Action Spectrum for Interaction between Visible and Far-Red Light on Face Chloroplast Orientation in *Mougeotia*

Received for publication October 6, 1987 and in revised form March 22, 1988

ZBIGNIEW LECHOWSKI AND JAN BIAŁCZYK
Department of Plant Physiology and Development, Jagiellonian University, Al. Mickiewicza 3, 31-120
Kraków, Poland

ABSTRACT

The orientation of chloroplasts from profile to face position in *Mougeotia* can be controlled in two ways: by a typical phytochrome-mediated system or by continuous, simultaneous irradiation with far-red and visible light. In experiments with dichromatic irradiation of *Mougeotia*, the light conditions applied prevented the formation of a far-red-absorbing form of phytochrome gradient in the cell. An unpolarized background of far-red light and linearly polarized monochromatic light of different wavelengths and vibrating parallel to the cell axis, if given by themselves, were completely ineffective in producing any changes in chloroplast orientation. Given together, however, changes in chloroplast orientation were induced. The action spectrum for this interaction between constant far-red and variable visible light was maximal at 620 nanometers. The chloroplast response in these dichromatic light conditions required a prolonged duration of exposure to continuous simultaneous irradiation of high fluence energy. The vibrating plane of linearly polarized 620 nanometer light had no significant influence on interaction with far-red light in chloroplast movement. The results obtained are different from the typical low energy phytochrome-mediated chloroplast orientation. This new type of chloroplast photoresponse might be mediated by an unknown sensory pigment.

Light-oriented chloroplast movement in *Mougeotia* has been shown to be controlled by R² and BL. The low-fluence-rate chloroplast orientation (profile to face position) is most effectively induced by R (14), with phytochrome being involved (for detailed information, see Refs. 1, 6, and 7). The action spectrum for the low-fluence-rate chloroplast response also shows weak action in the near UV and BL (5). This activity has been attributed to a BL-absorbing sensory pigment other than phytochrome, because of its independence of a strong FR background irradiation given simultaneously (3).

The low-fluence-rate chloroplast movement is governed by a local gradient of P₄ (6, 7) or by a photoexcited BL-absorbing sensory pigment (2, 19). In the case of phytochrome-mediated chloroplast orientation, the edge of the chloroplast in the cytoplasm always turns away from the regions of highest P₄ concentration (6, 7). Experiments with linearly polarized light pointed to a strong action dichroism of phytochrome molecules in *Mougeotia*. Absorbance dipoles of P₄ molecules are oriented parallel to the cell surface and follow helical lines around the cell, the effect of which is equal absorption all around the cell of polarized light vibrating parallel to the cell axis. This absorption neither brings about a P₄ gradient, nor changes chloroplast orientation. In contrast, absorbance dipoles of P₄ molecules are oriented normal to the cell surface (e.g., see Refs. 1, 6, and 7).

The chloroplast response to the plane of polarization of GL is the same as that for R. Polarized GL vibrating parallel to the cell axis did not result in any changes in chloroplast orientation. GL of this vibrating electrical vector, which has no effect alone, led to chloroplast reorientation when it was given together with strong FR (11). Under such conditions, no P₄ gradient should arise in the cell. These results might suggest that apart from a P₄ gradient that controls the low-fluence-rate chloroplast orientation in *Mougeotia*, there also exists a photosystem-absorbing GL whose activation both depends on simultaneous FR and GL action and requires a high fluence energy of GL (11).

In the present study, the action spectrum for this unknown type of *Mougeotia* chloroplast photoresponse was determined. The technique of simultaneous dichromatic irradiation developed by Hartmann (4) was used in the present study. In the combinations applied, continuous simultaneous dichromatic irradiation was included that should make the development of a P₄ gradient in *Mougeotia* impossible. That is, unpolarized strong FR background which should maintain a constant low level of P₄ in the cell (3), was given together with linearly polarized monochromatic light vibrating parallel to the cell axis.

MATERIALS AND METHODS

Plant Material. *Mougeotia* sp. was collected from a natural pond in Poland and was maintained in our laboratory for 2 years. It was cultured at 17 °C in Petri dishes in a liquid medium composed of 200 mM KNO₃, 15 mM KH₂PO₄, 80 mM MgSO₄·7 H₂O and 1% soil extract, as was described previously (11). The growth medium was changed every 10 d by transplanting the filaments into Petri dishes with sterile medium. Cultures were maintained under a photoperiod of 12 h light/12 h dark. Fluorescent lamps ("Flora" 40-W, LF-F, Polamp, Poland) were used as a source of light. The photon fluence rate of the light, measured in the range of 400 to 700 nm, was 5 μmol·m⁻²·s⁻². The filaments of algae for the experiments were taken from liquid cultures after 7 d. The filaments of *Mougeotia* were placed on slides parallel to one another in the same medium in which they were cultured. They were oriented normally to the long axis of the slides. The ends of the filaments were fastened to the surface of the slide with Vaseline. Cover glasses were sealed onto the slides with Vaseline. Moreover, all experiments were performed between 4 and 8 h after onset of the light phase, thereby minimizing possible diurnal fluctuations.

