Fluence and wavelength dependence of photoinhibition in the brown alga *Dictyota dichotoma*

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ABSTRACT: Photosynthetic O_2-production of *Dictyota dichotoma* was measured under constant conditions with an oxygen electrode after pre-illumination with light of different fluence rates and wavelengths. The extent of photoinhibition depends on both duration and fluence rates of irradiation, as well as on the wavelengths. The action spectrum shows photoinhibition maxima in the green (542 nm) and red (672 nm), corresponding with the ranges of wavelengths absorbed by the xanthophyll fucoxanthin and the red band of chlorophyll *a*, respectively. Blue light is far less effective in proportion to its absorption by the chlorophyll *a* and carotenoids other than fucoxanthin absorbing these wavelengths. Wavelengths above 700 nm absorbed only by PS I do not cause significant photoinhibition. These findings suggest that effective radiation is mainly absorbed by PS II. PS I seems not to be involved. If photoinhibited algae are transferred to light of low fluence rates, photosynthetic O_2-production recovers fully within about 1 h; in darkness full recovery is markedly delayed. The relatively fast recovery implies that photoinhibition in *D. dichotoma* is an adaptation to strong light exposure protecting the algae from photodamage.

INTRODUCTION

Excess light energy causes an inhibition of photosynthetic activity in plants, a phenomenon known as photoinhibition (Powles 1984). It is well known that during daytime the phytoplankton near the water surface is significantly photoinhibited, whereas photoinhibition decreases with increasing depth. Land plants also display photoinhibition. The extent of photoinhibition depends on several different factors. Shade plants show photoinhibition at lower fluence rates than sun plants (Powles & Critchley 1980). Reduced atmospheric CO_2 and O_2 tensions increase the extent of photoinhibition (Powles 1984). Other environmental stress factors such as low temperature and water stress increase the extent of photoinhibition of land plants (Ögren & Öquist 1984). Extremely high irradiances cause photodamage and, finally, oxidative death of the photosynthetic cells (Abeliovich & Shilo 1972). Powles & Critchley (1980) supposed that the electron flow through Photosystem II (PS II) is primarily affected. In addition Critchley & Smillie (1981) demonstrated by *in vivo* fluorescence measurements that the site of photoinhibition is the oxidizing side of PS II, while PS I reactions are not inhibited. Contrary to these findings Barényi & Krause (1985) suggested that the activities of both photosystems are affected by visible light. Harvey & Bishop (1978) investigated photoinhibition in mutants of the green alga *Scenedesmus obliquus.* These authors postulated that the initial phase of inactivation caused by irradiation is located in PS I. Only prolonged illumination of the cells causes the disappearance of PS II activity.

In order to explain the molecular mechanism of photoinhibition, Ohad et al. (1984) correlated the *in vivo* turnover of the 32 kDa protein (so called herbicid-binding-protein) to the extent of photoinhibitory damage in flagellate *Chlamydomonas reinhardtii* thylakoids. Matoo et al. (1984) postulated that the rate of breakdown of the 32 kDa protein depends on the intensity of illumination. Photoinhibition results from a rapid degradation of the 32 kDa protein. Cleland & Critchley (1985) suggested a different mechanism according to which a very efficient quencher is supposed to be generated by photoinhibition. This quencher may be a modified form of Q_A, a plastoquinone of PS II.

Action spectra of photoinhibition were not measured

in the past. Nilson & Danielson (1984) compared the photoinhibitory effects of only 3 different wavelengths. Most recently, Agel et al. (1987) investigated the wavelength dependence of photoinhibition in the cyanobacterium *Anabaena variabilis* in the range 411 to 711 nm. Only radiation between 520 and 680 nm absorbed by the phycobiliproteins and short wave chlorophyll *a* caused significant photoinhibition, whereas wavelengths < 520 nm were ineffective. Thus, for further discussions there was a need to determine the effects of different wavelengths for seaweeds also.

As shown by Nultsch et al. (1981), the photosynthetic activity of *Dictyota dichotoma* is strongly influenced by pre-illumination at high fluence rates but is independent of the chromatophore arrangement. The depression of the photosynthesis rate caused by high intensity irradiation was stronger in the red than in the blue, though red light is ineffective in causing the strong light arrangement of the phaeoplasts. Consequently, Zurzycki's (1955, 1975) hypothesis that the function of the chromatophore arrangement is regulation of photosynthetic activity does not hold true. Therefore, a more detailed study of photoinhibition in *D. dichotoma* seemed to be necessary.

