FEBS Letters 370 (1995) 146–148 FEBS 15892

A photoreceptor with characteristics of phytochrome triggers sporulation in the true slime mould *Physarum polycephalum*

Christine Starostzik*, Wolfgang Marwan

Max-Planck-Institut für Biochemie, 82152 Martinsried, Germany

Received 7 June 1995; revised version received 6 July 1995

Abstract Phytochrome is a ubiquitous photoreceptor in plants that controls a variety of responses to light, including gene expression, differential cell growth and intracellular movement of organelles. All phytochromes analysed so far are reversibly interconverted by light between an inactive and an active conformation, each of which has a different and characteristic absorbance spectrum. Based on photophysiological measurements we provide evidence, that a photoreceptor with these unique properties of phytochrome triggers sporulation in the true slime mould *Physarum polycephalum*.

Key words: Phytochrome; Photomorphogenesis; Sporulation; Signal transduction; Slime mould

1. Introduction

Plasmodia of *Physarum polycephalum* are giant cells that contain many thousands of nuclei, exhibiting perfect synchrony with respect to cell cycle and differentiation [1]. Naturally, *P. polycephalum* feeds on bacteria and other micro-organisms and continues cell growth as long as nutrition is available. Starving plasmodia take a developmental decision: either they encapsulate by forming macrocysts or, alternatively, the plasmodial mass differentiates to form sporangia each of which contains hundreds of spores, allowing the mould to spread. Visible light induces sporulation and inhibits macrocyst formation [2]. Although the effectiveness of blue and red light has been reported qualitatively [3], no action spectrum has been worked out.

2. Materials and methods

The albino strain LU897 × LU898 [4] was grown in the dark as microplasmodia in axenic shaken cultures [5]. After 4 days of growth at 26°C the plasmodial mass was harvested and transferred to starvation agar [6] plates (4.5 cm ∅) as described [7]. Plasmodia were starved for six days in complete darkness at 22°C to obtain competent specimens. Plasmodia were exclusively handled in complete darkness if not indicated otherwise. Irradiation was performed on a thermostated stage at 22°C. Light of a 450 W xenon lamp (Leica, Wetzlar, Germany) was filtered through bandpass and interference filters with 10–20 nm half-bandwidth (Schott, Mainz, Germany) and the monochromatic beam passed through an array of beam splitters (Spindler & Hoyer, Göttingen, Germany). This arragement allowed a batch of plasmodia to be simultaneously irradiated with different intensities of monochromatic light to give a fluence response curve out of three experiments. The setup was crucial for managing the many plates that had to be irradiated. Plasmodia were returned to the dark after a one hour exposure. The percentage of sporulated plasmodia was estimated on the next day.

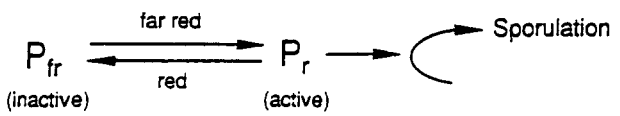
*Corresponding author. Fax: (49) (89) 8578-3777.

3. Results and discussion

Plasmodia of the albino strain LU897 × LU898 [4] were starved on minimal agar plates for six days in complete darkness to give competent specimens. When irradiated with a pulse of monochromatic light, sporulation of a plasmodium is an all-or-none response, i.e. the entire plasmodial mass is converted into sporangia if induction was sufficient. The percentage of sporulated plasmodia was a function of light intensity. Blue and far-red light were most effective in a sense that all plasmodia sporulated if enough light was applied. At other wavelengths, however, only part of the plasmodia sporulated even under saturating irradiation (Fig. 1). This phenomenon of wavelength-dependent saturation levels of stimulus–response curves in general can be the result of alternative molecular mechanisms: (1) a photochromic photoreceptor, the signalling state of which is formed by light of one wavelength and destroyed by another; or (2) two separate, antagonistically acting photoreceptor molecules, one activating, the other repressing sporulation.

When plasmodia were irradiated with far-red light followed by a red light exposure, no response was obtained. In contrast all plasmodia sporulated, if the sequence of irradiation was inverted. Red light was active in inhibiting sporulation only if far-red light has been applied before (Fig. 2). This result clearly suggests that a red light and a far-red light-absorbing intermediate are reversibly interconverted upon irradiation as it is the case in the plant photoreceptor phytochrome [8,9].

