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Light-stimulated phosphorylation of proteins in cell-free extracts from *Trichoderma viride*

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When illuminated by visible light, cell-free extracts from the fungus *Trichoderma viride* catalysed the phosphorylation of at least two proteins with molecular masses of 18 and 114 kDa which were practically absent when the phosphorylation was performed in the dark. The effect of light could be substituted by 3mM cyclic AMP, not only in the cell-free extract, but also in the separated cytosol. It is concluded that the process of photoinduced conidiation in *Trichoderma* involves phosphorylation of conidiation-specific proteins by (a) cyclic AMP-dependent protein kinase(s) present in the cytosol.

Protein phosphorylation; Photoinduced conidiation; cyclic AMP; (*Trichoderma viride*)

1. INTRODUCTION

One of the diverse effects of light in some fungi is the induction of conidiation [1,2]. In *Trichoderma viride* short pulses of visible or UV light induce conidiation in colonies that had been grown in the dark [3]. The process of conidiation, which may be considered to be the termination of the fungal life cycle, involves the formation of aerial mycelium and conidiphores bearing at their ends initially white, but later dark-green, conidia. The biochemical pathways leading from the reception of light signals to microscopically and macroscopically observable morphological changes and differentiation remain largely unknown. Previously, we have found that illumination of dark-grown mycelia of *T. viride* induces rapid transient changes in the intracellular levels of ATP, cyclic AMP (cAMP), and the membrane potential [4,5].

In this paper we show that light stimulates phosphorylation of at least two protein species of 18 and 114 kDa in cell-free extracts from *T. viride* and that the effect of illumination could be largely substituted by addition of 3 mM cAMP.

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2. MATERIALS AND METHODS

2.1. *Fungus*

Trichoderma viride Person ex S.F. Gray, strain no. F-534 from the Czechoslovak Collection of Microorganisms (J.E. Purkyně University, Brno), was used. The fungus was maintained on agar slants containing salts, glucose and yeast autolysate [5].

2.2. *Photoinduction*

Photoinduction of the mycelium was performed as described previously [4].

2.3. *Preparation of membrane and cytosolic fractions*

The membrane and cytosolic fractions from mycelia of *T. viride* were prepared from 24-h-old cultures grown in shake flasks on basal medium 5 at 28°C. Mycelia were filtered through a nylon cloth and washed with 20 mM Tris-HCl buffer, pH 7.5, containing 3 mM $MgCl_2$ and 1 mM EGTA (buffer A). The washed mycelia were mixed with buffer A containing 1 mM dithiothreitol, 1 mM phenylmethylsulphonyl fluoride (buffer B) and the suspension was homogenized with Ballotini beads no.8 for 15 min in a rotary disintegrator immersed in an ice bath. All subsequent steps were performed at 4°C. The homogenate was filtered through a nylon filter. The filtrate was centrifuged at 7000 × *g* for 20 min. The supernatant was used

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as cell-free extract. One half of the supernatant was centrifuged at 100000 × *g* for 1 h. The supernatant was used as a cytosolic fraction and the sediment (membrane fraction) was resuspended in buffer B.

2.4. *Phosphorylation of membrane and cytosolic proteins*

50 μl cell-free extract or its fraction (i.e. membranes or cytosol) containing 0.1–0.2 mg proteins were incubated with 0.37 MBq 5-[γ-^{32}P]ATP (1.11×10^{11} Bq/mmol) and with 10 mM NaF in a total volume of 55 μl at room temperature for 30 s in the dark or under 40 W white fluorescent lamp. The reaction was stopped by addition of 100 μl electrophoresis sample buffer containing 20 mM Tris-HCl (pH 7.8), 6% SDS, 6% β-mercaptoethanol, 45% glycerol and 0.006% bromophenol blue tracking dye followed by heating at 100°C for 2 min. The proteins were separated by SDS-polyacrylamide gel electrophoresis [6]. The gels were stained with Coomassie brilliant blue R-250 and destained with 7% acetic acid containing 10% methanol. The ^{32}P-labeled phosphoproteins in dried stained gels were detected by autoradiography with Kodak X-Omat SO 282 films.

