Photostimulated oxygen uptake in *Trichoderma viride*

ZDENA SULOVÁ,* MÁRIA HRMOVÁ and VLADIMÍR FARKAŠ

Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Czechoslovakia

(Received 15 May 1990; revised 23 July 1990; accepted 3 August 1990)

Exposure of dark-grown mycelia of *Trichoderma viride* to white light elicited a transient burst of respiratory activity manifested as increased O$_2$ consumption which was not paralleled by a corresponding increase in CO$_2$ production. The period of increased uptake of O$_2$ lasted for 5–10 min and was independent of the duration of illumination. The inhibitors of respiration tested, antimycin A and mucidin, and the antioxidant, dithiothreitol, effectively suppressed the photostimulated uptake of O$_2$, whereas rotenone, amytal and salicylhydroxamic acid were without effect. It is concluded that the illumination of mycelia caused irreversible photo-oxidation of an as yet unidentified compound, and that the electrons released by the photochemical event were accepted by a NAD-independent flavin dehydrogenase and further transferred to atmospheric O$_2$ via the cytochrome electron-transport chain coupled with the formation of ATP.

**Introduction**

Photoresponses in fungi include diverse processes such as changes of metabolism, inhibition or stimulation of growth, pigment synthesis, tropism, morphogenesis and differentiation (Gressel & Rau, 1983; Gressel & Horwitz, 1982; Rau, 1980; Dahlberg & Van Etten, 1982). In the soil saprophytic fungus *Trichoderma viride* a brief illumination of dark-grown colonies induces conidiation. Under experimental conditions, a ring of conidiophores with dark green or brown conidia is formed about 24–36 h after the illumination, in places that were at the growth perimeter of the colony at the time of irradiation (Gressel & Rau, 1983; Betina, 1985). Thus, after the fungus is transferred from dark to light, the light signal triggers in vegetative mycelia the formation of dormant spores, which can resist extreme environmental conditions. Simultaneously, emergence of the fungus on the soil surface makes possible effective multiplication by dissemination of conidia.

O$_2$ seems to play an important, as yet unexplained role in the process of photoinduced conidiation. It has been observed (Gressel *et al.*, 1975; and our unpublished results) that the photochemical event itself and the conidiation can be artificially separated. While the photoinduction can take place anaerobically, the presence of O$_2$ is essential for the conidiation to proceed.

Here we describe an investigation of the effect of illumination on O$_2$ consumption by dark-grown mycelia of *T. viride* and the effect of respiratory inhibitors on this process, in an attempt to elucidate the role of the respiratory chain in the transient burst of respiratory activity induced by light.

**Methods**

*Trichoderma viride* strain CCM F-534, from the Czechoslovak Collection of Micro-organisms, T. G. Masaryk University, Brno, Czechoslovakia, was grown in the dark at 30 °C on Whatman GF/A glass-fibre paper rings (3 cm in diameter with a 0.5 cm central hole) laid on the surface of agar medium, pH 5.5, containing, per litre: glucose, 20.0 g; yeast extract (Difco), 3.0 g; KH$_2$PO$_4$, 2.0 g; and agar 20.0 g. Respirometric measurements were performed in a standard Warburg manometric apparatus at 30°C. The manometric vessels contained 2.5 ml of the agar medium at the bottom, with or without inhibitors. The rings with the dark-grown mycelia were placed on the surface of the agar in the manometric vessels under the red safelight and preincubated in the dark at 30 °C for 3 h. After equilibration, the exchange of gases was recorded for the initial 60–80 min in the dark. The vessels were then illuminated by light generated by white fluorescent lamps (1.5 klux intensity) and the exchange of gases was recorded for another 50–60 min. The rates of O$_2$ uptake and CO$_2$ production were related to the amount of mycelial protein in each individual vessel as determined by the Lowry method after alkali extraction of the mycelia.

**Results and Discussion**

Since in our strain of *T. viride*, conidiation could be induced by light only in those mycelia that were in immediate contact with air (Farkaš *et al.*, 1984), the...
Warburg manometric technique proved to be the most suitable method for measuring the gas exchange in cultures grown on the surface of solid medium. Exposure of the dark-grown mycelia to light elicited within seconds a dramatic transient increase of the rate of O₂ consumption, whereas the rate of production of CO₂ remained similar to that of the dark-grown mycelia. The 'respiratory burst' took place for a short period of 5–10 min, after which the rate of O₂ consumption by mycelia returned to its original value. The period of increased O₂ consumption was independent of the duration of the exposure to light. When, after the first 10 min exposure, the mycelia were illuminated again after 2 h in the dark, the effect of the illumination on the rate of O₂ consumption was much less pronounced (Fig. 1a).

