Mycoscience 37: 295–299, 1996

Lag period for phototropism in *Pilobolus crystallinus* sporangiophores

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Accepted for publication 15 July 1996

The lag period for the second positive curvature was examined in *Pilobolus crystallinus* sporangiophores. The lag period for curvature development was 20–30 min at lower fluence rates than 6.32 nmol/m²s but greatly extended at higher fluence rates. When a 20-min symmetrical irradiation with blue light was applied before a 20-min unilateral blue light irradiation, sporangiophores bent as much as those unilaterally and continuously irradiated for 40 min. However, when a 20-min unilateral irradiation was followed by a 20-min symmetrical irradiation, sporangiophores did not show any curvature. That is, the reaction during the first 20 min of the lag period is independent of light direction. This light-direction-independent lag period is considered to be the duration required for adaptation. The lag period for phototropism was also extended when fluence rate was reduced after the start of irradiation. These results suggested that an adaptation process is involved in phototropism of *Pilobolus*.

Key Words——adaptation; blue light; lag period for phototropism; phototropism; *Pilobolus crystallinus*.

Phototropic fluence-response curves in higher plants and algae show two types of phototropic reaction: the first positive curvature, which is induced by pulse irradiation; and the second positive curvature, which is caused only by prolonged irradiation (Briggs, 1960; Kataoka, 1977; Steinitz and Poff, 1986). Sporangiophores of the coprophilous fungus *Pilobolus crystallinus* (Wiggers) Tode also show the first and second positive curvatures. Time courses of curvature show that the length of the lag period for the second positive curvature depends on the fluence rate, while that for the first positive curvature is independent of the fluence rate (Kubo and Mihara, 1988, 1996). Thus, the second positive curvature seems to be more complex, and analysis of the lag period for the second positive curvature induced by longer irradiation is essential to elucidate the mechanisms involved.

The lag period for phototropism in *Phycomyces* is extended when the fluence rate of continuous irradiation of the sporangiophore is suddenly decreased or increased (Bergman et al., 1969; Galland and Russo, 1984). Such extension of the lag period is considered to be required for the sporangiophore to adjust to the new intensity of light. That is, an adaptation process is involved in the lag period for phototropism of *Phycomyces*.

In this study, we analyzed in detail the lag period for the second positive curvature induced by continuous irradiation to clarify the involvement of an adaptation process in the lag period of *Pilobolus crystallinus* sporangiophores.

Materials and Methods

Pilobolus crystallinus strain IFO 8561 was obtained from the Institute for Fermentation, Osaka. Sporangiospores were inoculated on MYC agar (1% malt extract, 0.2% yeast extract, 0.2% casamino acids and 1.2% agar) and incubated at 23±1°C under continuous white light of about 8 W/m² (fluorescent tubes, FL20SSD/18; Mitsubishi, Tokyo). Four or five days after inoculation, agar blocks, each with many trophocysts, were cut out from the culture plate and placed in a screw-capped glass tube (12×125 mm) (Kubo and Mihara, 1996). The tube was placed in a dark box with a pin hole on the top for 24–28 h, to allow the sporangiophores to grow straight upward. When many young sporangiophores (stage 2) had formed, the tube was transferred into an experimental chamber under a red safety light and kept there for 20 min in darkness. In the experiments shown in Figs. 1, 2 and 4, the experimental chamber was placed under a stereoscopic microscope and blue light, generated by a Xenon lamp fitted with a grating monochromator (Jasco CT-25, Japan Spectroscopic, Tokyo), was applied from one side. For the curvature measurement, a photograph was taken every 10 min using red safety light.

