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Photoinhibition of Chloroplast Reactions. I. Kinetics and Action Spectra[1]

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Summary. A study was made of photoinhibition of spinach chloroplast reactions. The kinetics and spectral characteristics of the photoinhibition over a range between 230 and 700 mμ have been examined. The decline of activity due to preillumination was independent of wavelength, and dependent upon the number of quanta applied, not upon the rate of application. The effectiveness spectra of photoinhibition indicate that active ultraviolet light is absorbed by a pigment which is not a normal light absorber for photosynthesis and acts with a high quantum efficiency (> 0.1) for photoinhibition.

Active visible light is absorbed by the pigments which sensitize photosynthesis (chlorophyll, carotenoids). A very low quantum efficiency (about 10^{-4}) was observed for the photoinhibition with visible light.

The action spectrum of the photoinhibition of dye reduction by chloroplasts and lyophylized *Anacystis* cells indicated that the damage caused by visible light is due to quanta absorbed by photosystem II. However, since system I might not be involved in dye reduction, the spectra may reflect only damage to photosystem II.

Photoinhibition of photosynthesis has been defined as the debilitating effect of high intensities of visible light upon the photosynthetic capability of green organisms (17). The term is extended in this study to include the effects of ultraviolet light upon the photosynthetic reactions of chloroplasts. The kinetics of photoinhibition in whole cells have been studied using visible (16, 19, 24, 27) and ultraviolet light (2, 13, 25). In both cases, secondary metabolic effects prevented a quantitative analysis. Chloroplasts are more suitable for such a study because of their relative independence from nonphotosynthetic metabolic processes. Definitive data concerning the spectral characteristics of pigments which sensitize photoinhibition are lacking, although attempts to collect these have been made (9). This study was undertaken to fill this gap in the understanding of the damaging effects of large doses of light of selected wavelengths upon the photochemical activity of chloroplasts.

Methods

Spinach chloroplasts were prepared as described by Hill and Walker (12) from leaves grown in a greenhouse or obtained in local markets.

Except where otherwise noted, the same reaction vessel was used for preliminary exposure of the particles to photoinhibitory light and the subsequent assay of activity. The microvessel was made from an aluminum block, 35 × 25 × 3 mm. A slot was cut 3 mm wide by 18 mm deep and covered at the sides with quartz coverslips. A 30 μl aliquot of chloroplasts filled the slot 3.3 mm deep yielding a 0.1 cm^2 illuminated area with an optical path 3 mm in length. The small surface area increased the incident intensities attainable with available sources. The relatively large amount of aluminum in contact with the suspension greatly increased the rate of heat removal from the sample.

Light for photoinhibition was obtained from a 2000-w Xenon arc lamp. For experiments using filters to isolate desired wavelength bands, a series of quartz lenses were used to collimate the beam. The beam was passed through 6.5 cm of H_2O, the filters, and then focussed onto the microvessel. Incident light energy, as well as the fraction absorbed by the chloroplasts, was measured with a thermopile placed directly behind the vessel. The thermopile

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[3] Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPIP, 2,6-dichlorophenolindophenol; PQ, plastoquinone; PMS, phenazine methosulphate; PPNR photosynthetic pyridine nucleotide reductase (ferredoxin).

was calibrated at 328 mμ using a ferrioxalate actinometer fluid (10) in the same microvessel that was used in all experiments. The rear quartz window of the microvessel was frosted in an attempt to reduce the effect of scattering in the fractional absorption (α) measurement. In other experiments, the Xenon arc was focussed on the entrance slit of a 500 mm focal length B and L grating (blazed at 300 mμ) monochromator. The optical frame of the entrance condenser lens was focused at the front surface of the microvessel. For measurements of incident energy, the microvessel was removed and the thermopile placed in the same position.

Photosynthetic activity was assayed in a dual beam spectrophotometer which recorded the OD of the sample during actinic illumination (22). Light intensity during the assay was varied by neutral density filters in the actinic light beam. The measuring beam was 600 mμ for DPIP (ε_m = 20,000/cm pH 7.6) and at 340 mμ for NADPH (ε_m = 6200/cm)[3].

