PHOTORECEPTOR PIGMENT FOR BLUE LIGHT RESPONSES IN *Neurospora crassa*

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SUMMARY

Irradiating the mycelium of *Neurospora crassa* with blue light causes the photoreduction of a *b*-type cytochrome. The action spectrum for the photoreduction of the cytochrome *b* is similar to action spectra for the photoactivation of carotene synthesis and photoinhibition of the circadian rhythm of conidiation in *Neurospora*.

Light in the blue and near ultraviolet regions of the spectrum controls a number of metabolic and physiological processes in a wide variety of organisms from bacteria (1) and fungi (2) to higher plants (3) and animals (4). It may be assumed from the similarity of the action spectra for these various responses that a common photoreceptor pigment system controls these "blue light responses" (4,5). On the assumption that the photoreceptor pigment might change in response to light, photoinducible absorbance changes were sought in different photoresponsive organisms. Light-induced absorbance changes which had an action spectrum similar to that for the physiological "blue light responses" were found in the mycelium of the fungus *Phycomyces blakesleeanus* and in the cells of the slime mold *Dictyostelium discoideum* (6). The light-inducible absorbance change in both of these organisms was identified as the photoreduction of a *b*-type cytochrome, possibly mediated by a flavin.

The work reported here was undertaken in an attempt to find similar light-induced absorbance changes in an organism in which a circadian rhythm is under photocontrol. In the band strain (bd) of *Neurospora crassa*, spore formation (conidiation) is expressed as a circadian rhythm. This rhythm is sensitive to light in that its expression as a rhythm is inhibited

by continuous irradiation with low intensities of blue light (7). The phase of the circadian rhythm can also be advanced or retarded by brief irradiation periods given at appropriate times in the cycle (7,8) but the action spectrum for the phase shift in Neurospora is not known.

EXPERIMENTAL PROCEDURES

The mycelium of an albino band strain (al-2, bd) of Neurospora crassa was grown on 15 cm Petri dishes under conditions (9) in which the circadian rhythm of conidiation is expressed. This strain was isolated from a cross between al-2 (FGSC #99) and bd (FGSC #1859). Both strains are available from the Fungal Genetics Stock Center, California State University, Humboldt, Arcata, Calif. After 48 hr of growth (the first 18 hr in white fluorescent light, the last 30 hr in darkness), mycelium from the outer conidiating region was harvested under red safelight and 0.3 g of the tissue were pressed evenly onto the window of a cylindrical spectrophotometer cuvette to give a uniform sample about 2 mm thick. All operations and subsequent experiments were carried out at room temperature (23 $\pm 1^{\circ}$C).

Absorption spectra were measured with a single-beam spectrophotometer on line with a small computer (10). Kinetic measurements were made with a double-beam spectrophotometer in which the two measuring wavelengths were defined by interference filters (11). Actinic light was obtained from a xenon lamp in conjunction with interference filters and heat blocking filters. The measuring beams and the actinic light passed through the sample in the vertical direction from top to bottom. The difference spectrum of the light-induced absorbance change was determined by measuring the absorption spectrum of the sample before and immediately after a one minute irradiation with actinic light, during which time the phototube shutter was closed. Twelve seconds were required to open the shutter and scan the spectrum from 400 to 570 nm (i.e., the spectral region of interest) after the end of the actinic irradiation. The light-induced absorbance change decayed to some extent during the measurement but the general fea-

tures of the absorbance change are retained in the light-minus-dark difference spectrum. Absorbance measurements at 560 nm relative to a reference wavelength at 578 nm were made before, during, and after the actinic irradiation with the double-beam spectrophotometer with blocking filters placed between the sample and the phototube to protect the phototube from the actinic light ($\lambda < 530$ nm).

RESULTS AND DISCUSSION

Mycelial samples of Neurospora become fully reduced, as judged by the absorption spectra of the cytochromes, when first placed in the cuvette as a packed mass of mycelium but subsequently, over a period of hours, slowly oxidize. The absorption spectrum of a mycelial sample of the albino band strain which had been in the cuvette for 4 hr is shown in Fig. 1. The spectrum shows the presence of the reduced forms of a, b, and c-type cytochrome (α bands at 610, 560 and 550 nm, respectively). The difference spectrum (not shown) between the freshly prepared sample and the 4 hr-old sample indicated that the cytochromes in the 4 hr-old sample were 20 to 30% oxidized.

Irradiation of the partially oxidized, 4 hr-old sample with a moderate dose of actinic light (450 nm, 26 nEinsteins cm^{-2} sec^{-1} for 1 min) caused the absorbance changes shown in the light-minus-dark difference spectrum (L-D) in Fig. 1. The light-induced absorbance changes clearly indicate the photoreduction of the b-type cytochrome. If the freshly prepared sample (i.e., fully reduced) is irradiated with the same dose of actinic light, no change in the absorption spectrum is apparent.