For the symmetrical irradiation of the sporangiophores in the experiment shown in Fig. 3, a glass tube containing many sporangiophores was rotated around its axis at a speed of 2 rpm, and irradiated from one side and from the top. Blue light (450 nm, 75 pmol/m²s) generated by a spectrophotometer (QV-50; Shimadzu, Kyoto) was applied from one side, and weak blue light (7.5 pmol/m²s) emitted by a fluorescent lamp (FLR40SD; Mit-

subishi, Tokyo) through a glass filter (VB-46; Toshiba, Tokyo) was applied from the top. The light from the top induced the sporangiophores to grow straight upward during the rotation period, but did not prevent the curvature caused by the lateral irradiation when the rotation was stopped. That is, sporangiophores were irradiated symmetrically during the rotation, and from one fixed side and the top after the rotation was stopped. The irradiation during the rotation period is here called 'symmetrical' irradiation and that without rotation 'unilateral' irradiation. A photograph was taken after the sporangiophores had been left in the dark for a further 20 min to allow the phototropic response to reach completion.

Results

Sporangiophores of *P. crystallinus* were irradiated continuously from the side at various fluence rates, and the time courses of the bending were examined (Fig. 1). Bending started after a lag period of 20-50 min, and curvature increased linearly with time in all experiments. Bending rate increased with increasing fluence rate from 63.2 fmol/m^{2}s to 63.2 pmol/m^{2}s, but was nearly the same for the fluence rates higher than 0.632 nmol/m^{2}s. The lag period for curvature development was 20-30 min at fluence rates lower than 6.32 nmol/m^{2}s, but at higher fluence rates it increased greatly with the increase of fluence rate. These results agree with those reported previously (Kubo and Mihara, 1996). Thus, the effect of fluence rate on the bending rate differed from that on the length of the lag period, suggesting that the factor determining the lag period is different from that determining the bending rate.

In the next experiment, glass tubes containing many sporangiophores were first irradiated from one side (blue light; 64 pmol/m^{2}s) for 10, 15, 20, 22, 25 or 30 min, then turned 180° to receive the light from the opposite side, and the curvature toward the light after the turn was measured every 10 min (Fig. 2). The control sporangiophores, which were illuminated from the same side throughout the experiment, started to bend after a lag period of about 30 min. The curvature increased almost linearly with the duration of the irradiation beyond 30 min. The sporangiophores turned 10 or 15 min after the start of irradiation showed similar time courses of the curvature development to the control, but those turned after 20 min started bending 5-10 min later. These results suggest that the photoreaction during the first 15-20 min is light-direction-independent. This lag period is here called the 'light-direction-independent lag period' (LIL). When the sporangiophores were turned 25 or 30 min after the start of irradiation, they first bent toward the source of irradiation before the turn, then reversed towards the unilateral light applied after the turn. That is, sporangiophores sense light direction during the last 10-15 min of the 30-min lag period without showing any bending response. This lag period is called the 'light-direction-dependent lag period' (LDL).

The above experiment suggested that the lag period

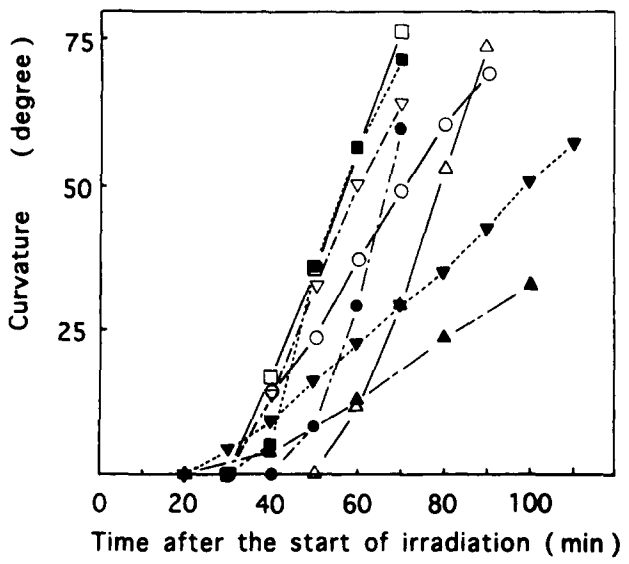

Fig. 1. Time course of phototropic bending caused by unilateral irradiation with blue light.
Sporangiophores were continuously irradiated at 63.2 fmol/m^{2}s (▲), 0.632 pmol/m^{2}s (▼), 6.32 pmol/m^{2}s(○), 63.2 pmol/m^{2}s(▽), 0.632 nmol/m^{2}s (□), 6.32 nmol/m^{2}s (■), 31.6 nmol/m^{2}s(●) and 63.2 nmol/m^{2}s (△).

