Arch Microbiol (1988) 150:529–533

Archives of
Microbiology

The kinetics of photoinhibition and its recovery in the red alga *Porphyridium cruentum**

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Abstract. When *Porphyridium cruentum* cells were illuminated with high fluence rate between 1900 and 4800 μmol photons m^{-2} s^{-1}, a decrease in the photosynthetic activity of the cells was observed. Within the time frame of 20 min, and under the fluence rates studied, the sum of photons to be absorbed by cells (mg of chlorophyll (Chl), sufficient to initiate photoinhibition was calculated to be 9235.8 μmol. The minimal specific light absorption rate to initiate photoinhibition in *P. cruentum* ranges between 2.29 and 4.26 μmol photons s^{-1} mg^{-1} chl.a. There was a linear relationship between the specific rate of photoinhibition and the specific light absorption rate. A photon number of 2.56×10^4 μmol mg^{-1} chl.a photoinhibited photosynthesis instantaneously. At 15°C, no photoinhibitory effect was observed at 2300 μmol photons m^{-2} s^{-1} even after 45 min of illumination. At the other extreme of 35°C, 84% inhibition of photosynthetic activity was observed within 10 min of exposure to 2300 μmol photons m^{-2} s^{-1}. Between 20 and 30°C, the photoinhibitory effect was comparable. Photoinhibited *P. cruentum* cells recovered readily when transferred to low light (90 μmol photons m^{-2} s^{-1}) and darkness, and the specific rate of recovery was independent of the light intensity to which the cells were exposed, during the photoinhibitory treatment.

Key words: Photosynthesis – O_2 evolution – Photoinhibition – Recovery – *Porphyridium cruentum* – Biomass

Photoinhibition has been defined as the reduction in the capacity of photosynthesis induced by exposure of photosynthetic organisms, structures, or organelles to visible light, without changes in bulk pigment composition (Powels 1984). Photoxidative damage may eventually ensue if photosynthesis is allowed to deteriorate further (Powels 1984; Krinsky 1976). Photoinhibition phenomenan have been also reported in algae (Richardson et al. 1983; Kyle and Ohad 1986) and was claimed to be an important factor in determining the productivity of cell mass in outdoor cultures of algae exposed to full sunlight (Vonshak and Guy 1988). Photoinhibited cells of many algae recover in dim light (Ogren and Oquist 1984; Samuelsson et al. 1985, 1987; Vonshak et al. 1988), a process which may be crucial in preventing the onset of the irreversible photooxidation in prolonged photoinhibited algal cultures.

The red alga *Porphyridium cruentum* has been suggested as a potential source of polysaccharide (Percival and Foyle 1979; Thepenier and Gudin 1985), polyunsaturated fatty acids (Ahern et al. 1983; Vonshak et al. 1985; Lee and Tan 1988) and phycoerythrin (Curtin 1985). In order to be able to develop an economically feasible system for such a process, efficient photosynthetic production of biomass at high fluence rate has to be achieved. It is the aim of this work to shed light on both the kinetics of inhibition by high fluence rate and recovery of the photosynthetic activity of *P. cruentum* cells.

Materials and methods

Alga and growth conditions

P. cruentum strain ld was obtained from the Göttingen Algal Culture Collection and cultivated in the chemically defined medium described by Jones et al. (1983). 100 ml of cultures in 250 ml Erlenmeyer flasks were incubated in an enclosed shaker incubator at 25°C under white light (day light fluorescent) with an intensity of 150 μmol photons m^{-2} s^{-1} (400–700 nm). The atmosphere of the incubator contained 5% CO_2.

Photoinhibition experiments

P. cruentum cells in log phase were harvested and suspended in fresh medium to give a chlorophyll (chl.) a concentration of 26 or 13 mg l^{-1}. *P. cruentum* cultures (6 ml) were placed in a thermoregulated double-jacket cylindrical glass vessel with a surface to volume ratio of 0.1. Cells were illuminated from one side by a high-intensity halogen lamp (O. Hedler, Runkel/Lahn, De Luxe 2000, 1000 W, FRG). The light intensity between 400 and 700 nm at the surface of the cultures was measured by a LICOR quantum pyranometer (UK). At time intervals, samples were withdrawn and their photosynthetic activity was measured.

