rundel, 1978) or retained in the medulla, where, as yet, their physiological function is unknown. Even the action of light has been simplified to a thermal effect on illuminated lichen thalli (Hamada, 1983).

Recently, some evidence about the role of phytochrome in the control of certain activities of lichens has been reported. Avalos and Vicente (1985) found that algal nitrate reductase is induced by P_{fr} , the far-red absorbing form of phytochrome, in *Evernia prunastri*, whereas the fungal enzyme seems to be independent of such a control mechanism. It is necessary to point out that although responses of damage protection of yeast cells against UV light seems to be related to phytochrome (Frainkin et al., 1973), the involvement of this photoreceptor in current fungal physiological processes is still discussed (Briggs, 1976; Gressel and Rau, 1983). Thus, it may be possible to consider photobionts as the sole (or main) source of phytochrome in lichens. However, it is generally accepted that lichen phenolics are fungal products (Mosbach, 1973). The function of the algal partner is reduced to the metabolic control of the amount of newly synthesized phenolics (Culberson and Ahmadjian, 1980).

On this basis, we attempt to probe whether the action of light on the accumulation of phenols in *Evernia prunastri* is mediated by phytochrome or other photoreceptors.

2. Materials and Methods

Plant material

Evernia prunastri (L.) Ach., growing on *Quercus pyrenaica* Lam. and collected in Valsain (Segovia, Spain) was used throughout this work. Thalli were dried in air at room temperature and stored at 7°C in the dark, in polythene bags until required, no longer than two weeks.

Incubation conditions

Samples of 1.0 g of air-dried thalli were floated on 10 mM sodium acetate in 75 mM phosphate buffer, pH 6.9, for 10 hr at 26°C in the dark. When indicated, each hour of incubation was started by 10 min red light or 10 min red followed by 10 min far-red light, with a photon flux rate of $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the level of plants. The light source was a 60W tungsten lamp. Red light was obtained by filtering white light through one layer of Cokin A003 (Chromofilter, France). Far-red light was obtained by filtering white light through one layer of Cokin A020 and one layer of Cokin A003. The pattern

of the spectral energy distribution of both red and far-red light was as has previously described (Avalos and Vicente, 1985).

Quantification of lichen phenolics

To identify lichen phenolics, lichen samples were dried with filter paper after incubation, macerated with methanol (HPLC grade) and centrifuged at $20000\times g$ for 30 min at 4°C . Supernatants were used to estimate lichen phenols. Quantitative determination of lichen phenolics was achieved by reverse-phase HPLC according to Legaz and Vicente (1983) by using a Varian 5000 liquid chromatograph equipped with a Vista CDS 401 computer. Chromatographic conditions were as follows: column, 300×4 mm i.d. packed with MicroPak MCH-10; mobile phase, acetic acid: water (2:98 v/v) methanol (20:80 v/v) (Legaz and Vicente, 1983); flow rate, 1.0 ml min^{-1} ; temperature, 20°C ; range of absorbance of the detector, 0.05; pressure, 115 atm; detector, UV set at 254 nm; internal standard, salicylic acid, 0.1 mg ml^{-1} ; external standard, evernic acid, 0.1 mg ml^{-1} .

3. Results

The time-course of accumulation of phenolics in *E. prunastri* thalli is biphasic in the dark. Two maxima are always found, one of them at 2–3 hr incubation and the other at 8 hr incubation. Red light pulses greatly enhance the first maximal value of both atranorin (Fig. 1) and chloroatranorin (Fig. 2) concentration in the thallus but this effect is completely reversed by far-red light pulses even at values below those found in the dark control. The enhancement effect is clearly diminished for D-usnic acid although the action of far-red light persists (Fig. 3). However, the second maximum of phenol accumulation found at 8 hr incubation is not enhanced by red light over the values obtained in the dark. Far-red light following red light pulses enhances phenols accumulation although the amount of these compounds produced in this way is also lower than that found in the dark (Figs. 1–4).

