Photo-inhibition of Phytoplankton Photosynthesis as a Function of Exposure Time*

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Abstract: The photo-inhibition of phytoplankton photosynthesis at higher intensities was examined with a cultured marine diatom, *Phaeodactylum tricornutum*, and natural samples. The question was to determine whether photo-inhibition results from excretion of photosynthetic products from cell or from an actual decrease in photosynthetic rate.

*P. tricornutum* cultured at 15 klux showed very little photo-inhibition up to 70 klux, and, in the sample cultured at 1 klux, most marked photo-inhibition was observed in 3 hours experiment. Extracellular release was less than 30% of particulate fixation, and did not show any extreme increase to supplement photosynthesis depression at higher light intensities. When the photosynthesis was measured during 10 minutes, both samples showed no photo-inhibition. The photosynthesis by low light sample lost the linearity of time-course with prolonged exposure at high light intensity. Observed photo-inhibition, therefore, we explained with the actual decrease in photosynthetic rate. Similar photo-inhibition could be seen in marine phytoplankton samples concentrated by filtration.

1. Introduction

Characteristics of the light-photosynthesis give an information necessary for understanding of physiological status of photosynthetic organisms, and make possible the indirect estimation of primary production. Many studies on this line have shown that photosynthetic responses to light can be classified into sun and shade patterns. These characteristics of photosynthesis have been known to change with growth conditions (Ryther and Menzel, 1959; Steemann Nielsen and Hansen, 1961) and species (Jörgensen, 1969). Another characteristic phenomenon on this line is the inhibitory effect of high light upon photosynthesis observable in natural waters (Goldman et al., 1963; Ichimura and Aruga, 1964; Ichimura et al., 1968) and algal cultures (Ryther, 1956). This phenomenon of photo-inhibition is important, when we consider the photosynthesis of microorganisms under field conditions where microorganisms sometimes receive strong sunlight. Though we have some analytical reports on the photo-inhibition (Myers and Burr, 1940; McAllister, 1961; Jones and Kok, 1967), the mechanism has not yet been explained satisfactorily.

The present authors sought to determine whether the photo-inhibition of phytoplankton results from the excretion of photo-synthesized products from cells or from an actual decrease in photosynthetic rate.

2. Methods

*Phaeodactylum tricornutum* was used for laboratory experiments, and was cultured with ASP 2 medium (Provasoli et al., 1957) autotrophically under conditions of continuous illumination at a light intensity of 1 klux or 15 klux at 20°C. The air containing 0.5% CO₂ was also continuously supplied. Algal samples were harvested by centrifugation at the middle of their logarithmic growth phase. The latter was determined from the cell number. After washing the algal sample twice with fresh
medium, it was resuspended into new medium containing about 400 mgC/l as sodium carbonate to avoid carbonate deficiency during the photosynthesis experiment. The concentration of cells was adjusted to 5 ~ 10 x 10^6 cells/ml.

Field experiments were done with marine and fresh water samples. Marine samples were collected from Sagami Bay on 2 ~ 9 June, 1970, during the research cruise KT-70-5, and were brought back to Shimoda Marine Biological Station of Tokyo Kyoiku University and experiments performed within a few hours after sampling. A portion of water sample was concentrated 10 to 20 times with gentle suction (< 5 cm Hg) through AA type Millipore\textsuperscript{®} Filter (pore size: 0.8\(\mu\)) by using a specially designed funnel (filtering diameter: 80 mm); the photosynthetic rate was then measured on the concentrated sample. Fresh water samples were collected from a eutrophic pond, Go-no-ike, Ibaragi Prefecture, on 7 July, 1970. Photosynthetic rate was measured with both concentrated and natural samples using the same method as with the marine samples.

Photosynthesis was measured at 20°C using the \(^{14}\text{C}\) and oxygen electrode techniques. Before start the photosynthetic experiments, all samples were kept in the dark for 1 ~ 6 hours.

In \(^{14}\text{C}\) method (Watt, 1966), the light source was a 500W incandescent lamp (Iwasaki Electrics Co. Ltd., 3200°K). Between the light source and the experimental vessel, a water filter (5 cm thickness) was placed to exclude heat radiation. The light intensity was regulated by use of fine metal mesh screens, and was measured with a Toshiba No. 5 selenium photometer. After exposure of phytoplankton to \(^{14}\text{C}\) carbonate, the particulates in 2 ~ 3 ml suspension was removed with gentle suction (< 5 cm Hg) through HA Millipore\textsuperscript{®} Filter (pore size: 0.45\(\mu\)). During the filtration care was taken, as far as possible, to exclude further light. The filtrate was then put into a Thunberg tube and a few drops of 35% HCl were added; the side arm of the Thunberg tube contained 10 N NaOH. After closing the top and shaking, the Thunberg tube was left for 30 minutes to remove inorganic \(^{14}\text{C}\). The radioactivity of each fraction, particulate and soluble, was measured by liquid scintillation counter (Aloka LSC-601).