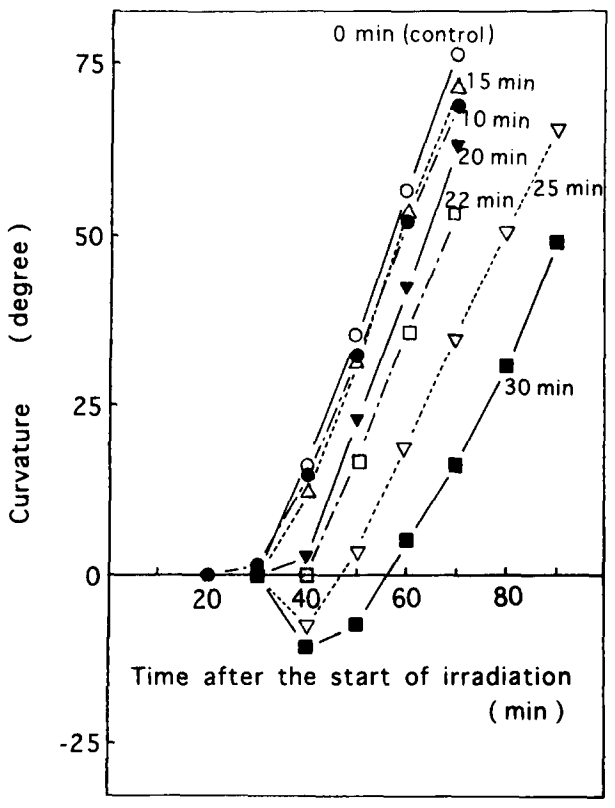

Fig. 2. Time course of phototropic bending after the direction of the irradiation was changed.
The sporangiophores were first irradiated from one side (blue light, 64 pmol/m^{2}s) for 0 (control), 10, 15, 20, 22, 25 and 30 min, and then turned 180° to receive the light from the opposite side. The curvature toward the direction of irradiation after the turn was measured every 10 min.

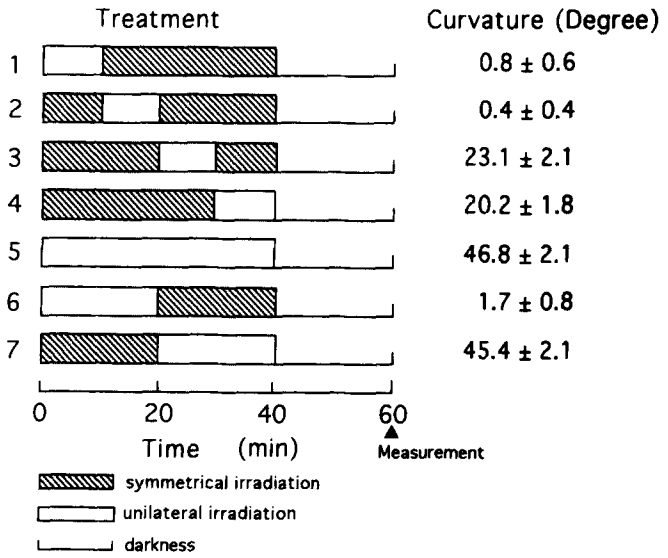

Fig. 3. Effect of symmetrical irradiation on phototropic curvature.
Sporangiophores were exposed to unilateral and symmetrical blue light (72 pmol/m²s) for the times indicated. Curvature was determined 20 min after the end of irradiation, when the curvature development was completed.