MATERIAL AND METHODS

Dictyota dichotoma was cultivated in a modified Schreiber solution (without earth and organic matter). In order to increase the growth rate, the cultures, grown in white light under a 10:14 h light(3.78 W m^{-2}):dark regime, were aerated. For pre-irradiation 'Prado' projectors (Leitz, Wetzlar, FRG) equipped with quartz iodine lamps were used. Fluence rates were varied by inserting neutral density filters. For light exposure, discs of 5 mm in diameter were cut out of the thallus and transferred to a small dish. The temperature during pre-irradiation was 15°C. Monochromatic radiation was produced with the aid of interference filters (Schott, Mainz, FRG), with an average half-band width of 12 nm. For the action spectrum of photoinhibition a constant photon fluence rate was used calculated on the basis of 10 W m^{-2} at 672 nm. Measurements were repeated 6 times for each wavelength. One individual alga could be used only 3 times for measurements. The relative oxygen production of photosynthesis was measured with an oxygen electrode (Hydro Bios, Kiel, FRG) mounted in a seawater flow-through cuvette. The pre-irradiated alga was transferred into this cuvette. Natural seawater was used as flow-through medium and was held at a constant temperature of 15°C. Red light of 1 W m^{-2} (672 nm SFK filter; Schott, Mainz, FRG) was used to measure photosynthetic oxygen production ('measuring light'). In order to study the

recovery of photosynthetic activity in weak light, the algae were either kept in continuous measuring light for 60 min, or exposed to a 5:5 min measuring light: dark cycle. Photoinhibition is expressed as O$_2$-production by the alga in percent of the value measured before treatment.

The absorption spectrum was measured with a Shimadzu (Kyoto Japan) MPS 5000 recording spectrophotometer.

RESULTS

White light experiments

In a first series of experiments *Dictyota dichotoma* was pre-irradiated with white light of different fluence rates and exposure times. As expected, photoinhibition increased with the duration of illumination (Fig. 1). In principle, it is a relatively fast process. After 10 min of exposure 20 % inhibition was measured, and almost 50 % was reached after 30 min.

Fig. 2 shows that photoinhibition, within certain limits, depends on the total fluence (fluence rate × time) of pre-irradiation. Up to 40 W m^{-2}, equal total
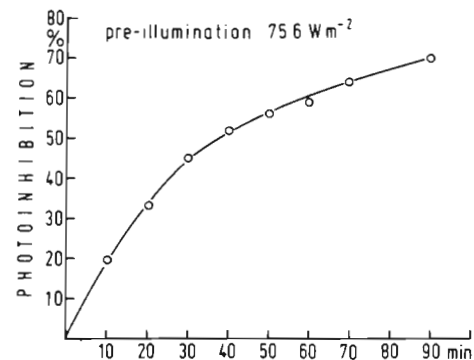
Fig. 1. Relative photosynthesis rate after different times of pre-illumination (75.6 W m^{-2}). Abscissa: time of pre-illumination; ordinate: photoinhibition in %

Fig. 2. Influence of fluence rate and duration of exposure on photoinhibition. White light was used for pre-illumination. A: 5.4 W m^{-2}, 240 min; B: 10.8 W m^{-2}, 120 min; C: 21.6 W m^{-2}, 60 min; D: 43.2 W m^{-2}, 30 min
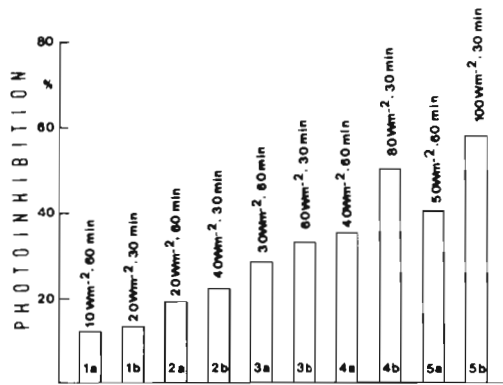
Fig. 3. Influence of fluence rate and duration of exposure on photoinhibition. White light was used for pre-illumination. Variation of exposure can be taken from the figure. Columns with the same number have the same total fluence

influences caused about the same extent of photoinhibition. However, above 40 W m^{-2} doubling the fluence rate caused greater photoinhibition than doubling the duration of pre-illumination (Fig. 3).