The oxygen electrode technique was carried out by use of a Clark type electrode (Yellow Springs Instruments Model 4004). Signals were recorded on a Riken Denki high speed recorder model SP-H5V. The experimental vessel was made of lucite plastic and was a cube of 1 cm in light pass. The electrode was inserted into the vessel one side. On the other side, was applied parallel light from an iodine lamp (Ushio, JCD 650). A couple of water filter (5 cm thickness) and infrared-cut filter was inserted between the vessel and the light source. The color temperature was adjusted by changing the applied voltage to the lamp so as to take equal to that in the isotope experiments.

3. Results

**Photosynthetic characteristics of P. tricornutum**

In Fig. 1 the light-photosynthesis curves of

![Graph](image)

*Fig. 1. Light-photosynthesis relations of P. tricornutum cultured at continuous illumination of 1 and 15 klux at 20°C, measured by \(^{14}\text{C}\) technique within 3 hours exposure. Symbols, Extracellular, Particulate and Total, by each curve mean extracellular release, particulate fixation and the sum of former two, respectively.*
*P. tricornutum* cultured at 15 and 1 klux are shown. These curves were obtained from 3 hours incubation by the $^{14}$C technique; solid line represents the photosynthesized $^{14}$C-fraction retained in cells. Aquatic ecologists have measured this fraction as photosynthesis of phytoplankton. The dotted line is the $^{14}$C lost from cells to the surrounding medium by either excretion or cell-lysis. We called the former and the latter particulate fixation and extracellular release in accordance with Watt (1966), and sum of the two was termed "total". The particulate fixation in samples grown under high light intensity showed light saturation at 10–20 klux, with very little photo-inhibition up to 70 klux, maximum intensity used. In the case of the samples grown under low light intensity, light saturation occurred at a light intensity of about 3 klux, and photo-inhibition was evident at only 7 klux. The particulate fixation in the latter sample decreased to 50 and 2% of the maximum value at 14 and 56 klux light intensity, respectively. During 3 hours of incubation, extracellular release was less than 30% of the particulate fixation at each light intensity, except at 56 klux in the latter sample, where the fraction of extracellular release became a high percentage because of the low particulate fixation. The pattern of light-extracellular release was similar to those of particulate fixation in each experiment. We, therefore, can also see the photo-inhibition in the total fraction curves for the samples grown under low light intensity. These results show that the observed photo-inhibition cannot be explained by increases in extracellular release of photosynthesized carbon. This is also to be expected from the results of Watt (1966) and Hellebust (1965).

Fig. 2 shows the light-photosynthesis curves of the same samples with the same cell density used for Fig. 1 experiment. They were measured by the oxygen electrode technique within 10 minutes exposure at each light intensity. Sun- and shade-type responses were again evident, but neither gross- nor net-photosynthesis showed any photo-inhibition even at 40 klux. The differences between two experiments, Figs. 1 and 2, were two; the methods for measurement of photosynthesis, and exposure time. Myers and Burr (1940) reported that the oxygen method gave lower results than real photosynthesis at higher light intensities because of oxygen consumption by photo-oxidation, and that the photo-oxidation became active with prolonged time. McAllister (1961), in 4 hours experiments, observed that photo-inhibition appeared more pronounced in results obtained with the oxygen method than with the $^{14}$C method. Considering their results, our data measured by oxygen method (Fig. 2) may show

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**Fig. 2.** Light-photosynthesis relations of the same sample used in Fig. 1 experiment at 20°C, measured by oxygen electrode technique within 10 minutes exposure. Symbols, $P_g$, $P_n$ and $R$ mean gross-photosynthesis, net-photosynthesis and respiration, respectively.
Fig. 3. Time course of photosynthesis of the same sample used in Fig. 1 experiment.

the initial lack of inhibition of photosynthesis because of short exposure time.

