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Short Communication

Spectral Characterization of the Photoreducible *b*-Type Cytochrome of *Dictyostelium discoideum*[1]

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ABSTRACT

Irradiation of a soluble extract from broken cells of *Dictyostelium discoideum* causes the photoreduction of a *b*-type cytochrome. The cytochrome *b* can be separated from cytochrome *c*, which is also present in the extract, by column chromatography on Brushite, but the cytochrome *b* is no longer sensitive to light after separation on the column. Low temperature spectroscopy shows that reduced form of the photoreducible cytochrome *b* has a Soret band at 423 nm and a split α band with maxima at 558 and 551 nm similar to the *b*-type cytochrome in complex II of beef heart mitochondria.

We reported previously, from experiments designed to measure light-inducible absorbance changes in photoresponsive organisms, the photoreduction of a *b*-type Cyt in the mycelium of *Phycomyces blakesleeanus* (8), in dense cell suspensions of *Dictyostelium discoideum* (8), and in the mycelium of *Neurospora crassa* (7). The action spectrum to reduce the Cyt *b* appears to be the same in these different organisms (7, 8) and very similar to the action spectra for various physiological responses which include phototropism (2) and sporangiophore initiation (1) in *Phycomyces* and the photostimulation of carotene synthesis (12) and the photocontrol of a circadian rhythm (10) in *Neurospora crassa*. The action spectrum to reduce the Cyt *b* shows that a photoreceptor pigment absorbing at about 470 nm (possibly a flavin) absorbs the light and mediates the reduction of the Cyt. The purpose of the work reported here is to characterize spectrally the Cyt *b* in *Dictyostelium* which can be photoreduced by blue light.

Dictyostelium was chosen as the source material in this work, even though the physiological function of the blue photoreceptor pigment is unknown in these cells, because of the comparative ease of obtaining the photoactive Cyt *b* system in soluble extracts from the broken cells. Our initial work indicates that the mycelial tissue is more refractory for purposes of isolation. *Dictyostelium* cells also have another photoresponsive pigment (a high molecular weight hemoglobin (9)) which appears to be the photoreceptor pigment for the phototactic response of this slime mold. This latter pigment, however, should not be manifest in the measurements reported here.

MATERIALS AND METHODS

Dictyostelium discoideum A3 was grown in flask culture with rapid swirling at 24 C in a medium containing 10 g of proteose peptone, 5 g of yeast extract, 10 g of glucose in 1000 ml of 2 mM potassium phosphate buffer at pH 6.5 (9). Cells were grown to maximum stationary phase of growth at 1.2 to 1.6×10^7 cells/ml, collected by centrifugation, washed three times with a salt solution modified from that described by Bonner (3) to contain 0.6 g of NaCl, 0.75 g of KCl, 0.15 g of $CaCl_2$, 0.93 g of KH_2PO_4, 0.54 g of K_2HPO_4, and glass-distilled H_2O to 1000 ml of solution, and resuspended in this salt solution to 10^8 cells/ml. The cells in this suspension were broken in a Ribi press at 12,000 p.s.i. and the subcellular particles eliminated by centrifugations at 1000*g* for 5 min and 12,000*g* for 30 min. The soluble supernatant was then collected following a centrifugation at 144,000*g* for 60 min and frozen to −20 C until use. This soluble supernatant was used without further treatment for the initial low temperature spectroscopy, or was concentrated about 50-fold by pressure dialysis with a Diaflo UM-10 membrane for column chromatography.

Brushite (calcium phosphate) was prepared by a modification of the method described by Siegelman and Firer (11) by slowly (100 ml/hr) combining 1 M $CaCl_2 \cdot 2H_2O$ and 1 M K_2HPO_4 with constant stirring. The Brushite, aged in excess of 1 month, was washed eight times with glass-distilled H_2O to remove the fine particles and charged with a final concentration of 0.3 M K_2HPO_4 with stirring overnight. The charged Brushite was washed three times with the modified Bonner salt solution and poured into a 2.5 × 20 cm column which was further washed overnight with modified Bonner salt solution.

Absorption spectra were measured with a single beam spectrophotometer on line with a PDP8/I computer (5). Samples were either frozen to −196 C in darkness or while being irradiated with actinic light (1 mw/cm^2). In the latter case the sample was also irradiated for 1 min at room temperature before the cooling process began. A cylindrical cuvette and Dewar with optical windows on the bottom (4) were used for the absorbance measurements at −196 C.