Light Source. The microscope was placed in a completely lightproof box. The specimen was irradiated on the microscope
stage through a condensor lens. Two independent optical systems were built for simultaneous irradiation with two wavelengths of monochromatic light. Both optical systems were set according to the Köhler principle and were so adjusted that the light spots formed in the place of the preparation were superimposed, as was previously reported in detail (11). Light provided by 1000-W Tungsram lamps was first filtered through a heat cut-off filter (a 5-cm-thick layer of a 4% aqueous solution of CuSO4). White light provided in this way was used for preirradiation of the algal cells.

For monochromatic irradiation, light was first filtered through filters eliminating heat effects (distilled water of 5-cm thickness and KG-1 of 4-mm thickness, Schott, Jena, GDR) and then through a selected interference filter of the SIF type (Zeiss, Jena, GDR) that was placed in the light path. The interference filters, which were in the range of 405 to 727 nm, had half-band widths of 8 nm or less. The irradiance was regulated by neutral-density glass filters (Schott, Jena, GDR) and measured in the plane of the algal filaments. Monochromatic light intensities were measured with a BX-91 photodiode (Siemens, FRG) calibrated by reference to a wavelength independent CA-1 detector (Kipp and Zonen, Delft, The Netherlands). Light was polarized by a Ber- nothar (Zeiss, Jena, GDR) linear polarizer inserted into one of the optical systems.

In these experiments FR was given as monochromatic 727-nm light.

**Experimental Procedure.** The preparation was placed on the microscope stage together with a thermostatted chamber that kept the filaments at 17 ± 1°C. For each spectral range investigated, the temperature of the medium was measured with a constantan-cupric thermocouple placed in the center of the light spot. The specimen was incubated for 1 h under conditions of weak white light (about 5 μmol·m⁻²·s⁻¹) and then used for the experiments. As a starting point for experiments, the chloroplasts were oriented in profile position by pretreating the cells with white light of 250 μmol·m⁻²·s⁻¹ (measured in the range between 400 and 700 nm). The preparation was kept in these light conditions for 1 h. More than 95% of the chloroplasts attained the profile position, after which the preparations were treated with monochromatic light.

For chloroplast observation at very weak irradiance and with FR, a microscope attachment was constructed with a built-in convertor (an 'Image Intensifier' type XX 1380, Mullard, England), as was described in a previous paper (11). Chloroplast counting was carried out at the beginning and at intervals of 5, 10, or 30 min. The time during which chloroplasts were counted was about 30 s. Chloroplasts were counted under the same light conditions that were maintained during the experiments. The position of 70 to 80 chloroplasts was determined for each measurement. The response was calculated as the percentage of chloroplasts seen in the face position. Each point on the curves is based on 6 to 7 different preparations, which is therefore, the average response of about 500 cells.

**RESULTS AND DISCUSSION**

**Action Spectrum.** Continuous dichromatic irradiation with a constant background fluence rate of unpolarized, strong FR, and with simultaneously given, linearly polarized monochromatic light vibrating parallel to the cell axis, was used for determination of the active spectral range. Dose-response relationship was tested at 11 wavelengths between 500 and 675 nm (Fig. 1). An essentially linear correlation between chloroplast orientation and the logarithm of photon fluence rate at all wavelengths tested was obtained. At saturating irradiances with monochromatic light in the range from 500 to 550 nm, the full extent of chloroplast response was not obtained in the 30-min period tested. In these cases, longer exposure was necessary for complete expression

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**Fig. 1.** Dichromatic dose-response curves for face chloroplast orientation in Mougeotia. Continuous monochromatic light was given simultaneously with unpolarized FR (727 nm, 12 μmol·m⁻²·s⁻¹) background irradiation. The numerals beside the curves represent wavelengths of the polarized monochromatic light vibrating parallel to the cell axis. Data were obtained after 30 min of irradiation.

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**Fig. 2.** Action spectrum for face chloroplast orientation in Mougeotia at a simultaneous continuous treatment with unpolarized FR (727 nm, 12 μmol·m⁻²·s⁻¹) background irradiation. Data taken from Figure 1.