Measurement of photosynthetic activity

Photoinhibited or control cultures were diluted to a final concentration of 2.6 mg chl.a l^{-1} with fresh medium con-

* Publication No. 28 of the Microalgal Biotechnology Laboratory

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Abbreviations: Chlorophyll; QL, specific light absorption rate

taining 1 g l^{-1} $NaHCO_3$. Their photosynthetic activity was assayed by measuring the rate of O_2 production using a Clark type O_2 electrode (YSI, USA) in a double-jacket thermo-regulated (25° C) glass vessel as described above, and illuminated by a slide projector lamp providing 700 µmol photon m^{-2} s^{-1} (400–700 nm).

Recovery experiments

Samples of photoinhibited *P. cruentum* cultures were diluted to a concentration of 2.6 mg chl.a l^{-1} with fresh culture medium, and incubated in dim white light (90 µm photon m^{-2} s^{-1}, 400–700 nm) and in complete darkness. At time intervals, the photosynthetic activity of the cultures was measured.

Determination of chlorophyll content

Chlorophyll a in *P. cruentum* cells was extracted and measured according to Vonshak et al. (1985).

Results

Photoinhibition of photosynthetic activities

A culture suspension of *P. cruentum* ld containing 26 mg chlorophyll (chl.)a l^{-1} was exposed to fluence rates (400–700 nm) of 4800, 3500, 2300 and 1900 µmol photon m^{-2} s^{-1}. Only at fluence rate above 1900 µmol photon m^{-2} s^{-1}, decreases in the oxygen production rate were observed, and these decreases were initiated only after a lag period (Fig. 1). The length of this lag period and the degree of inhibition appear to be functions of the fluence rate.

When a more diluted culture of *P. cruentum* (13 mg chl.a l^{-1}) was used, the effect of photoinhibition was enhanced, reflected in a greater degree of inhibition and a shorter lag period (Fig. 2).

Assuming that the fraction of transmissed and reflected light by a light-limited cell suspension of *P. cruentum* were small, as was the cause in other algal system (Iehana, 1987) of comparable cell concentrations, the rate of light being absorbed by cell (expressed as per mg chl.a) could be estimated, i.e. the specific light absorption rate QL = Fluence rate × surface area/chl. content × culture volume, having the units of µmol photon s^{-1} mg^{-1} chl.a. When the QL was plotted versus the reciprocal of the lag period (Fig. 3), a linear relationship was observed. The slope of the plot is 9235.8 µmol photon mg^{-1} chl.a (correlation 0.944), and may represent the accumulated number of photons absorbed by 1 mg of chlorophyll before the deactivation of photosynthetic activity (photoinhibition) commences, under the present experimental conditions. The value of 1/lag would approach 0 when the lag is infinitely large, i.e. photoinhibition does not occur. Thus the intercept of the plot on the ordinate (4.26 µmol photon s^{-1} mg^{-1} chl.a) gives a numerical estimate of the minimum light absorption rate to initiate photoinhibition in a *P. cruentum* culture.

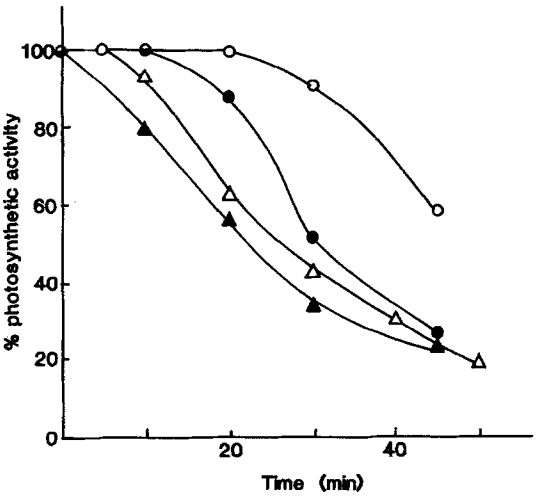

Fig. 2. Photosynthetic activity of *P. cruentum* cultures exposed to various fluence rates. The chlorophyll concentration was 13 mg l^{-1}. (○) 1900 µmol photon m^{-2} s^{-1}, (●) 2300 µmol photon m^{-2} s^{-1}, (△) 3500 µmol photon m^{-2} s^{-1}, (▲) 4800 µmol photon m^{-2} s^{-1}, 100% activity corresponds to 625 µmol O_2 h^{-1} mg^{-1} chl.a