Some peculiarities have been observed about the time-course of evernic acid production (Fig. 4). At 2 hr incubation, evernic acid accumulates in the dark at a level higher than that obtained after red light pulses. This

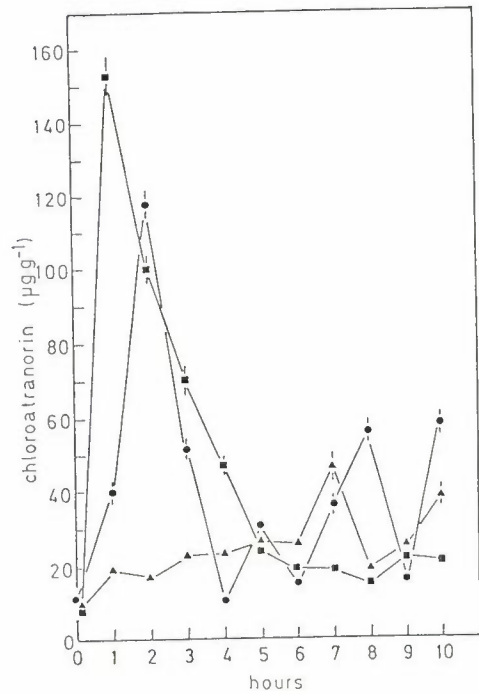
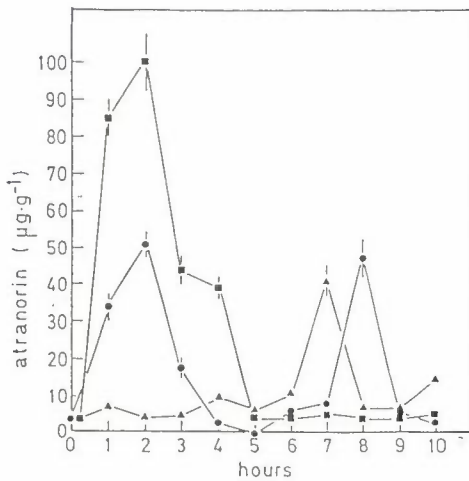
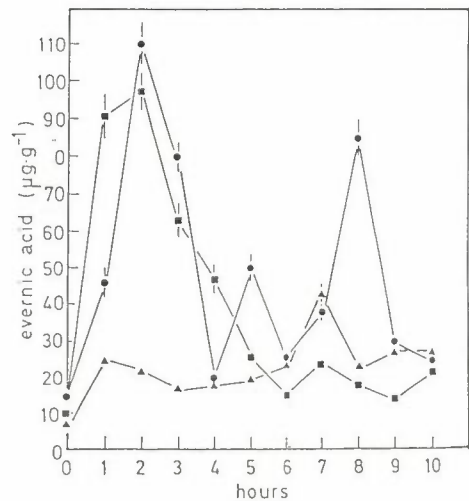
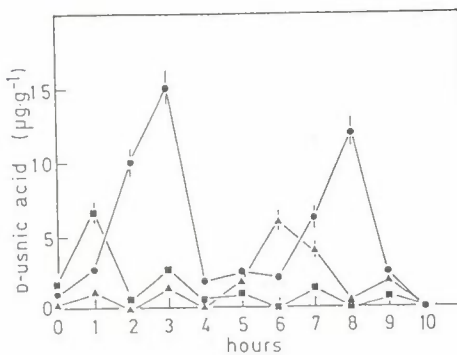


Figure 1. Time-course of atranorin accumulation in *Evernia prunastri* thalli floated on 10 mM sodium acetate in the dark (●), after red light pulses (■) or red + far-red light (▲) pulses at the start of each hour of incubation. Values are the mean of three replicates. Vertical bars give standard error where larger than the symbols.

Figure 2. Time-course of chloroatranorin accumulation in lichen thalli floated on 10 mM acetate. Symbols as in Fig. 1. Values are the mean of three replicates. Vertical bars give standard error where larger than the symbols.



Figures 3-4. Time-course of D-usnic acid and evernicin acid accumulation in *E. prunastri* thalli floated on 10 mM acetate. Symbols as in Fig. 1. Values are the mean of three replicates. Vertical bars give standard error where larger than the symbols.

