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Short Communication

Phycomyces: Electrical Response to Light Stimuli[1]

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ABSTRACT

Electrical signals have been detected in response to light excitation of the fungus *Phycomyces blakesleeanus*. These signals are related to the wavelength and intensity of the stimulus and the growth stage of the fungus. A relationship between the signals and the possible photoreceptor-pigment system is explored.

The fungus *Phycomyces blakesleeanus* sporangiophore develops in stages designated as I through IVb. These growth stages are related to the elongation of the sporangiophore and to the development of the sporangium. The sporangiophore is a single cell that can grow to centimeters in length. The growth zone, from 0.1 to just beyond 2 mm below the sporangium, is indicated as the sensory region (6). Sensitivity of the sporangiophore to a variety of physical stimuli has been documented, and the most extensively studied response is the phototropic response (3). To date no specific photoreceptor structure has been identified and it is still inconclusive whether the photoreceptor molecule is a flavin or carotenoid (7, 10). Detection of electrical signals from the sporangiophore in response to light excitation has not been reported despite efforts to obtain such data. We have now detected electrical signals upon light excitation and would like to report these experimental results.

MATERIALS AND METHODS

The experimental apparatus consisted of a two-chambered plastic well across which the sporangiophore was placed after it had been plucked and its base had been crushed. The sporangiophore made contact with the same solution in each chamber, 10 mM KCl, 12% sucrose. These chambers were connected to Ag-AgCl electrodes by agar-KCl bridges. The well (including the amplifier) was housed in a light-tight copper box, with a 2-cm hole and sliding cover to admit light. The output was fed through a Model A-35 electrometer amplifier (Medistor, Seattle, Wash.) and recorded simultaneously on a storage oscilloscope for photographic records and on a Varian chart recorder. Light sources consisted of a Zeiss microflash, Sylvania fluorescent bulbs, and a Bausch and Lomb monochromator. Sources were used with and without water filters, and Corning filters were used with the microflash. *Phycomyces blakesleeanus*, wild type G(+5) dark-grown stages I, IVa, and IVb were used. The sporangiophore was dark-adapted at least 20 min before each exposure to light.

RESULTS AND DISCUSSION

The response of *Phycomyces* sporangiophore to light stimuli was complex, and the sporangiophore, particularly in stage I, was quite active. An early receptor potential type response similar to that found by Arden *et al.* (2) and Ebrey (5) appeared sporadically in the data. This response occurred in stage I preparations, but was not observed in stage IV.

The most prominent response for all stages tested was a graded receptor potential. This response in *Phycomyces* consists of a positive wave whose amplitude is from 2 to 10 mv and duration 2 to 10 sec (Figure 1a). A second positive wave similar to the first may occur shortly after the first. Latency of response varied from 0.5 to 2 sec after a light pulse from the microflash, or exposure to light from the monochromator, and increased in some cases to 7 sec for exposure to the fluorescent light source. The largest amplitude potentials were seen in response to stimulation by the fluorescent light source (250 μw/cm^2 calibrated intensity reaching the sporangiophore). This is most likely due to the combination of exciting wavelengths present in the spectrum of the fluorescent light source. At equal intensities, the amplitude is slightly larger in response to illumination with 485 nm than to either 420 or 385 nm. A receptor potential could be obtained in response to illumination of 500 nm in stage I, but so far has not been obtained in stage IV.

One or more negative going spikes occurred on an average of 2.5 min after exposure to the microflash source. These spikes reached amplitudes of 8 to 20 mv and durations at about 0.5 sec (Fig. 1b). They have been observed to occur only in stage I in response to the microflash and in response to monochromatic light of 420 nm with reduced amplitude. Using a series of Corning filter combinations with the microflash, 420 nm was seen to be effective in producing repeated spiking. No spiking could be observed above 537 nm, and spiking occurred only occasionally between 442 and 537 nm.