(e.g., at 550 nm it was about 40 min (11). Using the fluence rate response curves of Figure 1, the reciprocal of the quantum flux density required for 50% response was plotted against wavelength, being normalized to the response observed with 620-nm light (Fig. 2). In this action spectrum, a sharp maximum was obtained at 620 nm. Although the effectiveness of the response was gradually reduced with decreasing wavelengths, the whole band of GL was active in the interaction with FR. The application of light at wavelengths exceeding 620 nm brought about a rapid reduction of chloroplast response and its complete disappearance at about 675 nm.

**Interaction between Linearly Polarized 620-nm Light and FR.** The effects on face chloroplast orientation of linearly polarized 620-nm light of different fluence rates and with parallel or normal vibrating plane, applied by itself or simultaneously with unpolarized strong FR background irradiation, were compared (Fig. 3). The precise action dichroism of phytochrome in Mougeotia is the basis of perception of light direction (e.g., Refs. 1, 6, and 7). Given by themselves, linearly polarized 620-nm light vibrating parallel to the cell axis (Fig. 3) or unpolarized FR (Fig. 4,
solid symbols) was ineffective in producing any change in chloroplast orientation. Continuous simultaneous exposure of Mougeotia to strong FR and to polarized 620-nm light vibrating parallel to the cell axis reoriented the chloroplast from profile to face position. The extent of the response depended on the intensity of 620-nm light (Fig. 3) and on the time of exposure (Fig. 4). This kind of photoresponse required higher intensities in comparison with the response induced by 620-nm light vibrating normal to the cell axis given by itself (Fig. 3). Under such dichromatic light conditions, chloroplast orientation also depended on the intensity of FR (Fig. 5). At the saturating intensity of 620-nm light, which in these experiments was given as a constant (3.3 μmol·m⁻²·s⁻¹) background, the full extent of the response was reached at 5.8 μmol·m⁻²·s⁻¹ of FR. A manifestation of the activity of chloroplast orientation during simultaneous treatment with FR probably indicates a conjunction with phytochrome action. The maximum response occurred at very low level values of the Pₜ/Pₜ₉₀ photoequilibrium ratio, resulting in the maintainance of a very low level of P₉₀ over a prolonged period of exposure time with strong FR. The vibration plane of the polarized 620-nm light had no significant influence on the interaction with FR on chloroplast orientation; the fluence response curves were superimposed in both cases (Fig. 3). The maximal activity at 620 nm in interaction with FR in high energy irradiation and no preference for the plane of polarization of the incidence visible light indicates that phytochrome is not the photoreceptor controlling this kind of chloroplast movement. The data may suggest the presence of a second sensory pigment in Mougeotia that absorbs this spectral range, as was earlier postulated by Tanada (15, 16) in his results with dichromatic irradiance of Albizzia leaflets.

The necessity for chloroplast orientation of simultaneous irradiation with FR and with 620-nm light was shown in experiments with different sequences and times of light treatment (Table 1). As the time courses of chloroplast orientation presented in Figure 4 show, at saturating fluence rates of both kinds of light no response to dichromatic irradiation occurred in the first 10 min. After the initial phase, chloroplasts started to change position. The full face orientation was reached after about 30 min.

The application of dichromatic irradiation at saturating fluences during the first 5 min, followed by treatment of Mougeotia with only one kind of light, did not cause any change in chloroplast orientation (Table 1). The chloroplast response required

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**Table 1. Effects of the Action of Continuous Irradiation with Unpolarized FR**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of Chloroplasts in Face Position</th>
</tr>
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<tbody>
<tr>
<td>I 5 min FR → 25 min OL</td>
<td>0</td>
</tr>
<tr>
<td>10 min FR → 20 min OL</td>
<td>0</td>
</tr>
<tr>
<td>II 5 min OL → 25 min FR</td>
<td>0</td>
</tr>
<tr>
<td>10 min OL → 20 min FR</td>
<td>0</td>
</tr>
<tr>
<td>III 5 min FR + OL → 25 min OL</td>
<td>0</td>
</tr>
<tr>
<td>5 min FR + OL → 25 min FR</td>
<td>0</td>
</tr>
<tr>
<td>IV 30 min FR + OL</td>
<td>100</td>
</tr>
</tbody>
</table>
prolonged duration of simultaneous action of both kinds of light. The data do not conform to the known properties of the low energy phytochrome effects that are induced by small total light fluxes (low irradiance times short time) and that show full or nearly full reversibility during successive exposure to R and FR (9, 10). In the case of low energy phytochrome-mediated chloroplast response in Mougeotia, flash durations of a few milliseconds are enough to induce chloroplast orientation (10). Further experiments with application of alternating FR and visible light flashes are necessary to fully characterize chloroplast movement of the new type that is presented here.