Using the $^{14}$C method, we followed the time courses of photosynthesis with the samples used for Figs. 1 and 2. Results are shown in Fig. 3. The photosynthesis of 15 klux cultured sample followed a linear time course at both 3.4 and 70 klux. Extracellular release also increased with time, and its amount fluctuated between 5 and 30% of particulate fixation. The sample cultured at 1 klux also showed a linear time course of photosynthesis at 1 klux. At 24 klux, however, the photosynthetic rate decreased with time from prolonged exposure to high light intensity, and after 3 hours it became about 1/10 of the initial rate. About 2 hours after the incubation, the release of extracellular $^{14}$C became high, but only 20% of the apparent inhibition could be attributed to extracellular release. When the rate (1,250 cpm/30 minutes) of particulate fixation after the first 30 minutes of incubation at 24 klux was extrapolated to 3 hours, a rate of 7,750 cpm/3 hours was expected. This rate was similar to the maximum rate of photosynthesis of P. tricornutum cultured at 1 klux (i.e. 7,750 cpm/3 hours) (Fig. 1). Thus, if the rate in the initial 30 minutes incubation could be maintained throughout exposure time (3 hours), the pattern of light-photosynthesis curve would be identical with that obtained from the electrode experiment. Considering these results, it became clear that photo-inhibition at higher light intensities occurred due to a decrease in photosynthetic rate with time, and that this phenomenon was observed preferentially in samples cultured under low light condition.

Photosynthetic characteristics of natural phytoplankton

The first field experiments used marine samples from Sagami Bay. The light intensity in 30 m layer was few percent of surface illumination. The chlorophyll content of samples was 0.5~5 Chl a mg/m$^3$, and the dominant species were Chaetoceros sp. or Exuvella marina. Photosynthesis was measured under two conditions, non-concentrated and concentrated, by the $^{14}$C method for 3 hours. The concentrated sample was prepared just before the experiment. Results are shown in Fig. 4, where the data on the non-concentrated sample were plotted relatively after adjusting the highest value to that of the concentrated one. Photonsynthetic rates in non-concentrated samples incubated at 40 klux were 0 (0 m) to 30% (30 m) less than the maximum rate. Occurrence of photo-inhibi-

Fig. 4. Light-photosynthesis relations of samples taken from 0, 10 and 30 m layers of Sagami Bay on 3 June 1970, measured by $^{14}$C technique within 3 hours exposure. Symbols as in Fig. 1. Circles are the data from concentrated samples and triangles from non-concentrated samples.
tion was not marked in either sample. However, the inhibitory effect appeared more clearly in the 30 m sample than the surface one.

More striking photo-inhibition was observed in concentrated samples, where the depression fraction reached about 30% of photosynthesis maximum at 26 klux and 80~90% at 56 klux. However, the effect of photo-inhibition was not significantly different in the samples obtained from various depths. It must be also noted that extracellular release was 30~160% of the particulate fixation, strikingly high compared with that in cultured samples (c.f. Figs. 1 and 3). These phenomena were most marked in the sample of 10 m on June 1970, which is shown in Fig. 5. Photosynthesis in the non-concentrated sample was scarcely suppressed at 40 klux. The photosynthesis of concentrated sample, however, was suppressed to 10~20% of the maximum value under light intensities greater than 20 klux.

Time course of photosynthesis was also examined with the concentrated sample at two light conditions, 3 and 54 klux (Fig. 5). Under 3 klux, the time course was linear during 3 hours of incubation; at 54 klux, however, photosynthesis slowed down after 30 minutes of incubation as similarly to those of *P. tricornutum* grown under low light conditions. Further, the photosynthetic rate estimated from the experiment for the initial 30 minutes of incubation was only 35% of the maximum value obtained with the same sample. This indicates that the slow-down of photosynthesis began before 30 minutes of incubation had elapsed.

The second field experiment was carried out with fresh water samples collected from the surface and 70 cm at Go-no-ike. The light penetrating to a 70 cm depth was about 10% of the surface value. The chlorophyll concentration at 0 and 70 cm was 100 and 50 Chl a mg/m³, respectively. *Chlamydomonas* sp. and *Anabaena* sp. were dominant in both samples. In both samples the photosynthetic rate was measured by ¹⁴C and oxygen electrode methods. In the former method, a non-concentrated sample was used and incubated for 3 hours. In the latter method, a non-concentrated sample was used and incubated for 3 hours. In the latter method, the sample was concentrated 10 to 20 times by centrifugation, before measuring the photosynthesis. Results are shown in Fig. 6. The light-photosynthesis curves observed by the electrode method were the sun-type, though the sample from 70 cm showed a higher photosynthetic rate than that of 0 cm at low light region. In either sample, no photo-inhibition was observed up to 50 klux. The results from ¹⁴C method are plotted on a relative scale after adjusting the highest
rate to that of the electrode method. They agreed well with the data of the electrode method especially in the 70 cm sample, and showed no photo-inhibition even at 50 klux. In the time course experiment, the photosynthesis of both samples, 0 and 70 cm, was linear with time at 50 klux during 4 hours.