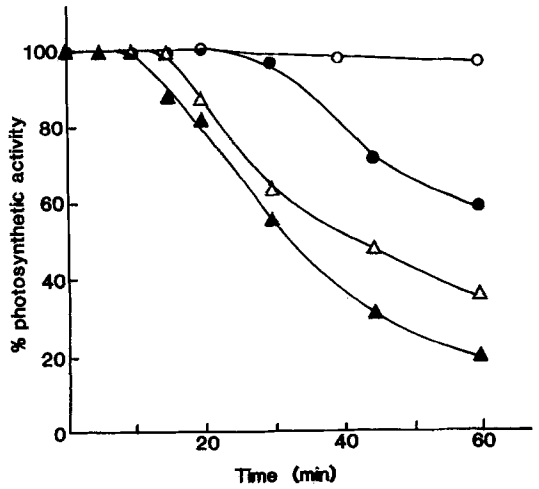

Fig. 1. Photosynthetic activity of *P. cruentum* cultures exposed to various fluence rates. The chlorophyll concentration was 26 mg l^{-1}. (○) 1900 µmol photon m^{-2} s^{-1}, (●) 2300 µmol photon m^{-2} s^{-1}, (△) 3500 µmol photon m^{-2} s^{-1}, (▲) 4800 µmol photon m^{-2} s^{-1}. 100% activity corresponds to 617 µmol O_2 h^{-1} mg^{-1} chl.a

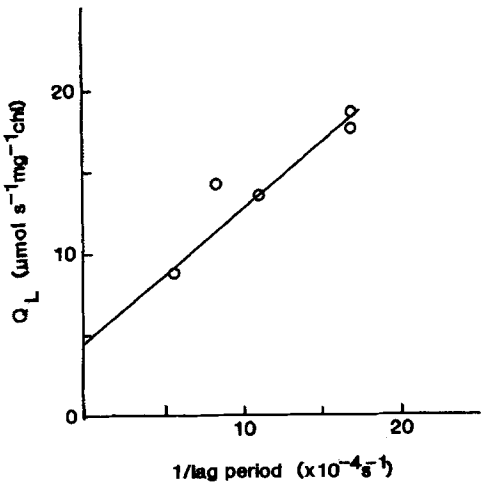

Fig. 3. Specific light absorption rate (*QL*) vs. the reciprocal of the lag period before the commencement of photoinhibition in *P. cruentum* cultures exposed to various fluence rates

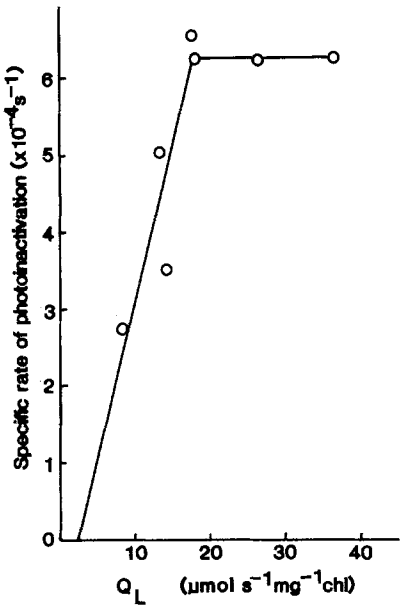

Fig. 4. Specific rate of photoinhibition of *P. cruentum* cultures vs. the specific light absorption rate. The specific rate of photoinhibition was calculated from the slope of the plot between ln (Oxygen Production Rate) and the time of exposure to strong light