In a typical experiment, 30 μl of chloroplast suspension containing 1 μg of chlorophyll in Tris-HCl buffer (0.05 M, pH 7.6) was pipetted into the microvessel at room temperature. The suspension was irradiated with the desired dosage of inhibitory light, then 10 μl of the appropriate assay mixture were added and activity measured in the spectrophotometer. A new sample was used for each exposure period. Unless otherwise stated, the final reaction mixture of 40 μl total volume for the DPIP reduction assay contained, in μmoles: 1.5 Tris-HCl (pH 7.6) and 0.01 DPIP. The 40 μl reaction mixture for the assay of NADP reduction contained, in μmoles: 1 Tris-HCl (pH 7.6); 0.02 NADP; 0.050 ADP; 0.070 $MgCl_2$; 0.050 K_2HPO_4; and saturating PPNR (35 μl/ml of final mixture).

In experiments concerning the effect of O_2, a 1-ml sample was placed in a quartz Thunberg cuvette. O_2 was removed before irradiation by adding 10 μmoles glucose and 100 μg glucose oxidase, quickly evacuating the cuvette and flushing with nitrogen. After repeating the evacuation and flushing 10 times, the vessel was placed in the dark at room temperature for 15 minutes before irradiation. After irradiation the vessel was opened and the dye added and mixed aerobically.

Preparations of *Anacystis nidulans* were made from 2- to 4-day old cultures which were grown at 25° under fluorescent light of about 1000 ft-c intensity and were bubbled with 1 % CO_2 in air. Lyophylization procedures followed closely those used by Schwartz for *Chlorella* (26). Cells from the culture were washed twice in distilled water, suspended in a minimum of distilled water and frozen in a dry ice-acetone bath. Lyophylization usually took 1 to 2 hours. For use, 1 mg of the dried preparation was resuspended in 1 ml of reaction medium (0.5 M sucrose 0.05 M Tris-HCl and 0.01 M NaCl at pH 7.2). Irradiation and the assay of dye reduction activity were carried out as for chloroplasts.

Results

Before studying the kinetics and wavelength dependence of photoinhibition some characteristics of photoinhibition under the conditions used in these experiments were determined.

Photoinhibition of chloroplast reactions has been reported to occur at liquid nitrogen temperature, but at a rate about 5 times slower than at room temperature (21). The lower rate of photoinhibition at liquid nitrogen temperature was thought to be caused by the difference in light path due to the ice crystals in the suspension. Experiments run at 23° and 3° using light of different wavelength regions were found to have Q_{10}'s of from 1.0 to 1.06 (table I). Photoinhibition therefore, does not involve a temperature-dependent dark reaction.

Table I. *Temperature Independence of Photoinhibition of Chloroplasts in Various Wavelength Regions*

Assay: DPIP reduction in saturating, red actinic light at room temperature. Numbers given are half-times of decay in seconds, derived from semilog plots of the decay curves. Wavelength regions were isolated by filters.

Wavelength region	23°	3°	Q_{10}
230–390 mμ	1.0	1.2	1.06
360–525 mμ	7.0	7.0	1.0
>570	7.5	7.5	1.0

The time course of photoinhibition in UV and visible light is independent of O_2 concentration (table II). The mechanism of photoinhibition therefore, cannot be a simple photooxidation involving molecular O_2. This lack of O_2 dependence clearly distinguishes photoinhibition from the bleaching of the chlorophyll which is dependent upon O_2 concentration (6, 27).