In contrast to the low energy, phytochrome-mediated chloroplast orientation in Mougeotia, the photoresponse characterized in the present and earlier work (11) showed a number of different properties which can be summarized as follows: (a) chloroplast response appeared only in the case of simultaneous irradiation with FR and visible light, (b) strong irradiance with both kinds of light is necessary to obtain a response, (c) prolonged duration of exposure to high irradiance of light is required to reach full face position, and (d) chloroplast orientation was independent of the vibration plane of polarized light.

This type of chloroplast interaction shows a number of similarities to the HIR (12). However, none of the four classes of HIR photo-orientational processes described by Mancinelli (12) required this kind of simultaneous dichromatic irradiation to obtain a response. Nonetheless, the variability of the spectral sensitivity of the HIR in different systems is possible. Vincente and Garcia (18) found that in Trifolium repens seedlings simultaneous FR and GL irradiation restored hypocotyl growth, which was inhibited by FR given by itself.

The physiological mechanism of the interaction of FR and visible light in the range from 500 to 675 nm on chloroplast movement in Mougeotia is not known. Similar results were obtained in experiments concerning the interaction between FR and GL on leaflet movement in Albizia (15, 16) and concerning the generation of a bioelectric field potential of the soybean hypocotyl (17). Tanada (16, 17) suggested that this kind of interaction may be active in the modification of the properties of membranes, leading to changes in their permeability. The low energy, phytochrome-mediated chloroplast and the response characterized here lead to the same kind of chloroplast movement (to face position), but it seems that they are mediated by different transduction chains. This conclusion is consistent with the results showing that this type of chloroplast response is strongly dependent upon irradiance in the FR (low P3/P3 ratio) (11, 18) while in the low energy phytochrome-mediated response it occurs in the R (high P3/P3 ratio) (9, 10).

**Effect of Interaction between BL and FR**. Under BL the low-fluence-rate chloroplast orientation in Mougeotia is a response to the complex of light reactions mediated by a minimum of two independent pigments. Obviously, the interpretation of action spectra reciprocity and other light studies in the blue region is fraught with difficulties. Under conditions of low irradiance in the blue region, the predominant system in operation appears to be phytochrome (5) although the existence of another pigment, a 'cryptochrome,' absorbing only UV-BL may be assumed (2, 8, 19, 20). The absorption of BL by phytochrome and originates the Pfr gradient in the alga. A part of the BL effect is independent of the direction of light ('tonal effect') (13) and since the action of this pigment is not negated by FR even at high irradiance (3), it is distinctly different from phytochrome.

In order to test the effect of BL on the type of chloroplast response that is being characterized currently, experiments with two kinds of irradiation were carried out. Linearly polarized BL (405 and 450 nm) vibrating parallel to the cell axis was given by itself or simultaneously with unpolarized strong FR background irradiation. This combination of light conditions eliminates the development of a Pfr gradient in the cell, while simultaneously revealing the part played by the BL and FR interaction. In Mougeotia, the maximum absorption of a cryptochrome has been postulated to be at about 450 nm (2, 19). The chloroplast response curves as a function of fluence rate, obtained with 450-nm light given by itself or simultaneously with FR, were similar (Fig. 6b). In the case of 405-nm light given simultaneously with FR, the response was higher by about 9 to 14% as compared to FR. This is the same as the 405-nm light. A similar weak increase in the low fluence rate chloroplast response in Adianium protonema was obtained by Yatsuhashi et al. (21) in experiments with continuous and simultaneous treatment with BL (in a wide spectral range) and FR, but no difference was found when BL and FR were given alternately.

The differences in the rates of chloroplast response in BL given by itself and BL given simultaneously with FR might be brought about by activation of a photochemical response different from those mediated by phytochrome and a cryptochrome. There is also a possibility that the R fluorescence emitted by BL-irradiated chloroplasts converts Pfr to P3 in their vicinity, thus producing a Pfr gradient in the cell. On the other hand, the simultaneous action of strong FR eliminated this possibility. Interaction between BL and FR irradiation on face chloroplast orientation is much less effective than in the case of FR and 620-nm light.

The bright range of sunlight (FR and visible light) is a necessary condition for the expression of this type of chloroplast response. In the natural environment, algae are exposed daily to prolonged periods of high irradiance of different spectral range of light. This might suggest that in Mougeotia spp., there exists a physiological mechanism that allows them to reach face position of chloroplasts even in high irradiance, indirectly modifying the rate of photosynthesis.
LITERATURE CITED