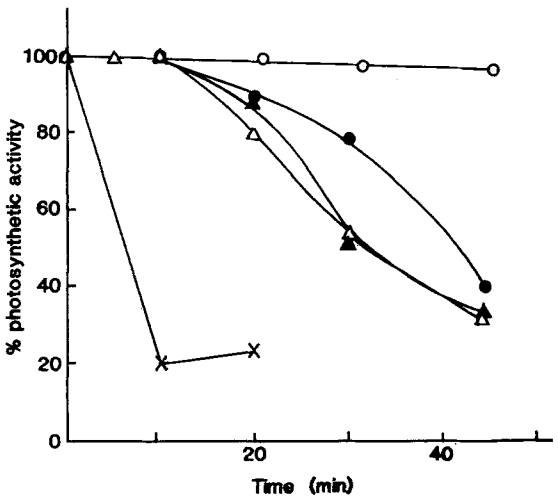

Fig. 5. The effect of temperature on the photosynthetic activity of *P. cruentum* cultures exposed to 2300 μmol photon m^{-2} s^{-1} light. Chlorophyll concentration was 13 mg chl.a. (○) 15°C, (●) 20°C, (▲) 25°C, (△) 30°C and (×) 35°C. 100% activity corresponds to 600 μmol O_2 h^{-1} mg^{-1} chl.a

The rate of reduction in photosynthetic activity due to high fluence rate as described in Figs. 1 and 2 appears to be first-order kinetics (exponential). The specific rate of photoinhibition could thus be estimated from the slope of the plot between ln (Oxygen Production Rate) and the time of exposure to high light. The plot in Fig. 4 is an attempt to correlate the specific rate of photoinhibition and the specific light absorption rate. As shown in Fig. 4, below a light absorption rate of 18 μmol photon s^{-1} mg^{-1} chl.a a nearly linear relationship is observed (correlation 0.888). The reciprocal of the slope (2.56×10^4 μmol photon s^{-1} mg^{-1} chl.a) gives the estimated photon required to inactivate instantaneously 1 mg of chlorophyll a. The plot intercept at

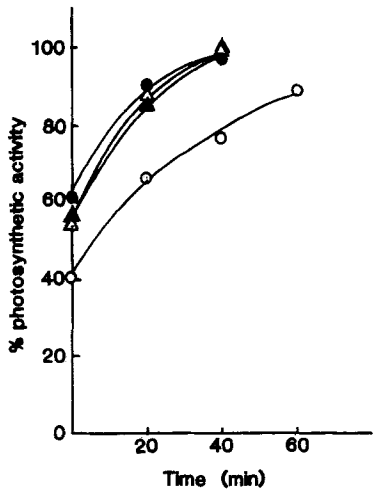

Fig. 6. Recovery of photosynthetic activity of photoinhibited *P. cruentum* cultures in low light (90 μmol photon m^{-2} s^{-1}), after exposure to various high fluence rates. (▲) 26 mg chl.a l^{-1}, 2300 μmol photon m^{-2} s^{-1}, (△) 26 mg chl.a l^{-1}, 3500 μmol photon m^{-2} s^{-1}, (○) 26 mg chl.a l^{-1}, 4800 μmol photon m^{-2} s^{-1}, (●) 13 mg chl.a l^{-1}, 4800 μmol photon m^{-2} s^{-1}

2.29 μmol photon s^{-1} mg^{-1} chl.a on the abscissa indicates the maximal light absorption rate where no photoinhibition can be detected. Above the light absorption rate of 18 μmol photon s^{-1} s^{-1} chl.a, the specific rates of photoinhibition remained constant at 6.2×10^{-4} s^{-1}, implying that light saturation of the cells occurred at these light absorption rates and that part of the light passed through the cell suspension.