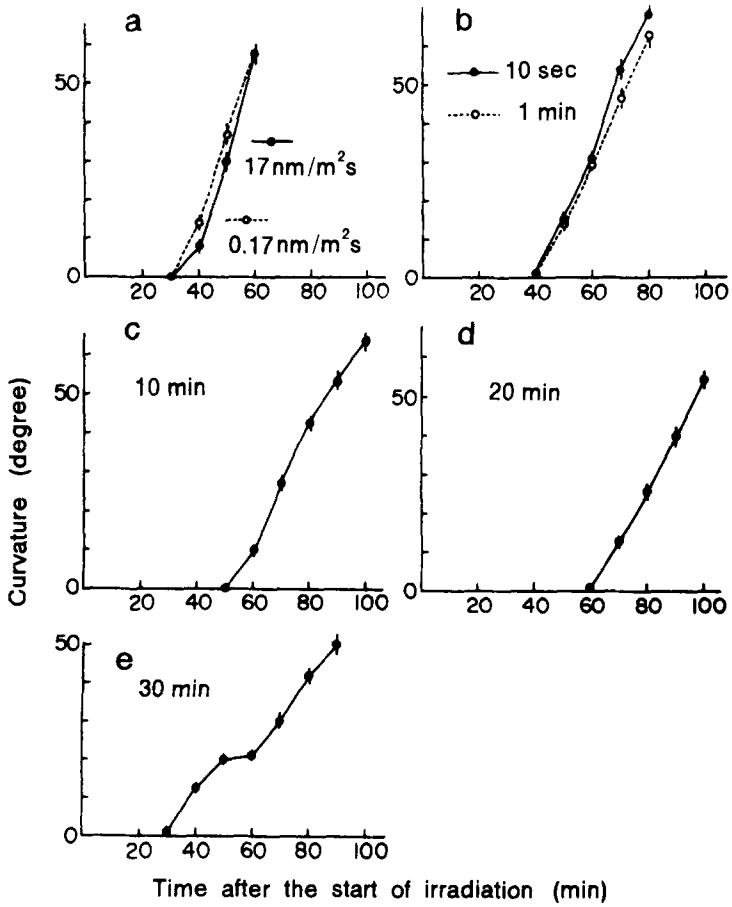

Fig. 4. Effect of a step-down of the fluence rate on the time course of phototropic bending.
(a) Sporangiophores were irradiated continuously with blue light at the fluence rate of 17 nmol/m²s or 0.17 nmol/m²s. (b–e) Sporangiophores were irradiated with blue light at 17 nmol/m²s for 10 s (b), 1 min (b), 10 min (c), 20 min (d) or 30 min (e), followed

for phototropic bending consists of two phases, LIL and LDL. To confirm this possibility, the sporangiophores were irradiated symmetrically and/or unilaterally with blue light (72 pmol/m²s) for 40 min as shown in Fig. 3, and the curvature was determined 20 min after the end of the irradiation.

When the sporangiophores were first irradiated unilaterally for 20 min, and then symmetrically for another 20 min, almost no bending occurred. However, when the sporangiophores were first irradiated symmetrically for 20 min and then unilaterally for another 20 min, as much bending occurred as after unilateral irradiation for 40 min (Fig. 3). Unilateral irradiation for 10 min during the first half of the 40-min irradiation period did not cause any bending when symmetrical irradiation followed. However, 10 min of unilateral irradiation during the last half did cause bending, although the curvature was about a half of that caused by 40 min of unilateral irradiation. These results support the assumption that the effect of irradiation during the first 15-20 min is independent of its direction.