To our knowledge, this is the first direct evidence that light stimulates O₂ uptake in a fungus. The stimulation of respiration by light has been previously observed in other systems, e.g. in chlorophyll-less and colourless mutants of *Chlorella* (Kowalik & Gaffron, 1967; Schmid & Schwarze, 1969). In the latter cases, however, it was the metabolism-coupled respiration that was stimulated by light, since the increased O₂ consumption was paralleled by an increased CO₂ production. Other differences from our system were that in *Chlorella*, there was a 10–20 min lag period between illumination and the onset of increased respiration and that the stimulated respiration persisted for a relatively long time under the continuous light. The authors explained this effect as being due to increased amino acid oxidation by an FMN-dependent oxidase (Schmid & Schwarze, 1969).

Previously we have found that rotenone, antimycin A, cyanide and the antioxidant dithiothreitol (DTT) were effective inhibitors of conidiation and growth in *T.*

---

**Fig. 1.** Effect of illumination on the rate of CO₂ production and O₂ consumption in mycelia of *T. viride*. At the times indicated by arrows the mycelia were exposed to light for 10 min. (a) No inhibitors present. ■, Illuminated mycelia; ○, dark control. (b) ●, Control without inhibitor; △, in the presence of 50 μM-rotenone. (c) ●, Control without inhibitor; △, in the presence of 20 μM-antimycin A. (d) ○, Control without inhibitor; △, in the presence of 1 mM-DTT.
viride. Of these compounds, antimycin A, cyanide and DTT at an appropriate concentration showed a greater inhibitory effect on conidiation than on the growth whereas rotenone inhibited growth more than conidiation (Z. Sulová & V. Farkaš, unpublished).

Both antimycin A (Fig. 1c) and DTT (Fig. 1d) prevented the increase in the rate of O\textsubscript{2} consumption caused by the illumination. The same effect has been found with the mucidin (data not shown). On the other hand, rotenone and amytal, which block transfer of electrons from NADH to coenzyme Q (see Fig. 2) in the respiratory chain, were without effect on the light-induced stimulation of respiration (Fig. 1b). SHAM, which inhibits the alternative respiratory pathway in yeasts and fungi (Alexander & Jeffries, 1990), was also ineffective in suppressing the photoinduced 'respiratory burst' (not shown).

Several investigations on photoreception in fungi indicate that in many species, including T. viride, the pigment involved in photoreception is a flavin (Betina, 1985; Horwitz et al., 1984). In Neurospora crassa it was shown that the illumination causes a flavin-mediated reduction of cytochrome \(b\) (Muńoz et al., 1974; Muńoz & Butler, 1975), thus indicating the participation of the cytochrome respiratory chain in the transduction of the light signal. At present, the identity of the compound yielding electrons upon illumination is unknown. Nevertheless, the transient nature of the light effect upon respiration indicates that the electron-yielding compound is present in the cells only in a limited amount and that its oxidation during the photochemical event is irreversible. In this context it would be of interest to reconstitute the system \textit{in vitro} using various subcellular fractions and other substitutes to investigate the existence, function and localization of a hypothetical electron-yielding redox compound. The existence of such a compound \((X)\) participating in the process of photoinduced carotenogenesis in Fusarium was proposed by Rau (1980).

The inability of rotenone, amytal and SHAM to suppress the light-induced 'respiratory burst' indicates that the electrons released by the photochemical event are funnelled into the respiratory chain at the coenzyme Q site and that the SHAM-sensitive alternative respiration does not play a role. On the other hand, simultaneous addition of cyanide and SHAM prevented the light-induced 'respiratory burst'; hence, the normal respiratory pathway is involved (see Fig. 2).

Under normal conditions, the transfer of electrons via the cytochrome pathway is accompanied by the production of ATP at the phosphorylation sites II and III (Fig. 2). For this reason, it can be expected that the light-stimulated respiration would cause an increased production of ATP. This assumption is strongly supported by our previous observation that the illumination of dark-grown mycelia of T. viride causes an approximately 100% increase in the intracellular level of ATP (Farkaš et al., 1984; Grešík et al., 1988). Thus it seems highly probable that in T. viride the electrons released by the photochemical event are used to drive oxidative phosphorylation during the short period of photoinduction.

Fig. 2. The respiratory chain of T. viride, showing inhibition sites (bold arrows) and phosphorylation sites I II III.

References


2. Sulové, M., Hrmové and V. Farkaš


