Effect of Light on the Concentration of Adenine Nucleotides in 
Trichoderma viride

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Dark-grown colonies of the fungus Trichoderma viride exhibited an immediate transient increase 
(by about 100%) of the intracellular concentration of ATP when exposed to white light. ATP 
reached its maximum concentration after about 15 min and returned to its original value after 
about 30 min after the onset of illumination, whether the illumination was discontinued or not. 
On the other hand, only a slight decrease in the concentration of ADP, and practically no change 
in the concentration of AMP in the illuminated mycelia were observed. The increase of the ATP 
concentration in mycelia was reflected in the rise in concentration of total adenylates and in the 
transient increase of adenylate energy charge from 0.66 to 0.77. Randomly chosen non- 
sporulating mutants of the same strain of T. viride did not show any significant changes in the 
concentrations of adenine nucleotides after exposure to light.

INTRODUCTION

Many fungi in response to light initiate sporulation (Tan, 1978; Dahlberg & Van Etten, 1982) 
and Trichoderma viride is one of the best studied organisms in this respect (e.g. Bjornsson, 1959; 
Galun & Gressel, 1966; Betina, 1984). Conidiation in Trichoderma does not have circadian 
character and the concentric zones of sporulation observed on the colonies correspond to the 
alternations of light and dark periods (Betina & Zajacova, 1978). The wavelength maxima 
effective in the induction of sporulation in T. viride lie in the near-ultraviolet and blue region of 

Photoinduced sporulation represents an advantageous model to study biochemical aspects of 
differentiation in fungi since a simple natural effector, such as light, devoid of side-effects, is 
used to initiate a series of metabolic changes leading to sporulation. At present, however, the 
nature of these metabolic changes remains largely unknown.

In preliminary studies, so far not published in extenso (Farkaš & Betina, 1977), we have 
observed an increased concentration of ATP in illuminated mycelia of Trichoderma viride. In 
this paper we describe changes in the concentrations of the adenine nucleotides, ATP, ADP and 
AMP, as one of the primary consequences of exposure of mycelia of T. viride to light.

METHODS

Organism. Trichoderma viride Persoon ex S. F. Gray, strain No. F-534 from the Czechoslovak Collection of 
Microorganisms (J. E. Purkyně University, Brno) and its non-sporulating mutants M4, M6 and M8 were obtained 
from Professor V. Betina (Slovak Polytechnical University, Bratislava). The strains were maintained on potato 
extract/2% (w/v) glucose agar slants at 4 °C and subcultured at monthly intervals.

Cultivation. Conidia from 7-d-old cultures grown at room temperature were suspended in 0.1 
% Tween 80 and 
filtered through glass wool. The concentration of conidia was adjusted to 10⁶ ml⁻¹ and from this suspension 10 μl 
were pipetted onto the centres of glass fibre filter discs (Whatman GF/A, 25 mm diameter) layered on the surface 
of 2% (w/v) agar medium containing yeast extract (Oxoid), 3.0 g; KH₂PO₄, 2.0 g; MgSO₄·7H₂O, 2.0 g; and 
glucose, 20.0 g l⁻¹. After autoclaving, the pH of the medium was 5.5. The Petri dishes (20 cm in diameter) with the
operations requiring darkness were performed under a red safelight. The diameter of the colonies on the discs was about 20 mm. Smaller colonies were usually not competent for photoinduction. Non-conidiating mutants were inoculated on the discs as pieces of mycelia removed with a cork-borer from the mother colonies.

**Photoinduction.** Petri dishes with grown colonies were transferred to a dark-room and after 2 h, when their temperature was equilibrated with that of the surrounding air (25 °C), they were illuminated under a fluorescent lamp by a white light of 1.2 klx intensity for the indicated times. The control plates remained in the dark and all operations requiring darkness were performed under a red safelight.

**Sampling and extraction of adenine nucleotides.** At time intervals, before and after illumination, the discs with mycelial colonies were removed in triplicate from the surface of the agar medium and immediately placed in tubes containing 2 ml of prewarmed (96 °C) water and immersed in a boiling water bath for 10 min. The tubes with the extracted mycelia were kept at −20 °C for further analysis. According to preliminary tests, the extraction of adenine nucleotides with boiling water was as efficient as the use of dilute mineral acids.