RESULTS AND DISCUSSION

The absorption spectrum of the soluble supernatant from the broken cells frozen to −196 C in darkness (Fig. 1, curve

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D) shows a split α band with maxima at 548 and 545 nm and a Soret band at 413 nm. This absorption spectrum is characteristic of reduced Cyt *c* at −196 C. The absorption spectrum of a similar sample frozen to −196 C while being irradiated with blue light (Fig. 1, curve L) shows a maximum at 558 nm and a shoulder near 423 nm in addition to the absorption bands of Cyt *c*. The effect of light on the absorption by the sample can be seen more readily in the light-minus-dark difference spectrum (Fig. 1, curve L-D). It is apparent from this difference spectrum that the irradiation treatment causes the appearance of absorption bands at 558, 551, and 423 nm and a bleaching at 410 nm.

The absorption spectrum of the sample after chemical reduction with a few grains of sodium dithionite (Fig. 1, curve Dith) also shows the absorption band at 558 nm and a shift of the Soret band to longer wavelength (an increase in absorbance around 423 nm). The difference spectrum due to chemical reduction by dithionite (Fig. 1, Dith-D) shows reduction of the same *b*-type Cyt that is reducible by light (we interpret the bands at 558 and 551 nm to represent the split α band of a single *b*-type Cyt). A comparison of the two difference spectra in Figure 1 indicates that about half of the Cyt *b* was trapped in the reduced state by freezing during irradiation. The bleaching in the 440 to 490 nm region of the dithionite-minus-dark difference spectrum may indicate the presence of flavin that is not apparent in the light minus dark difference spectrum.

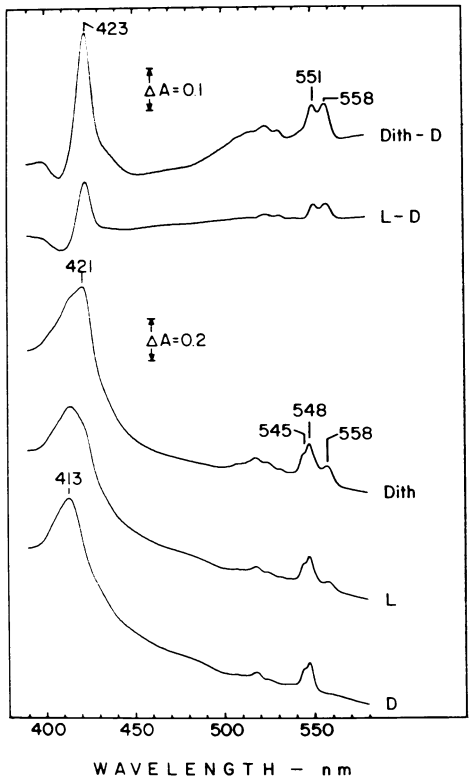

FIG. 1. Low temperature spectral characterization of a cell-free extract of *D. discoideum*. Spectra of a 144,000*g* soluble supernatant fraction from a cell-free extract of *D. discoideum* were measured at the temperature of liquid nitrogen. D: sample frozen to −196 C in darkness; L: sample frozen to −196 C while being irradiated with 1 mw/cm² actinic light at 470 nm; Dith: sample frozen to −196 C in darkness after the addition of a few grains of sodium dithionite. The light minus dark difference spectrum (L-D) and the dithionite minus dark difference spectrum (Dith-D) at a 2-fold increase in sensitivity.

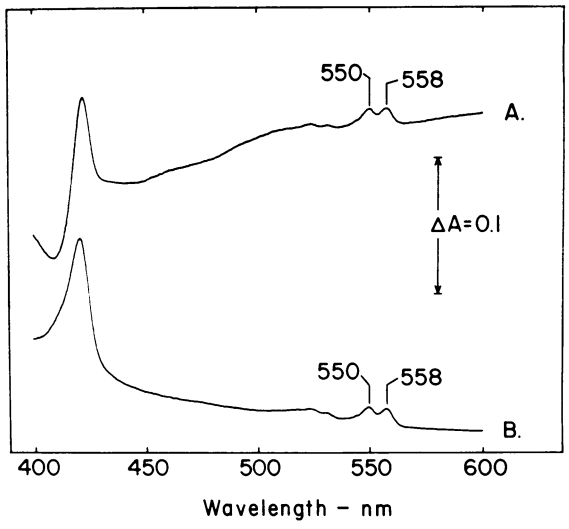

FIG. 2. Low temperature spectra of the partially purified *b*-type Cyt. Samples of the soluble supernatant were chromatographed on a Brushite column, eluted with modified Bonner salt solution, and reconcentrated about 10-fold by pressure dialysis. Spectra were measured at the temperature of liquid nitrogen. The Cyt was eluted off the Brushite column in its oxidized form and oxidized spectra were measured without further addition; reduced spectra were measured following addition of several grains of sodium dithionite. A: dithionite reduced minus oxidized difference spectrum; B: absolute absorption spectrum of a sample frozen following reduction with sodium dithionite.