From these results, an adaptation process is suggested to be involved in the lag period of the second positive curvature in *Pilobolus* sporangiophores. To verify this, the effect of a step-down of fluence rate on the duration of the lag period was examined (Fig. 4). When continuous blue light at 17 nmol/m²s or 0.17 nmol/m²s was applied unilaterally, sporangiophores started to bend about 30 min after the start of irradiation. Lag periods and bending rates were almost the same for both fluence rates (Fig. 4a). However, when the sporangiophores were first irradiated with blue light at 17 nmol/m²s for 10 s, 1 min, 10 min and 20 min followed by 0.17 nmol/m²s of blue light, bending started, respectively, 40, 40, 50 and 60 min after the start of irradiation (Fig. 4b-d). In other words, bending started about 40 min after the reduction of fluence rate in all experiments. When the fluence rate was reduced 30 min after the start of irradiation, bending started after a 30-min lag from the start of irradiation but the increase in curvature stopped temporarily after 15-20 min. After that, the curvature increased again at the same rate as that before it stopped (Fig. 4e). These results suggested that the reaction occurring before the step-down of the fluence rate was nullified by changing the fluence rate and that a new reaction subsequently began.

Discussion

The lag period for the first positive curvature induced by pulse irradiation was about 10 min (Kubo and Mihara, 1988). However, the lag period for the second positive curvature induced by continuous irradiation was 20-50 min, its length being dependent on the fluence rate (Fig. 1). This lag period is suggested to consist of two phases (Fig. 2). One is the LIL, which lasts 15-20 min after the start of irradiation at 64 pmol/m²s. The remaining 10-15 min of the 30-min lag period is the LDL, during which sporangiophores sense the light direction without showing any bending response. That is, the LDL is the lag period for the bending reaction itself, and the LIL is irrelevant to the direction of the bending reaction. The LIL is considered to be the period required for the sporangiophore to adjust to the new intensity of light (light adaptation), as shown in *Phycomyces* sporangiophores (Galland and Russo, 1984).

In the phototropism of *Phycomyces*, the lag period for bending is extended when the fluence rate is suddenly increased. Such step-up experiments showed that a light adaptation process is involved in the phototropism of *Phycomyces* sporangiophores. In this study, the length of the lag period was examined after the start of illumination rather than after the step-up of fluence rate. However, a similar extension of the lag period was observed. A similar light adaptation process may be involved in the phototropism of *Pilobolus* sporangiophores.

The extension of lag period in the wild type of *Phycomyces* was saturated at 22 min, but that in a *mad* mutant was extended to more than 70 min (Galland and Russo, 1984). In *Pilobolus*, the lag period was extended to more than 50 min when sporangiophores were continuously illuminated at higher fluence rate (Fig. 1). This large extension of the lag period is similar to that in the *mad* mutant of *Phycomyces*.

In the phototropism of *Phycomyces* sporangiophores, bending occurred in two steps when dark-grown sporangiophores were irradiated continuously (Galland and Lipson, 1987). It was suggested that three components with different lag periods were involved in this phototropism. The early and the late components operate at lower fluence rates but the intermediate component operates at higher fluence rates. In *Pilobolus*, the lag period was extended greatly at higher fluence rates than 6.32 nmol/m²s (Fig. 1). This delay of bending is similar to the intermediate component in *Phycomyces*. However, curvature increased linearly with time and did not occur in two steps. Therefore, the extension of the lag period at higher fluence rates is not due to the appearance of another component. Rather, the lag period may be extended because the sporangiophore requires a longer period to adapt to stronger light.

In *Phycomyces* sporangiophores, adaptation after a step-up of fluence rate (light adaptation) shows different kinetics from that after a step-down of fluence rate (dark adaptation) (Galland and Russo, 1984). The lag period in *Pilobolus* was also extended both by the onset of irradiation and by the step-down of fluence rate. However, it remains unknown whether the extensions of the lag period in these two cases are mediated by the same or different mechanisms. Kinetic study is necessary to determine whether two types of adaptation are involved in the phototropism of *Pilobolus*.

Acknowledgement—We wish to thank Dr. A. Takimoto for his critical reading of the manuscript and valuable suggestions.

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