Based on the kinetic minimal model of a photochromic photoreceptor



the light-dependent change in the concentration of the active species P_r is

$$\frac{d[P_r]}{dF} = \sigma_{fr} k_{fr} [P_{fr}] - \sigma_r k_r [P_r]$$

where F is the photon fluence, σ_{fr} and σ_r are wavelength-dependent absorption cross sections of the two intermediates and k_{fr} and k_r account for the quantum yield. When the concentration of P_r is low as compared to P_{fr}, the photochemical back-reaction to P_{fr} can be neglected. Under this condition, $d[P_r]/dF$ is proportional to the absorption cross-section σ_{fr}. This relationship was used to construct an action spectrum from stimulus-response curves recorded at different wavelengths. Since photoconversion of P_{fr} to P_r causes sporulation, P_r is referred

SSDI 0014-5793(95)00820-9

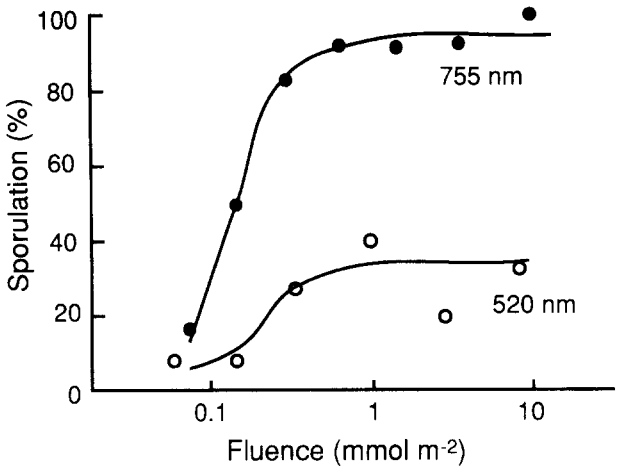


Fig. 1. Wavelength-dependent saturation levels of fluence–response curves. Plasmodia were irradiated for one hour with light of the wavelength indicated. For each data point 12 plasmodia were evaluated.

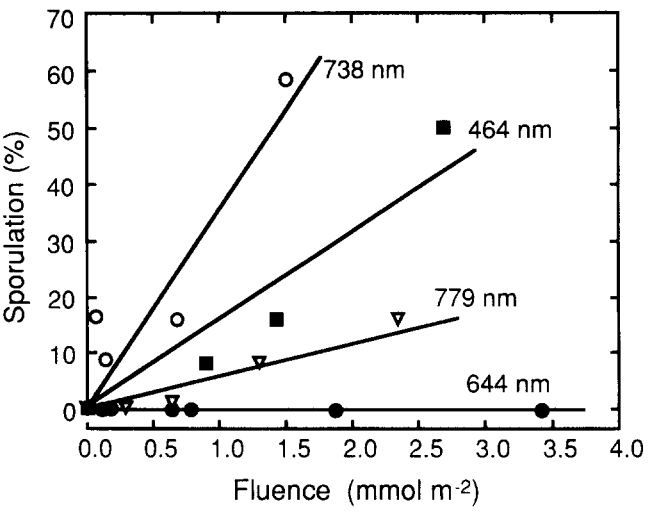


Fig. 3. Linear fluence–response relationships used to construct the action spectrum for the induction of sporulation. Plasmodia were irradiated for one hour at the wavelengths indicated. For each data point, 12 plasmodia were evaluated.

to as 'active' in the model. The alternative mechanistic possibility that P_{fr} actively represses sporulation and far-red irradiation reversibly inactivates the repressor cannot be excluded. However, the equation given above holds for either possibility.

The percentage of sporulated plasmodia (S) was plotted versus the photon fluence (F). As expected, a linear relationship was obtained for the initial part of the stimulus–response curves (Fig. 3). The slope (dS/dF) was estimated to give the relative photon effectiveness at each wavelength. The action spectrum obtained in this way peaks around 738 nm in the far-red and around 488 nm in the blue (Fig. 4, open circles). Since induction by blue light (unlike that by far-red) is only partially reversible by red light (not shown), an additional blue light-absorbing photoreceptor seems to be present.

To characterize the active intermediate P_r spectrally, plasmodia were first irradiated with far-red light choosing an intensity to produce a relatively high, but not saturating amount of the P_{fr} intermediate (i.e. 80–90% sporulation). Then a second irradiation at a different wavelength was applied and the inhibition of sporulation (I) recorded as a function of photon exposure (F). The action spectrum for the P_r intermediate was obtained by plotting the initial slope of the stimulus–response curves (dI/dF) against the wavelength of the second irradiation (Fig. 4, closed circles).