4. Discussion

When the photo-inhibition at high light intensity did not occur, light-photosynthesis curves measured by either CO₂-fixation or oxygen evolution took the same pattern in cultured algal cells (Figs. 1 and 2) and in those of natural habitat (Fig. 6). On occurrence of the photo-inhibition, the time course of the photosynthesis showed always a gradual decline in the velocity (Figs. 2 and 5). These clearly indicate that a different appearance of the photo-inhibition depending on two measuring methods (Fig. 1 vs 2) is not due to difference in the index measured, CO₂-fixation and O₂-evolution, but instead to the time dependence of the inhibitory effect of strong light. Many data on photo-inhibition of photosynthesis in the natural habitat have been reported (Goldman et al., 1963; Ichimura and Aruga, 1964; Aruga, 1965; Ichimura et al., 1968). However, in these studies the photosynthesis was measured using the incubation having time-spans from minute-scale to a full day. If the photo-inhibition always varies with time of strong light exposure, it, therefore, is difficult to compare data from different sources. We must be careful on the time course when we measure the photosynthesis and express it in terms of velocity.

In cultured P. tricornutum, occurrence of photo-inhibition is far more marked in cells grown under low light than those under high light (Fig. 1). Photosynthetic rates in weak light region, however, were far larger in cells grown under low light than those in cells raised under high light (Fig. 2). These two characteristics of photosynthesis seems a different appearance of the same event induced by strong light. Photosynthetic system of the cells grown under high light could have been photo-inhibited at the site of, or a site closely related to, the photochemical reaction in a way as suggested by Jones and Kok (1967) or by Steemann Nielsen and Jørgensen (1968) during algal growth. Thus, in experimental conditions, the photosynthesis by cells grown under high light would take a lower velocity in low light region, but it would be more resistant to strong irradiation than that of cells grown under low light. This agrees with the characteristics of photosynthesis in natural samples obtained from eutrophic pond (Fig. 6).

We also found morphological changes in plastids in vivo which probably related to the photo-inhibition phenomena. Before and after the experiment in low light, the plastids of P. tricornutum were examined by microscope. When a sample was put in 1 klux light, the plastids did not show any change in size, but those in 24 klux diminished to 65% of their initial size during 3 hours incubation. This shortened plastid was the same in size as that cultured at light intensities stronger than 10 klux. The shrinkage was also found reversible with dependence on light intensity.

In cultured P. tricornutum and natural samples, extracellular release had no quantitative relation to the photo-inhibition phenomenon. However, it is known that extracellular release depends on nutrient and light conditions, growth phase and the health condition of algae and species (Hellebust, 1965; Watt, 1966). These factors are also closely related to photo-inhibition, and consequently, extracellular release may have some qualitative relations to photo-inhibition, even though the present data did not show them.

After concentrating the natural marine phytoplankton by gentle filtration, photosynthesis showed striking photo-inhibition at higher light intensity in comparison with non-concentrated samples. Microscopical observation suggested that phytoplankton in concentrated samples was much damaged before the photosynthesis experiment. Physical damage of algal cells also increases susceptibility to the strong light.

In this experiment, samples from mesotrophic environments (Chaetoceros sp. or Exuvieilla marina dominant) were far more easily damaged by mild treatment (gentle filtration or centrifugation) than cultured diatoms or phytoplankton.
from cutrophic pond. Though photosynthetic rates of damaged cells did not decreased during at least 3 hours incubation under weak light, the extracellular release of photosynthetic products became remarkably greater. These results suggest that on measurement of oceanic photosynthesis with 14C method, we must be careful in the treatment of phytoplankton after light-exposure so as to avoid physical breakage of algal cells, and also that attention to solubilized photosynthetic products is necessary. These will be especially important in samples dominated with fragile algae which are known for easier broken by treatments such as filtration than diatom.

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References


植物プランクトン光合成の強光阻害

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要旨 強光照射によりプランクトンの光合成がしばしば著しく阻害される事は良く知られている。われわれは、種々の条件下で培養した藻類 Phaeodactylum tricornutum 及び、野外試料として、相模湾下で得た浮遊藻類 (Chaetoceros. sp.) 及び茨城県神ノ池で採集した緑藻、潮藻試料 (Chlamydomonas sp., Anabaena sp.) についてその光合成に対する強光照射の阻害作用を 14C 法及び酸素電極法を用いて調べた。

得られた結果は

1. 強光の阻害作用の発現には、単に光強度のみでなく、照射時間が強く関係すること、従って、強光阻害が見られる場合は光合成反応は非直線的に進行する。

2. shade-type の光合成速度 - 光強度関係を示す細胞で光合成の強光阻害が顕著に生ずるほか、細胞の物理的損傷も、強光阻害現象を誘起する。

3. 光合成産物の細胞外放出による見かけ上の光合成速度の低下において、強光阻害現象は説明出来ない。ことなど示した。これらの結果に基づいて、光合成の強光阻害現象の生因についても若干の議論を試みた。