Effect of temperature

Temperature had a profound effect on the sensitivity of the *P. cruentum* cells to high fluence rate (Fig. 5). Cells were grown at 25° C and incubated at different temperature during their exposure to high fluence rate. Incubation at low temperature (15°C) showed protective effect and no reduction in oxygen production rate was observed after 45 min of exposure to high fluence rate. When the incubation temperature was further increased to 35° C, a drastic incresae in the sensitivity of the cells to high fluence rate was observed, resulting in a drop by 84% after only first 10 min of exposure to 2300 μmol photon m^{-2} s^{-1}. Thereafter the photosynthetic activity was maintained at about the same level.

Recovery of photosynthetic activity

The ability of *P. cruentum* cells to recover from photoinhibition was investigated by incubating cells photoinhibited to about 50% of the original level of photosynthetic activity in dim light (90 μmol photon m^{-2} s^{-1}) and in total darkness. Oxygen production rate was measured at 20-min intervals. As shown in Fig. 6, the photosynthetic activity of cells subjected to light intensities of 2300 to 4800 μmol photon m^{-2} s^{-1} recovered steadily within the first 20 min of incubation in dim light. The same initial recovery rate was observed in cultures incubated in darkness (data not shown). The recovery of photosynthetic activities continued and was almost completed after 60 min of incubation in dim light. The specific rate of recovery of the photosynthetic activity

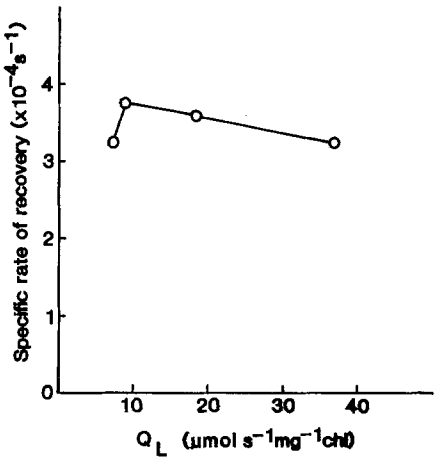

Fig. 7. Specific rate of recovery of photosynthetic activity of photoinhibited *P. cruentum* cultures (recovery conditions as in Fig. 6). Specific rate of recovery is defined as the fraction of recovery of photosynthetic activity over a unit period of time

defined as the fraction of recovery of photosynthetic activity per unit time of *P. cruentum* cells after exposure to various high fluence rate was estimated from the slope of the plot of ln (Oxygen Production Rate) vs time. The initial rates of recovery were around 3.5×10^{-4} s^{-1} in the first 20 min, and were independent of the light intensity to which the cells were exposured during the photoinhibitory treatment (Fig. 7).

Discussion

A lag period before the exponential decline in photosynthetic activity of algae cultures exposed to high fluence rate was also observed by other workers (Bjorkman et al. 1972; Jones and Kok 1966a, b; Kok 1956; Powles and Thorne 1981). However, the reason for such a lag in photoinhibition was not discussed. In the present study, a linear relationship between the specific rate of photon absorption (μmol photon s^{-1} mg^{-1} chlorophyll (chl.)a) by *P. cruentum* cells and the reciprocal of lag period before the commencement of photoinhibition is clearly demonstrated (Fig. 3). The slope of the plot (9235.8 μmol photon mg^{-1} chl.a) in Fig. 3 gives the theoretical photon number required to be absorbed by *P. cruentum* cells to initiate deactivation of the photosynthetic activity, resulting in apparent photoinhibition. This initial destabilisation process by light photons appears to be accumulative within the range of fluence rates and time frame studied (Figs. 1 and 2). In longer time frames, recovery from photoinhibition may set in and will complicate the matter. It has been proposed that photoinhibition of algae is caused by loss of the 32000 dalton herbicide-binding polypeptide at photosystem II (Kyle et al. 1983, 1984; Matto et al. 1984; Trebst and Draber 1986; Wettern and Galling 1985). Thus, the initial lag before apparent photoinhibition may represent the accumulative loss of the 32000 dalton polypeptide or other components of the PSII unit until a critical fraction was reached. The minimum specific light absorption rate required to initiate photoinhibition in a *P. cruentum* culture is 4.26 μmol photon s^{-1} mg^{-1} chl.a (Fig. 3). This value may represent the point where the rate of recovery from photoinhibition balances the rate of photoinhibition. We may deduce that in order to prevent photoinhibition in an outdoor *P. cruentum* culture at noon, when solar fluence rate is about 2000 μmol photon m^{-2} s^{-1}, the minimal cell concentration should be around 469.5 mg chl.a m^{-2}. Thus for an algal culture system with a volume to illuminated surface area ratio of 0.1 (a rectangular pond of 100 m × 10 m × 0.1 m), the minimal cell density to alleviate photoinhibitory effect is 4695 mg chl.a m^{-2} or 4.7 mg chl.a l^{-1}, provided the agitation in the culture system is sufficient to ensure an even distribution of light among the cells (Lee and Pirt 1981).