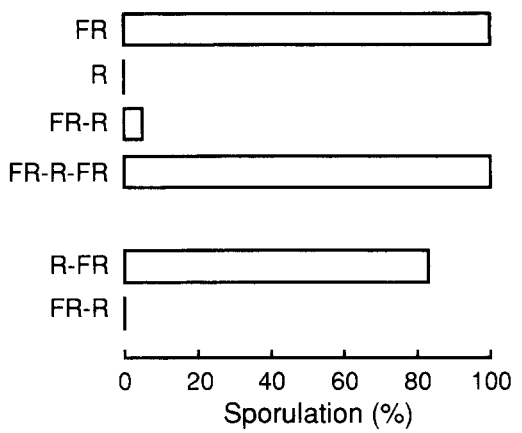


Fig. 2. Activation or inhibition of sporulation depends on the sequence of irradiation with far-red and red light. Competent plasmodia were exposed to a pulse of far-red (755 nm, 28 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 10 min) or/and red light (644 nm, 12 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 20 min) in the sequence indicated. Sporulation was assayed on the next day. In the experiment represented by the two lower bars, far-red (1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and red light (4.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was applied during a one hour exposure. Sixteen plasmodia were evaluated to give one data point.

The two action spectra for the induction of sporulation and its photoreversibility indicate that a photoreceptor with characteristics of phytochrome controls sporulation in *P. polycephalum*. The *Physarum* photoreceptor is synthesized as P_{fr} in the dark and photomorphogenesis occurs upon photoconversion to P_r. This is exactly opposite to what is known in plant phytochromes. Homologous phytochrome genes have been identified in higher plants, ferns, mosses and algae (for review, see [10]).

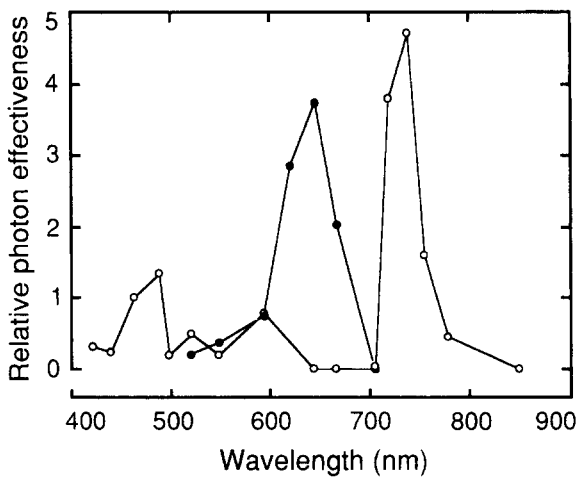


Fig. 4. Action spectrum for the induction (○) and inhibition (●) of sporulation. The relative photon effectiveness was calculated from fluence–response curves as shown in Fig. 3 in the way described in the text. For most of the data points a set of fluence–response curves that was obtained in independent experiments was evaluated. For each set a reference wavelength was included to correct for small variations in the sensitivity of different *Physarum* batches. The data points were connected by straight lines to guide the eye.

In the fungus *Aspergillus nidulans* a red/far-red light reversible photoreceptor has been shown to control conidiation [11]. Phylogenetically, slime moulds have emerged from the eukaryotic branch of life long time before the common anchestor of animals, plants and fungi evolved [12]. In this light, our results suggest that phytochrome may be an ancient eukaryotic photoreceptor.

Acknowledgements: We thank Kathrin Ueberholz for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (Ma 1516/1-2).

References

[1] Schedl, T., Burland, T.G., Gull, K. and W.F. Dove (1984) J. Cell Biol. 99, 155–165.
[2] Schreckenbach, Th. (1984) in: H. Senger (ed.), Blue Light Effects in Biological Systems, Springer-Verlag, Berlin, pp. 463–475.
[3] Nair, P. and Zabka, G.G. (1965) Mycopathol. Mycol. Appl. 27, 123–128.
[4] Anderson, R.W. (1977) Genet. Res. 30, 301–306.
[5] Daniel, J.W. and Baldwin, H.H. (1964) Methods Cell Physiol. 1, 9–41.
[6] Daniel, J.W. and Rusch, H.P. (1962) J. Bacteriol. 83, 234–240.
[7] Starostzik, C. and Marwan, W. (1994) J. Bacteriol. 176, 5541–5543.
[8] Hendricks, S.B., Butler, W.L. and Siegelman, H.W. (1962) J. Phys. Chem. 66, 2550–2555.
[9] Rüdiger, W. (1992) Photochem. Photobiol. 56, 803–809.
[10] Furuya, M. (1993) Annu. Rev. Plant Physiol. Plant Mol. Biol. 44, 617–645.
[11] Mooney, J.L. and Yager, L.N. (1990) Genes Dev. 4, 1473–1482.
[12] Woese, C.R. (1994) Microbiol. Rev. 58, 1–9.