Chlorophyll bleaching and photoinhibition are different processes. Figure 1 shows a comparison of the relative rates of the 2 reactions under identical aerobic exposure conditions. The decrease of chlorophyll absorption follows a logarithmic decline, similar to the NADP reduction activity, but at a rate about 50 times slower both in red and blue light. Also, as was earlier observed with whole cells (16, 17), there is an initial lag period in the bleaching process, during which the normal photochemical activity of the chloroplast is completely lost. Such

Table II. *O_2 Independence of Photoinhibition in Visible and UV Light*

Values are rates of DPIP reduction after exposure expressed as percent of the rate observed before exposure.

Preillumination	$+O_2$	$-O_2$
60 sec $\lambda > 400$ mμ	36 %	40 %
30 sec $\lambda < 400$ mμ	22	20

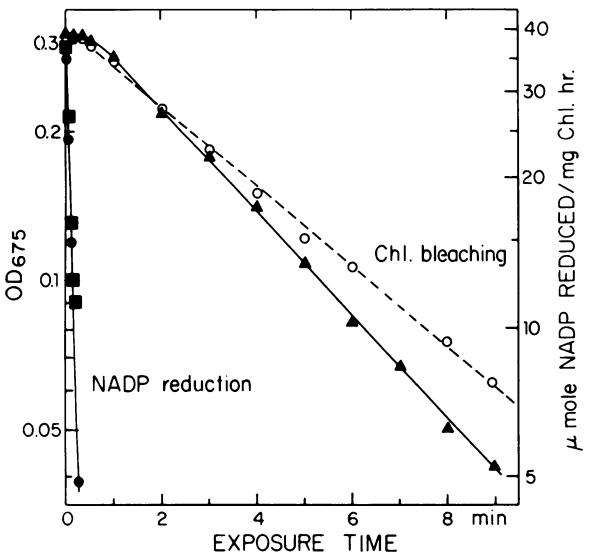

FIG. 1. Comparison of the decay of NADP reduction (closed circles, squares) and chlorophyll bleaching (open circles, triangles) during exposure to identical sources of strong visible light. Circles: blue light of 360–520 mμ, NADP decay curve from line 2, table III; triangles and squares: red light of > 570 mμ, NADP decay curve from line 5, table III. Assay: NADP as described in Methods. Chlorophyll bleaching measured in intact chloroplasts as decrease of absorbance at 678 mμ. Both assays using same preparation of chloroplasts at same concentration (1 μg Chl in 30 μl).

data support the contention that chlorophyll bleaching is a secondary reaction proceeding only after the photosynthetic capacity is lost.

Red light (> 570 mμ) causes a logarithmetic decrease in the absorption at 490 mμ with a half-time only slightly longer than that of the bleaching at 678 mμ. Thus, light absorbed by chlorophyll affects the bleaching of carotenoids and chlorophyll almost equally. This effect should be accounted for in proposals concerning the photoprotective function of carotenoids.

No evidence was found for the formation of a soluble inhibitory substance upon irradiation with UV light. Fresh chloroplasts added to an irradiated inactive sample were fully active. Thus, the effect of UV light is localized in a structure-bound chloroplast constituent. Forti and Jagendorf (7) found the same result in their study of the effect of visible light on photophosphorylation.

The manifestation of photoinhibition depends upon the rate of the photochemical reaction used to assay the activity of the photoinhibited chloroplasts. If the subsequent assay was made using weak light, the decline in activity brought about by exposure to the actinic beam began immediately and proceeded at a rate independent of the actinic intensity. If the subsequent assay of chloroplast activity was made with intensities above saturation, the decline in activity due to photoinhibition began only after a characteristic lag period and proceeded at a rate dependent upon the intensity: higher assay intensities showing higher activity for identical samples of photoinhibited chloroplasts. The lag period at high intensities has been reported for photoinhibition of O_2 evolution in whole algal cells (17). All experiments described in the following sections were carried out using weak intensities to assay photosynthetic activity, and as such, describe the decrease in the quantum yield.

Kinetics and Quantum Yield(s) of Photoinhibition. The rate of decline in activity of photosynthetic reactions has a logarithmic character with respect to time of exposure to the photoinhibitive light. This decline, studied previously by others, is in accord with the general exponential attenuation law:

$$R_t = R_o\, e^{-ct} \qquad \text{I}$$

where R_o is the initial rate, R_t is the rate after exposure to photoinhibitive light during time t; and c is a proportionality constant.