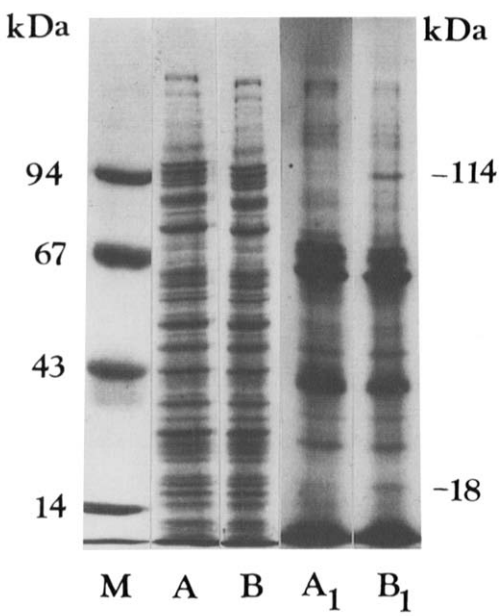

Fig.1. Light-induced phosphorylation of proteins in cell-free extracts from *Trichoderma viride* incubated with [γ-^{32}P]ATP. Lanes A and B are Coomassie-stained SDS-PAGE electrophoretograms of dark control and illuminated reaction mixtures, respectively. Lanes A_1 and B_1 are corresponding autoradiograms. Lane M shows the molecular mass markers. It should be noted that although all lanes in the figure are from the same gel, the Coomassie-stained lanes are presented at slightly different magnification than the autoradiograms. The molecular masses of labeled proteins were determined from the original gel. Proteins of 18 kDa and 114 kDa whose phosphorylation was stimulated by light are marked on the right.

3. RESULTS AND DISCUSSION

Phosphorylation of proteins is a powerful tool in regulation of various biochemical and physiological processes in the cells [7]. The enzymes responsible for this activity are protein kinases, either activated by Ca^{2+}-calmodulin or by cAMP [8].

Since we previously observed that illumination of dark-grown mycelia of *T. viride* is accompanied by the rapid transient increase of the intracellular concentrations of ATP and cAMP [5], we supposed that, as a consequence, phosphorylation of some proteins may occur.

Due to methodological complications occurring during work with the whole mycelium, we decided to follow the effect of light on the phosphorylation of proteins in vitro in the cell-free extracts, using [γ-^{32}P]ATP as the donor of phosphate. As can be seen in fig.1, illumination stimulated phosphorylation of at least two protein species with respective molecular masses of 18 and 114 kDa. The corresponding phosphorylated protein species were

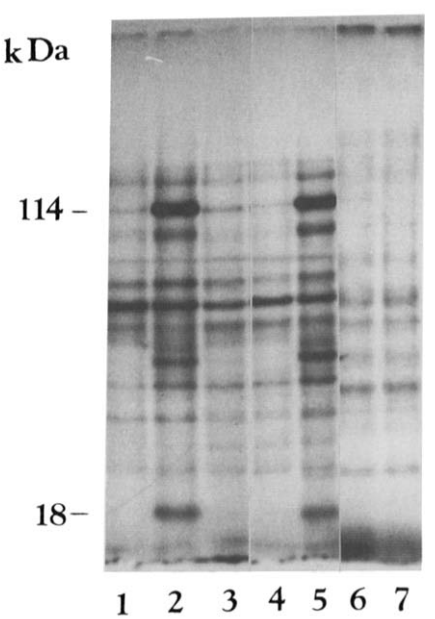

Fig.2. Autoradiograms of dark-incubated phosphorylation mixtures after SDS-PAGE electrophoresis showing the effect of cAMP and Ca^{2+}. Lanes: 1,2, crude extract; 3–5, cytosol; 6,7, membranes; 1, control (no additions); 2, plus 3 mM cAMP; 3, control; 4, plus 10 mM $CaCl_2$; 5, plus 3 mM cAMP; 6, control; 7, plus 3 mM cAMP.

almost undetectable in the reaction mixture that had been kept in the dark.

There are several protein kinases described in fungi, such as cAMP-dependent protein kinase [9,10], Ca^{2+}-calmodulin-activated protein kinase [11] and the one which is dependent on Ca^{2+} and phospholipids [12]. In order to ascertain which of these protein kinases is involved in the light-induced phosphorylation of proteins, we tried to substitute the light effect by 3 mM cAMP or calcium. As shown in fig.2, cAMP was able to mimic the effect of light by stimulating the phosphorylation of a whole spectrum of proteins including those of 18 and 114 kDa, not only in the cell-free extract, but also in the separated cytosol. On the other hand, Ca^{2+} at 10 mM inhibited the phosphorylation of all proteins. The separated membrane fraction was also capable of phosphorylating some of its endogenous proteins, however, neither light, nor 3 mM cAMP had any effect on their pattern.

The ability of cAMP to stimulate the phosphorylation of 18 and 114 kDa proteins, not only in the cell-free extract, but also in the cytosol prove that the cAMP-dependent protein kinase(s) and the corresponding protein substrates are of the cytosolic origin. The need for the presence of membranes for the light-stimulated phosphorylation to occur in the cell-free extracts may be explained by the assumption that the generator of cAMP, i.e. adenylate cyclase which is present in the membrane fractions is actually stimulated by light. Our preliminary results seem to support this assumption.

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