Experiments with monochromatic light

As mentioned above, action spectra of photoinhibition have been scarcely measured previously. In order to determine an action spectrum of *Dictyota dichotoma*, the thalli were exposed to monochromatic light of different wavelengths at a constant photon fluence rate of 56 μmol m^{-2} s^{-1}. For the blue region of the spectrum, SFK filters were used, with half-band widths of about 40 nm, since we did not have the means to produce radiation of sufficient high fluence rates with interference filters. For the same reason it was not possible to measure the action spectrum on the basis of fluence
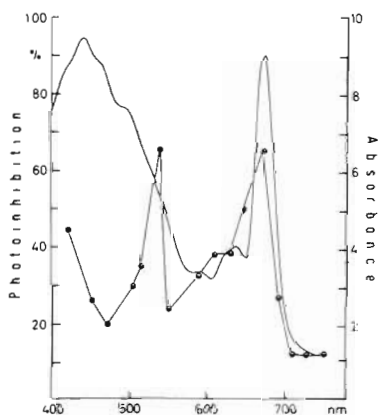

Fig. 4. Action spectrum of photoinhibition (•——•) and *in vivo* absorption spectrum (——). Pre-irradiation: (•) SFK filters; (ⓞ) interference filters, 56 μmol m^{-2} s^{-1}, 672 nm, 60 min. Abscissa: wavelength in nm, ordinates: photoinhibition in % and absorbance

rate-response curves. Fig. 4 shows the relative action spectrum of photoinhibition in comparison to the absorption spectrum of *D. dichotoma*. Nultsch et al. (1981) reported that in *D. dichotoma* depression of the photosynthesis rate caused by high fluence rates is stronger in the red than in the blue. This finding is confirmed by the present action spectrum. The lower effectiveness observed under blue light may be due to shading and/or photoprotection by carotenoids other than fucoxanthin, e.g. β-carotene. Wavelengths above 700 nm do not cause significant photoinhibitory effects. Thus, we may conclude that the photoinhibitorily effective radiation is absorbed mainly by PS II. PS I seems to be largely unaffected. In the range of radiation absorbed by chlorophyll *c* (present only in small amounts) no significant stronger activity causing photoinhibition was detected compared to adjacent wavelengths. However, irradiation around 540 nm, absorbed by the xanthophyll fucoxanthin, was about as effective in causing photoinhibition as that absorbed by the red band of chlorophyll *a*.

Recovery of full photosynthetic activity

If *Dictyota dichotoma* thalli are transferred from strong light back to weak light conditions, the photosynthetic activity recovers and, finally, reaches the initial level of oxygen production within about 1 h. The kinetics of this recovery of photosynthesis depend on the wavelength of pre-irradiation, as shown in Fig. 5 for 2 different wavelengths: one strongly (542 nm) and one less effective (691 nm) in causing inhibition. Recovery of full photosynthetic activity took about 50 min. Since pre-irradiation with the wavelength 542 nm was more effective than irradiation with 691 nm, the values of O$_2$-production are of course higher in the latter case.

A significantly delayed recovery, however, was observed in darkness. Therefore, the kinetics of recovery depend on the light-dark regime. This is shown in Fig. 6. The photosynthetic activity of thalli
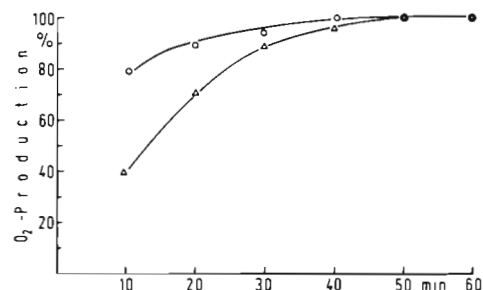

Fig. 5. Recovery of photosynthetic activity after pre-irradiation with 56 μmol m^{-2} s^{-1}. (△) 542 nm, 60 min; (ⓞ) 691 nm, 60 min. Measuring light: 672 nm, 1 Wm^{-2}, 60 min; light and darkness alternating every 5 min

which were pre-irradiated under the same conditions recovered faster in continuous weak light (1 W m^{-2}, 672 nm) than in a 5:5 min light (1 W m^{-2}, 672 nm): dark regime. The delay of recovery in darkness is shown in Fig. 7. If the organisms were kept after pre-irradiation for 30 min in darkness, almost no recovery of photosynthetic activity could be observed.
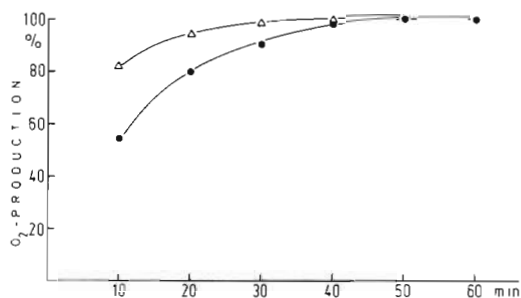