The Cyt *b* and Cyt *c* in the soluble supernatant from the broken cells were separated by column chromatography on Brushite. The Cyt *b* is eluted in the oxidized form and can be reduced chemically by dithionite but not by ascorbate. The Cyt *b* shows no sensitivity to light after separation on the column. The absolute absorption spectrum and the reduced minus oxidized difference spectrum of the partially purified Cyt *b* at −196 C are shown in Figure 2. Comparison of the absorbance of the Soret band of the Cyt with the 280 nm absorption band of the protein from room temperature spectra indicated that the Cyt *b* was purified about 12-fold by the chromatography on Brushite (data not shown).

So far as we can discern from the absorption spectra and from chromatography of the soluble supernatant from the broken cells, this soluble supernatant contains only one *b*-type Cyt which is photoreducible by blue light. The spectral characteristics of this Cyt are similar to those of Cyt $b_{557.5}$ in complex II of beef heart mitochondria (6) which has (in the reduced form at −196 C) a split α band with maxima at 557.5, and 550 nm and a Soret band at 422 nm. Attempts to solubilize Cyt $b_{557.5}$ from complex II result in a broadening of the α bands and a decrease of α_1 relative to α_2 giving an absorption spectrum even more similar to that of the soluble Cyt *b* from *Dictyostelium* cells (K. A. Davis and K. L. Poff, unpublished).

The Cyt *b* which is photoreducible in the crude supernatant from broken cells of *Dictyostelium* is not photoreducible following chromatography on Brushite. We suspect that the photoreceptor (possibly a flavin) which mediates the reduction of the Cyt b_{558} has been removed or disconnected by the chromatography. Attempts will continue to purify the pigment system in a photoactive form.

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LITERATURE CITED

1. Bergman, K. 1972. Blue-light control of sporangiophore initiation in *Phycomyces*. Planta 107: 53–67.
2. Bergman, K., P. U. Burke, E. Cerdá-Oldema, C. N. David, M. Delbrück, K. W. Foster, E. W. Goodell, M. Heisenberg, J. Meissner, M. Zalokar, D. S. Dennison, and W. Shropshire, Jr. 1969. Phycomyces. Bact. Rev. 33: 99–157.
3. Bonner, J. T. 1947. Evidence for the formation of cell aggregates by chemotaxis in the development of the slime mold *Dictyostelium discoideum*. J. Exp. Zool. 106: 1–26.
4. Butler, W. L. 1972. Absorption spectroscopy of biological materials. Methods Enzymol. 24B: 3–25.
5. Butler, W. L. and D. W. Hopkins. 1970. Higher derivative analysis of complex absorption spectra. Photochem. Photobiol. 12: 439–450.
6. Davis, K. A., Y. Hatefi, K. L. Poff, and W. L. Butler. 1973. The *b*-type cytochromes of bovine heart mitochondria: Absorption spectra, enzymatic properties, and distribution in the electron transfer complexes. Biochim. Biophys. Acta 325: 341–356.
7. Muñoz, V., S. Brody, and W. L. Butler. 1974. Photoreceptor pigment for blue light responses in *Neurospora crassa*. Biochem. Biophys. Res. Commun. 58: 322–327.
8. Poff, K. L. and W. L. Butler. 1974. Absorbance changes induced by blue light in *Phycomyces blakesleeanus* and *Dictyostelium discoideum*. Nature 248: 799–801.
9. Poff, K. L., W. F. Loomis, Jr., and W. L. Butler. 1974. Isolation and purification of the photoreceptor pigment associated with phototaxis in *Dictyostelium discoideum*. J. Biol. Chem. 249: 2164–2167.
10. Sargent, M. L. and W. R. Briggs. 1967. The effects of light on a circadian rhythm of conidiation in *Neurospora*. Plant Physiol. 42: 1504–1510.
11. Siegelman, H. W. and E. M. Firer. 1964. Purification of phytochrome from root seedlings. Biochemistry 3: 418–423.
12. Zalokar, M. 1955. Biosynthesis of carotenoids in *Neurospora*. Action spectrum for photoactivation. Arch. Biochem. Biophys. 56: 318–325.