**Reagents.** Standards of ATP, ADP and AMP as appropriate sodium salts, and desiccated firefly lanterns used for the preparation of crude luciferase (Stanley & Williams, 1969) were purchased from Sigma. Pyruvate kinase and myokinase were from Boehringer. The enzymes pyruvate kinase and myokinase were purified from low molecular weight compounds before use by chromatography through Sephadex G-25, as described by Carver & Walker (1983). All other reagents were of analytical grade, obtained from Lachema (Brno, Czechoslovakia).

**Analytical methods.** The concentrations of ATP, ADP and AMP in hot-water extracts from the mycelia were determined by the luciferin-luciferase assay essentially according to Carver & Walker (1983) using a liquid scintillation counter (Packard Tri-Carb 3330) for measurement of the intensity of light emission (Stanley & Williams, 1969). To assess the percentage recovery, known amounts of ATP were added to mycelia as internal standards before extraction. On average, the recovery was 94%, which value was used as the correction factor for calculating nucleotide concentrations. All determinations were performed in duplicate. Proteins in hot-water and/or alkali (1 M-NaOH) extracts from mycelia were determined with the Folin phenol reagent using bovine serum albumin as the standard; extracts from non-inoculated filter discs that were placed on the surface of nutrient agar during the growth of mycelia were used as blanks.

**RESULTS AND DISCUSSION**

The dark-grown colonies of *T. viride* exhibited a sudden increase in the concentration of intracellular ATP when exposed to light for a period of 10 min (Fig. 1a). The ATP concentration reached its maximum after about 15 min from the onset of illumination and returned to its original value after about 30 min. In the non-illuminated dark controls the concentration of ATP remained more or less constant during the whole experiment. Repeated illumination after the period of 2 h did not cause any further significant changes of the concentration of ATP in the mycelium. The same pattern as depicted in Fig. 1 was obtained when the mycelium was exposed to continuous light. Concurrently, the concentration of ADP decreased slightly, and practically no changes were observed in the concentration of AMP. At present, we are unable to explain why the increase of ATP concentration is not mirrored by an equivalent decrease of the ADP concentration, so that the sum of concentrations of the measured adenine nucleotides transiently increases (Fig. 1b). One possible explanation may be that the increased concentration of adenylates arises at the expense of rapidly decaying species of polynucleotides or mRNAs. The value of the total adenylate energy charge, (ATP + 0.5 ADP)/(ATP + ADP + AMP) calculated according to Atkinson & Walton (1967) increased transiently from 0.66 to 0.77 as a consequence of light-stimulated ATP accumulation.

It is necessary to stress here that the described changes in nucleotide pools were observed only with mycelia directly exposed to air. We did not detect any significant response to illumination in stirred, submerged cultures of *T. viride*. The technique of cultivation of mycelia on uniformly inoculated filter discs enabled us to remove multiple samples of mycelium of essentially equal amounts of biomass in a relatively short time.

The randomly chosen non-conidiating mutants M4, M6 and M8 did not respond to photostimulation at all: they had steady concentrations of adenine nucleotides during the whole experiment, regardless of whether the illumination was periodic or continuous (results not shown). It is possible that they lack or are defective in the photoreceptor or some other vital function which transduces the light stimulus to the level of the ATP generating system.
Light and adenine nucleotides in *T. viride*

Fig. 1. Changes of nucleotide pools following the illumination of dark-grown colonies of *T. viride*. (a) ATP (○), ADP (●) and AMP (△) concentrations. (b) Adenylate energy charge (■) and total adenylates (□). The bar indicates the period of illumination.

The explanation for the observed photostimulated increase of the ATP concentration in *T. viride* is, at this stage of the work, purely speculative. By analogy with *Neurospora crassa* (Muñoz et al., 1974; Paietta & Sargent, 1983), the photoreceptor could be a flavin or a flavoprotein (Horowitz & Gressel, 1983). If, as suggested by Muñoz et al. (1974), the light causes flavin-mediated reduction of cytochrome *b*, the oxidation of the latter coupled with phosphorylation should lead to an increase of the intracellular ATP content, as has indeed been observed in our experiments. It is difficult to explain, however, why the response of the ATP concentration to illumination is not repetitive. It is probable that the light induces irreversible metabolic changes in the organism so that repeated or continued illumination of the mycelium has no further effect. This would imply that the light stimulus is effective only once per life-cycle. Apparently, the sporulation-competent cells, having obtained the necessary light dose, switch their metabolism ultimately towards sporulation. Revealing the complex nature of these changes represents an interesting challenge for future research.

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REFERENCES