Table III. *Validity of Ixt Law for Photoinhibition of Dye Reduction and NADP Reduction by Red (λ > 570 mμ), Blue (360 to 520 mμ) and UV Light (328 mμ, half-band 15 mμ)*

Chloroplasts containing 1 μg of chlorophyll were irradiated in a 30 μl volume of 0.05 M Tris buffer (pH 7.6). After irradiation the remainder of the reaction mixture was added in 10 μl (reaction mixture as in Methods). Actinic light intensity was identical in all assays and was less than 20 % saturating. Saturated rate of NADP reduction, 162 μmoles/mg Chl hr.

The value of $\alpha\,\phi$ was calculated according to equation II.

μEin/min Inc.	Abs	Abs Quanta/ Chl/min	$t_{1/2}$ min	Quanta/ Chl for $t_{1/2}$	$\alpha\,\phi$ μEin^{-1}
		NADP Reduction			
Blue photoinhibitory light α = 47.5 %					
64.5	31.0	27,600	0.021	580	0.51
33.0	16.0	14,400	0.055	790	0.38
1.9	0.9	810	0.9	730	0.40
0.25	0.12	110	6.1	670	0.45
Red photoinhibitory light α = 23.5 %					
71.0	16.7	15,000	0.082	1230	0.12
3.9	0.92	830	1.67	1380	0.11
0.56	0.13	117	10.0	1170	0.12
		DCPIP Reduction			
Blue photoinhibitory light α = 52 %					
70.0	36.5	32,800	0.095	3120	0.10
35.0	18.2	16,400	0.2	3280	0.10
1.5	0.78	700	3.7	2600	0.12
0.22	0.115	105	10.9	1150	0.29
Red photoinhibitory light α = 21.5 %					
126.0	27.0	24,500	0.08	1950	0.07
68.0	14.6	13,200	0.155	2050	0.065
9.3	2.0	1,800	1.25	2250	0.06
2.0	0.43	390	4.8	1860	0.07
328 mμ photoinhibitory light α = 29 %					
0.097	0.0280	25	1.7	42.5	3.6
0.056	0.0168	15	3.1	46.5	3.45
0.034	0.0102	9.2	4.8	44.2	3.6
0.0175	0.00525	4.7	8.5	40.0	3.85

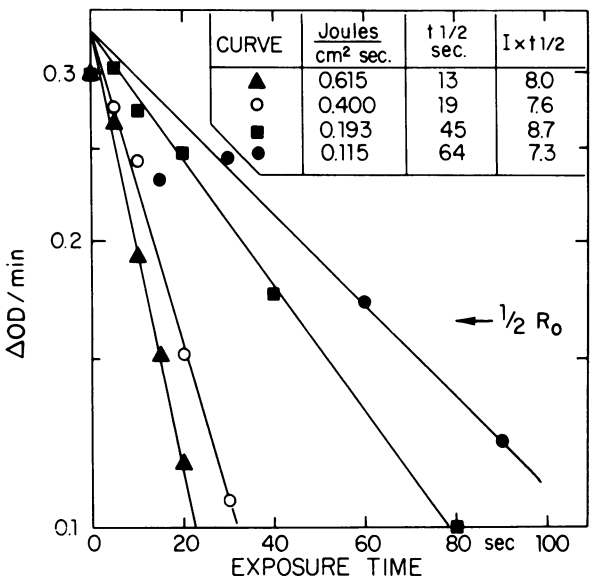

FIG. 2. Decay of dye reduction activity of chloroplasts during exposure to white light of various intensities. Dye reduction assayed as in Methods. Light from Xenon arc filtered through 4 layers of glass, 37 cm of water and wire screens. Intensity measured with calibrated thermopile.