The linear relationship between the specific rate of photoinhibition and the specific light absorption rate, as evident from Fig. 4, suggests a direct correlation between the two variables. It is evidently the quantity of light received by each algal cell, which matter, rather than the fluence rate measured at the surface of a culture, which has so often been used to describe the relationship between photoinhibition and light intensity (Kyle and Ohad 1986; Powles 1984). The reciprocal of the slope of the plot in Fig. 4 gives the number of photon required to completely inactivate instantaneously 1 mg of chlorophyll a in *P. cruentum* cells (2.5×10^4 μmol photon m g^{-1} chl.a). In practice it is not possible to achieve such high value. Thus there is always a possibility for photoinhibited *P. cruentum* cells to recover in a culture system which provides a low-light region. The intercept on the abscissa, in the plot in Fig. 4, gives the minimum specific light absorption rate where photoinhibition could be initiated in a *P. cruentum* culture. This value estimated from Fig. 4 as 2.29 μmol photon s^{-1} mg^{-1} chl.a, is lower than the value obtained from Fig. 3 (4.26 μmol photon s^{-1} mg^{-1} chl.a), but the difference is well within the limits of sensitivity of our measurements.

Incubation temperatures may have two effects on the sensitivity of *P. cruentum* cells to high fluence rate. Increasing temperatures may destabilise molecules of the photosynthetic apparatus and other vital cellular components. On the other hand, a photoinhibition effect has been attributed to the accumulation of light energy which cannot be dissipated by biological, biochemical or biophysical means (Powles 1984). A decrease in growth temperature would slow down biochemical reactions, resulting in more undissipated energy. The optimal growth temperature of *P. cruentum* ld is 25° C. The effects of temperature on the stability of structural molecules of *P. cruentum* seems to override the rate of light dissipation. Another possible explanation is that if the turnover of the photosynthetic components is a function of electrons transferred through the photosynthetic electron transfer chain, then the protective effect of low temperature (15° C) could be explained by the slower rate of electron passing through the photosynthetic component. At higher temperature, higher oxygen production rate was observed (Vonshak et al. 1985) indicating that more electrons were being transferred, thus resulting in faster degradation of photosynthetic component and stronger photoinhibitory effect. It is worth mentioning that at extremely low temperature (2° C) chilling-enhanced photoinhibition in isolated barley protoplast was observed (Horton and Hague 1986).

Photoinhibited *P. cruentum* cells recovered readily when they were transferred to low-light conditions (Fig. 6) or complete darkness and the initial rates of recovery of cells photoinhibited by various fluence rates were comparable (Fig. 7). This may imply that the repair mechanisms for photodenatured component of photosystem is not sensitive to photoinhibitory effect. The fast recovery from photoinhibi-

tion suggests that the photoinhibitory effect is at least partly due to inactivation rather than degradation of photosynthetic components, as suggested by other reports (Kyle and Ohad 1986; Samuelsson et al. 1985; Vonshak et al. 1988).

Acknowledgements. This collaborative research was made possible through the Exchange Programme between the Science Council of Singapore and the Israeli Academy of Science and Humanity. The authors wish to thank the International Center for Desert Studies in the Jacob Baustein Institute for Desert Research for supporting this work.

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Received November 7, 1987/Accepted July 7, 1988