The kinetics of the absorbance change measured at 560 nm (relative to a reference at 578 nm) induced by two intensities of 450 nm actinic light are shown in Fig. 2A. It is apparent that the initial rate of the absorbance change at the onset of irradiation and the total extent of the change are smaller with the lower intensity. Relative spectral response curves were determined by measuring the extent of the absorbance change induced

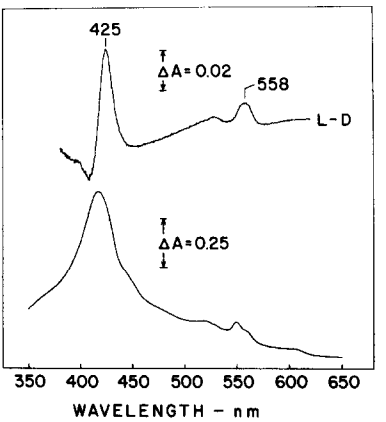

Figure 1. Lower curve: absorption spectrum of mycelium of the albino band strain of Neurospora crassa. The absorption spectrum of the mycelial mat (approx. 2 mm thick) was determined relative to a scattering reference consisting of 8 layers of tissue paper. Upper curve, L-D: light-minus-dark difference spectrum determined between the spectrum measured immediately after a 1 min irradiation with 450 nm light, 26 nEinsteins cm^{-2} sec^{-1} and that measured just before the irradiation.

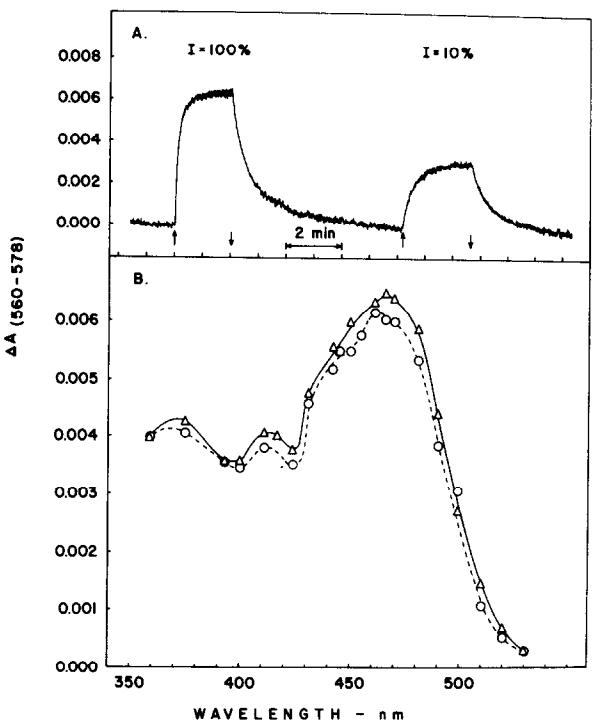

Figure 2. A: Kinetics of the light-induced absorbance change at 560 nm (relative to a reference wavelength at 578 nm) in the mycelium due to irradiation with two intensities of 470 nm light (100% = 6 nEinsteins cm^{-2} sec^{-1}). Actinic light on at upward arrows, off at downward arrows. B: Spectral dependence of the absorbance change measured at 560 nm relative to 578 nm. Extent of the absorbance change due to irradiation with different wavelengths of actinic light of equal quantum flux (6 nEinsteins cm^{-2} sec^{-1}).

by different wavelengths of actinic light of equal quantum flux (6 nEinsteins cm^{-2} sec^{-1}). Two such curves, determined with different samples, are shown in Fig. 2B. Care was taken to insure that the response did not approach saturation at any of the wavelengths tested. It is apparent that a pigment absorbing maximally at about 465 nm (possibly a flavin) is responsible for the photoreduction of a b-type cytochrome in the Neurospora mycelium. Photoreduction of the b-type cytochrome was also observed with mycelial samples from the wild-type strain of Neurospora crassa grown in darkness to limit carotene synthesis.

The results show clearly that the redox state of a b-type cytochrome is under photocontrol via a photoreceptor pigment. We would also infer, on the basis of the close similarity between the action spectrum for the photoreduction of cytochrome b and the action spectrum for the photoactivation of carotene synthesis in Neurospora (12) (as well as in other fungi [13]), that the same photoreceptor pigment controls other metabolic processes such as carotenogenesis, possibly via a photocontrol on respiratory metabolism. It also appears likely, on the basis of action spectra, that the photocontrol on the circadian rhythm of conidiation in Neurospora is via the same photoreceptor pigment. The action spectrum for the photoinhibition of the expression of conidiation as a rhythm (7) indicates a typical blue light response with a maximum at 465 nm and a sharp decrease of action at wavelengths longer than 520 nm. Unfortunately, an action spectrum for the actual phase shifting of the rhythm of conidiation was not determined but it is reasonable to assume that the action spectrum would be the same as that for the photoinhibition of the rhythm.

Action spectra for photobiological phenomena in animal systems are less well defined but some, nevertheless, are consistent with the typical blue light responses found in lower organisms and higher plants. The action spectra for the photoregulation of circadian thythms in Drosophila (4) and in Pectinophora (14) show a broad action maximum through the blue with

very little action at wavelengths longer than 520 nm.

The photoreduction of bytochrome b by blue light (action maximum at about 465 nm) has also been found in mycelial mats of Phycomyces and in dense cell suspensions of Dictyostelium (6). The photosensitive pigment thus appears to be common to different types of photoresponsive cells. After breaking Dictyostelium cells in a Ribi press, the photoresponsive pigment system was obtained in a soluble extract (6). Thus, the extraction, purification and characterization of the photoresponsive pigment system from Dictyostelium, Neurospora or other photoresponsive organisms appear feasible.

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