To test for the reciprocity of time and intensity for photoinhibition the experiments shown in figure 2 were performed. Decay curves were measured with intensities of white photoinhibitory light varying over a 6-fold range. The data show a good fit to a straight line on a semilog plot. In spite of the 6-fold difference in intensity, there was no difference, within experimental error, in the amount of total energy necessary to decrease the rate of dye reduction to one-half the initial rate.

Results of further experiments using photoinhibitory light of selected wavelength regions and a wider variation of dosage are shown in table III. The protocol used in these experiments was identical with that given in figure 2, except that the incident intensity is expressed in μEinsteins per minute. The value of energy per quantum was chosen for the wavelength for which the product of the filter transmission and the chloroplast absorption was maximal. Reciprocity of intensity and time is evident in all wavelength regions. For the range of intensities and times for which this reciprocity holds, equation I now may be modified to:

$$R_t = R_o\, e^{-\phi\alpha I t} \qquad \text{II}$$

where I is the incident light intensity, α, the fractional absorption of the suspension and ϕ, the quantum yield of the photoinhibition. A plot of $\alpha\,\phi$ vs. wavelength then is the action spectrum of photoinhibition.

If the absorption of the suspension (αI) is known, it becomes possible to calculate the quantum yield of photoinhibition; a plot of ϕ vs. wavelength now yields the quantum yield spectrum. This quantum yield is relative unless the concentrations of the absorbing moieties are known. The quantum number, thus, is the number of quanta absorbed per chlorophyll molecule necessary to decrease the activity of the chloroplasts to 37 % ($1/e$) of the original value.

Action and Quantum Number Spectra of Photoinhibition. The uniformity of the kinetics throughout the spectrum, the high intensity of the Xenon arc lamp, and the small cross-sectional area of the microvessel (0.09 cm²) made it possible to measure the relative sensitivity of the chloroplasts to monochromatic light of high purity. However, the thermal (or dark) decline in activity of the chloroplasts during the relatively long exposure times (4–6 min.) prohibited the measurement of complete decay curves at each wavelength.

An abbreviated method therefore, was used. At each wavelength, control samples and photoinhibited samples were alternately assayed. From the observed ratio R_o/R_t, we determined $\alpha\phi$ and $1/\phi$ (equation II) giving the spectra shown in figures 3 and 4. We obtained the photoinhibition action spectrum for wavelengths between 230 and 710 mμ (15 mμ half-band width) shown in figure 3 with a single preparation of chloroplasts. The UV spectrum, run with higher resolution (9 mμ half-band width), is also presented in figure 3 (inset). It shows a peak between 250 and 260 mμ, a shoulder around 280 mμ, and a pronounced minimum at about 240 mμ. Below 240 mμ the sensitivity rose very sharply, but light intensities sufficient for quantitative measurements at these short wavelengths were not available.

A more detailed action spectrum in the visible region is shown in figure 4. Because of the very low sensitivity of the chloroplasts to visible light precise data were difficult to obtain, even with the use of interference filters instead of the monochromator. However, the spectra clearly implicate chloro-

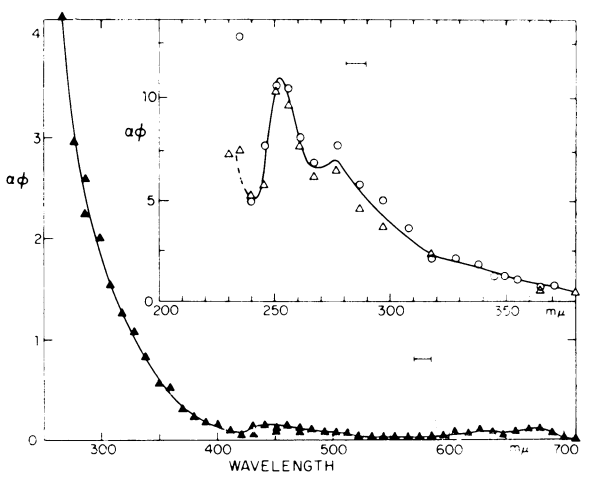

FIG. 3. Relative inhibitory activity of selected wavelengths of light upon dye reduction by isolated chloroplasts. Procedure and assay as described in text.