As the sporangiophore matures through stages II to IVb, the complexity and type of the observed electrical response appears to change. Although the receptor potential is seen for all stages studied, the sporadic early receptor potential and the spike response is not seen in these later stages. This bears a relationship to the pigment system of *Phycomyces*. Orientation of the photopigment molecule is related to the early receptor potential observed in photoreceptors and plants (2, 9). Experiments have shown that rod crystals, having a vertical orientation, can be observed in *Phycomyces* sporangiophore under polarized light (7, 10). These crystals appear to be aligned in the growth zone, but whether they are located in or near the membrane has not been determined. Spectroscopic and chemical analysis indicate

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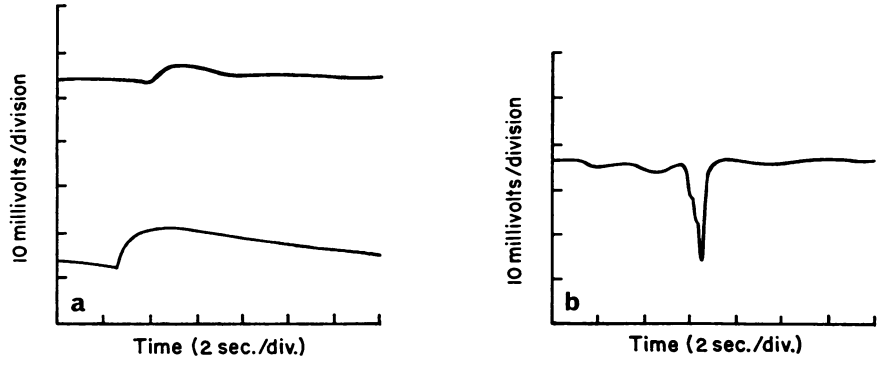

FIG. 1. a: Typical positive responses for stages I and IV sporangiophore after 2 sec exposure to light stimuli. The upper curve is an example of the response to 485 nm monochromatic light (150 μw/cm^2 intensity at sporangiophore). The lower curve is an example of the response to the fluorescent light source (250 μwatts/cm^2 intensity reaching sporangiophore). b: Spike response of stage I sporangiophore exposed to a pulse from the microflash (60 w/sec) in combination with Corning filters to give a peak at 420 nm.

that these crystals contain a flavin (7). The possibility of either a flavin or carotenoid, or both, as the photoreceptor pigment is indicated (11). The cell wall of the growth zone is a complex structure of up to seven layers (3). One or more of these membranes could also serve as a surface for one or both of these pigments.

The latency and time course of the receptor potential observed for *Phycomyces* is slow when compared to that of other photoreceptors (5, 9). The amplitude of the response is related to both wavelength and intensity. Wavelengths of 385, 420, and 485 nm correspond to absorption maxima in the action spectra for *Phycomyces* (4). Although 420 nm did not produce large amplitude receptor potentials, it was at this wavelength that spiking was seen in stage I sporangiophore. Spikes were observed for both monochromatic light and filters in combination with the microflash. The average latency (2.5 min) suggests a relationship with the transient growth response, which occurs around 3 min after exposure to light (3). The absence of the spike response in stage IV is also related to the pigment system of *Phycomyces*. It is possible that the photoreceptor-pigment system in stage IV may be in a different state than in stage I or that the receptor potential is mediated by a different pigment system than the early receptor potential and the spike response. Similar observations have been made by Ebrey (5) on the bean plant *Phaseolus vulgaris*. A slow positive response was seen after detection of an early receptor potential. This response varied in amplitude according to the amplitude of the early receptor potential and also among preparations. Ebrey (5) noted that these results suggested different pigments or different mechanisms mediated the early receptor potential and the later positive response.

Ahlquist and Gamow (1) hypothesize from their experiments that the growth zone and certain sensory properties may be transferred from the tip in stage I *Phycomyces* to the developing sporangium in stages II and III and back to the sporangiophore in stage IVa. It is possible that as the sporangiophore matures, alteration of the growth zone and receptor-pigment system takes place. The receptor-pigment system for the receptor potential in stage IV may function almost identically to that in stage I. The system responsible for the early receptor potential and the spike response, however, has been either photoreduced to the extent that it cannot be detected by the present method or altered in some other manner during the maturation process.

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