Fig. 6. Influence of different light conditions on recovery of photosynthesis. (△) Continuous measuring light of 1 Wm^{-2}, 672 nm, 60 min. (●) Alternation of light (1 Wm^{-2}, 672 nm, 5 min) and dark (5 min)
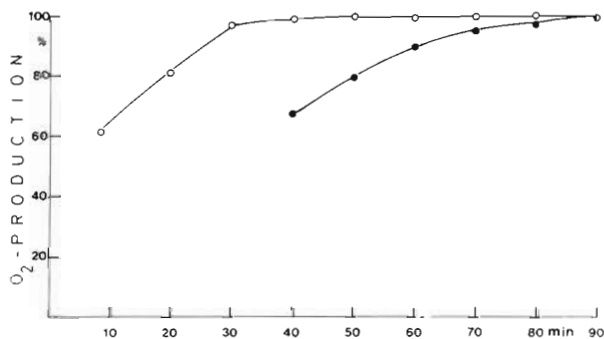

Fig. 7. Influence of darkness on recovery of photosynthetic activity. (●) Pre-illumination 43.2 Wm^{-2}, 60 min, white light. After exposure, the alga was kept for 30 min in darkness. Measuring light: 672 nm, 1 Wm^{-2}, 60 min; light and dark alternating every 5 min. First data of oxygen evolution could be measured after 40 min. (○) Restitution of photosynthesis rate after the same pre-irradiation. Measuring light: 1 Wm^{-2}, 60 min, light and dark alternating every 5 min

DISCUSSION

The extent of photoinhibition is a function of the duration of light exposure and the photon fluence rate. At fluence rates exceeding 40 W m^{-2} doubling the fluence rates is more effective than doubling the exposure time. Critchley & Smillie (1981) stated that a light intensity 5 times higher than that used for cultivation is high enough to generate photoinhibition. It is remarkable that Dictyota dichotoma is photoinhibited by exposure to as little as 5 W m^{-2}, i.e. only 1.3-fold the

light intensity used for cultivation. At an irradiance of 75.7 W m^{-2} 20 % photoinhibition occurs within 10 min.

In Lemna gibba, a water plant, Nilson & Danielson (1984) reported that the depression of photosynthetic activity caused by monochromatic light at 446 and 662 nm was approximately equal, whereas photoinhibition at 704 nm was significantly less than at 662 nm. In Dictyota dichotoma, however, blue light is much less effective than light of longer wavelengths in causing photoinhibition, as also observed by Agel et al. (1987) in the cyanobacterium Anabaena variabilis. The relatively small effect of the blue light may be due to shading and/or photoprotection by other carotenoids such as β-carotene. In both organisms, A. variabilis and D. dichotoma, red light absorbed by chlorophyll a is strongly active. The high effectiveness of the range around 542 nm in D. dichotoma points to participation of the carotenoid fucoxanthin in perception of the active light. Light absorbed by PS I, e.g. wavelengths above 700 nm, is almost ineffective. Thus, the hypothesis discussed in former reports that PS II is the site of photoinhibition is strongly supported for D. dichotoma by our action spectrum measurements.

Ohad et al. (1984) speculated that the turnover of the Q_B-protein (the so-called herbicid-binding-protein) is responsible for the extent of photoinhibition. According to this theory Matoo et al. (1984) proposed a regulation model for photoinhibition. They postulated that the 32 kDa protein is destroyed during high intensity irradiation, whereas it is synthesized again during recovery. This synthesis is light dependent. Ohad et al. (1984) observed in Chlamydomonas reinhardtii that photoinhibited chloroplasts recovered photosynthetic activity completely in weak light but only partially in darkness. In higher plants, Greer et al. (1986) found that the rate of recovery was substantially lower in darkness than in weak light. We observed similar effects in the marine alga Dictyota dichotoma. Darkness delayed the recovery of photosynthetic activity in comparison to continuous weak light irradiation. However, recovery depends also on the duration, the photon fluence rate and the light quality of pre-illumination. The rapid restitution of photosynthetic activity which takes place within 60 min points to the ecological importance of photoinhibition at least in this brown alga. Apparently, it is a protective mechanism from excess light energy rather than a photodamage. In this way, organisms are able to adapt to alterations of environmental light conditions relatively fast. Therefore, in future studies it would be of great interest to study photoinhibition of this alga in situ.

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