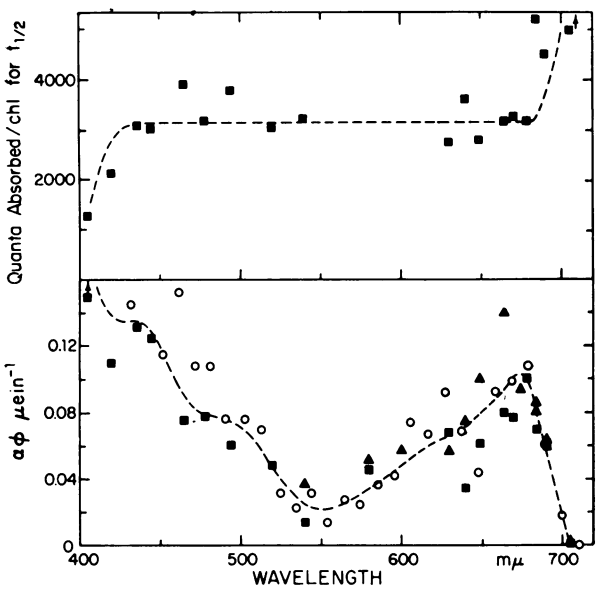

FIG. 4. Photoinhibition action and relative quantum number spectra. As figure 3, except monochromatic light isolated by interference and blocking filters. Different symbols are different experiments. Quanta absorbed per chlorophyll molecule for $t_1/_2$ calculated as in table III.

phyll as the sensitizing pigment. Plotted above the action spectrum is the number of quanta absorbed per chlorophyll molecule to give a one-half decrease in dye reduction rate. The quantum requirement appears to be constant over the visible spectrum, averaging around 3000 quanta/ chlorophyll for $t_1/_2$, in agreement with the data in table III ($1/\phi$ = 4-5000/Chl).

Photoinhibition in Lyophylized Anacystis nidulans Cells. The data of figure 4 shows a rise of quantum number at long wavelength. This suggests that the red drop of the quantum yield of photosynthesis found by Emerson and Lewis (5) might also be evident in the spectrum of photoinhibition. The rapidly diminishing absorption of chloroplasts at these wavelengths made accurate measurements of the light absorption of the chloroplasts in the microvessel impractical. To explore this point further, the action and relative quantum number spectra of photoinhibition in visible light were determined for the Hill reaction exhibited by lyophylized cells of *Anacystis nidulans*. The pigments of this blue-green alga lend themselves to a clear separation of the 2 pigment systems and their associated photoacts (15).

Lyophylized *Anacystis* preparations did not evolve or consume O_2 appreciably as measured polarographically unless a reduceable substrate was present in the light. With $K_3Fe(CN)_6$ as an oxidant, the preparations showed 30 to 40 % of the O_2 evolution of whole cells at all light intensities. The maximum rate observed was 18 cell volumes per hour. Similar rates of dye reduction were found spectrophotometrically. However, the lyophylized preparation also reduced the dye in the dark at a rate of 5 to 10 % of the maximum reduction rate in the light. The dark reduction continued for long periods of time and persisted after exposures to light. Attempts to annihilate the dark reduction by procedure variations failed. No simultaneous uptake of O_2 in the dark was found. Perhaps the dye substituted for O_2 as an electron acceptor in the disrupted respiratory system of the lyophylized cells. All rates of dye photoreduction were corrected for this dark reduction. Significant NADP reduction, as seen by Black et al. (4), could not be obtained in the same reaction mixture used for NADP reduction in chloroplasts (8).