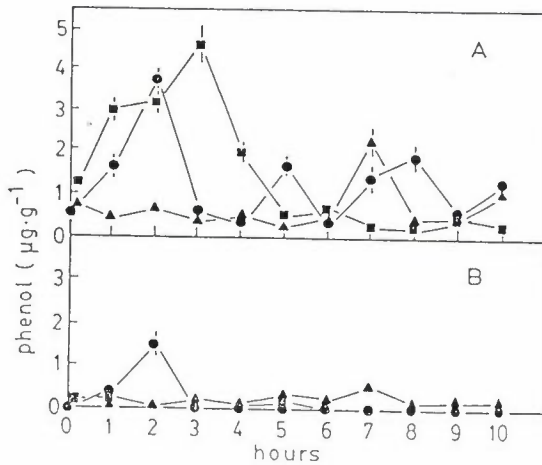


Figure 5. Time-course of both orsellinic (A) and evernicic (B) acids accumulation in lichen thalli floated on 10 mM acetate. Symbols as in Fig. 1. Values are the mean of three replicates. Vertical bars give standard error where larger than the symbols.

Some peculiarities have been observed about the time-course of evernicic acid production (Fig. 4). At 2 hr incubation, evernicic acid accumulates in the dark at a level higher than that obtained after red light pulses. This difference, when compared with the other phenolics, must be related to the distinct behaviour in the time-course of evernicic acid precursors, orsellinic and evernicic acids, shown in Fig. 5. Whereas the first phenol, orsellinic acid, shows the current bimaximal response, one of them induced by red light pulses at 3 hr incubation, the second one at 8 hr in the dark (Fig. 5A), evernicic acid is practically undetectable during the entire incubation time (Fig. 5B). Thus, it is possible that lower enhancement of evernicic acid production by red light could be explained by the limiting concentration of evernicic acid, which is not affected by red light.

4. Discussion

A two step response of phenol accumulation has been found in *E. prunastri* thallus using acetate as a precursor. When lichen thalli are incubated in the dark, phenolics are accumulated at 2 hr incubation and, later also at 8 hr. Synthesis of lichen depsides first requires the production of orsellinic acid catalyzed by an orsellinate synthase (Gaucher and Shepherd, 1968). Orsellinic acid is then transformed to evernicic acid after methylation. However,

orsellinic acid must also be transformed to methyl β -orcinol carboxylate (Vicente et al., 1984) to be used in the synthesis of both atranorin and chloroatranorin. This implies that the size of the pool of evernic acid (Fig. 5) acts as a limiting factor of evernic acid production. Thus, the last monocyclic phenol is exhausted when the orsellinate precursor is used to synthesize either evernic acid or methyl β -orcinol carboxylate (Figs. 1,2,4 and 5). The accumulation of D-usnic acid is not connected with orsellinic acid because methylphloroacetophenone, the natural precursor of usnic acids, is not produced through orsellinic, but by phloroglucinolic cyclasation (Culberson, 1969; Taguchi et al., 1969).

The use of a presumably sole pool of orsellinic acid to synthesize three different depsides in *E. prunastri* can explain that evernic acid accumulation does not respond to enhancement effect of red light, whereas both atranorin and chloroatranorin accumulation is strongly increased, under the same conditions, in short time periods (Figs. 1, 2 and 4). The first peak of evernic acid concentration is, therefore, lower after red light pulses than that obtained in the dark because of the accelerated synthesis of β -orcinol depsides.

Enhancement of phenols accumulation by red light could suggest phytochrome as an effector but red/far-red reversibility is not near to the level of the dark control. However, red light can act in a mixed control way. Algal phytochrome can regulate ribulose-1,5-biP carboxylase activity (Mohr and Kasemir, 1976) and, then, far-red following red light pulses diminish the amount of carbohydrates translocated from algal to fungal cells in short time periods. In this way, the supply of immediately metabolizable monosaccharides to the mycobiont to produce phenol precursors is levelled off. This action can be reduced after repeated red light pulses, and then low amounts of phenols can be produced at long time periods. However, far-red light might also inhibit remobilization of stored carbohydrates to explain why red/far-red treatments produce amounts of phenols lower than that observed in the dark.

If phytochrome control exists, it must be exerted from algal cells since we have found that mycobiont cells, isolated from lichen thalli by gradient centrifugation (Ascaso, 1980), do not show any absorbance variation after red or red + far-red light treatments. This is in agreement with the lack of reverse reaction on nitrate reductase activity when fungal preparations are used (Avalos and Vicente, 1985).

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