The activity of the *Anacystis* preparations showed a broad pH optimum between pH 6 and 8, the activity dropping to zero at pH 4 and 10. Although distilled water or 0.1 M phosphate buffer did not sustain full activity, the molarity of the sucrose in the suspending medium was not critical. No difference was found between samples prepared at 0° or at room temperature. If stored dry at -20° under nitrogen, the activity of the preparation was maintained for over a week.

The photoinhibition decay curves observed with this material after long periods of exposure departed from the usual logarithmic decay found with chloroplasts; the effectiveness was slightly less than expected. A dark period between exposure and assay also tended to lessen the effectiveness of the inhibitory light. Perhaps a small but significant amount of the repair reaction, typical of whole cells (see Discussion) is still active in lyophylized cell preparations. Aside from this slight deviation, the lyophylized cells showed the same relationship between

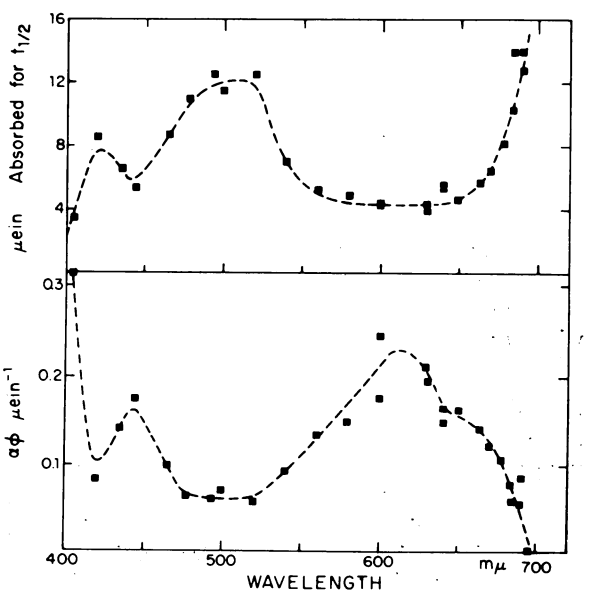

FIG. 5. Photoinhibition action and relative quantum number spectra. As figure 4 except for the use of lyophylized *Anacystis* cells. Procedures as described in text.

the intensity of visible preillumination light and the time of exposure as that found for chloroplasts. Action spectra of photoinhibition were run in a manner identical to those of chloroplasts, and an example is shown in figure 5.

The action spectrum ($\alpha\ \phi$) is very similar to action spectra of photosynthesis for this (15) and other blue-green algae (11), the highest activity being in the region where the accessory pigment phycocyanin absorbs most strongly. The maximum effectiveness ($\phi = 0.2/\alpha$) is very close to that found for chloroplasts ($\phi = 0.1/\alpha$, see fig 4) since the absorption of the *Anacystis* suspension used was about double that of the chloroplast suspension. The relative quantum requirement (fig 5, top) shows a broad minimum between about 550 mμ and 650 mμ, and a clear "red drop" at wavelengths above 650 mμ and below 550 mμ similar to O_2 evolution in *Anacystis* (15). The high requirement at long wavelengths and around 500 mμ indicates that chlorophyll a and carotenoids are relatively inactive. Both spectra show increasing sensitivity at 405 mμ similar to the UV effect encountered in chloroplasts. Pigment connected to system II thus seems to be predominant in the sensitization of photoinhibition by visible light of dye reduction.

Discussion

It should be emphasized that in all experiments reported here, exposure to photoinhibitory light was carried out in the absence of exogenous chloroplast oxidants. It is assumed, therefore, that even the lowest intensities are effectively above light saturation.

The use of chloroplasts without substrate greatly simplifies the study of photoinhibition. In whole cells, 2 processes compete with or oppose photoinhibition: A) photosynthesis itself, which diverts quanta in weak and moderate intensities and B) a temperature dependent restoration mechanism which acts during and after exposure to visible light (16). No such repair mechanism could be found in spinach chloroplasts, but a small activity of this type was observed in the lyophylized *Anacystis* preparations.

The observed reciprocity of intensity and exposure time, found with chloroplasts in visible light, is not consistent with previously reported results for whole green algae (17) which indicated proportionality with the square of the intensity. This difference can now be explained by the 2 opposing reactions in whole cells, lower light intensities appearing relatively less effective. Photoinhibition by UV light has been assumed to follow the reciprocity law (2, 13), but proof for this was again difficult because of secondary metabolic effects in whole cells.

The observed rate law precludes heating of the environment as the cause of inhibition. In this case the halftimes of photoinhibition would be dependent upon light intensity and not the absolute dose of quanta. A multiple hit mechanism, where 1 quantum prepares a sensitive locus for damage by a second quantum, would also show a deviation from the reciprocity law unless the half-life of this sensitive locus was long compared to the rate of quantum absorption. At the lowest intensity in which we observed validity of the reciprocity law, each chlorophyll on the average absorbed a quantum twice per second; the life time of the sensitive locus thus would have to be more than 0.5 second. If one assumes the damage occurs in a trapping center (see below) and that there is one trap per 40 to 400 chlorophylls, (system II or system I respectively) (20), each trap would then receive a quantum about once every 10 to 1 msec respectively, and the required life time of the sensitive locus would thus be correspondingly shorter. Even then, the damage caused by the second quantum would have to occur with a very low probability (quantum yield).

Quanta absorbed by the photosynthetic pigments, under circumstances when photosynthesis is taking place, are transferred to a unique pigment which is responsible for the photochemistry of the system (reaction center or trap). The lack of bleaching of the absorbing pigment during photoinhibition, the Q_{10} of one, and the concomitant decline of quantum yield, saturating rate and maximum flash yield (22) imply that the inactivation occurs in the reaction center or a related moiety. Thus, quanta causing inactivation must also be transferred to this reaction center rather than being disposed of as heat or fluorescence in the pigment bed. Photoinhibition by visible light thus is caused by a secondary photoreaction in the trapping center which occurs with a low but definite probability and which results in inactivation of the reaction center.

One can assume that photoinhibition occurs with the same quantum yield, regardless of intensity, or the presence of substrate. To just saturate NADP photoreduction by chloroplasts, each chlorophyll molecule absorbs one quantum every 4.5 second (assuming a quantum yield of 0.5 equation/Ein and a maximum rate of 220 μmoles/mg Chl hr). On this basis, the lowest visible light intensity used in our inhibition experiments (2 quanta absorbed per Chl-sec, table III) was about 10 times light saturation, whereas the highest intensity used (about 500 quanta absorbed/Chl-sec) was 2500 times saturation. The extrapolated half-time of photoinhibition in just saturating visible light equals about 2 hours for NADP reduction or about 4 hours for dye reduction.

The red drop in the action spectra of both chloroplasts and *Anacystis* (fig 4, 5) requires either: that the sensitizing pigments belong to photoact II, or that a balanced influx of quanta between the 2 photoacts is necessary for inhibition to occur, similar to the requirement for complete photosynthesis. An alternate explanation rests upon the assumption that dye reduction occurs via photoact II only, so that the data of figures 4 and 5 would pertain only to photoinhibition of system II. This question will be considered in more detail elsewhere (14).

As the absorption of the chlorophylls and carotenoids, as well as the action spectrum of photosynthesis decline below 430 mμ, the absorbing pigment for photoinhibition in UV light cannot be a photosynthetic light absorber. The wavelength at which the transition occurs from 1 type of inhibition to the other appears to be around 420 mμ.

The action spectrum of photoinhibition in the UV region follows the absorption spectrum of many compounds found in the chloroplasts (notably plastoquinone, linolenic acid, and perhaps a pteridine analogue). It also has a striking resemblance to the action spectrum of the bleaching of *Euglena* cells which was attributed to the destruction of a nucleoprotein (23). Identification of the pigment responsible for the inactivation of photosynthesis is difficult because most of the moieties absorbing in this region are probably adversely effected during exposure. The nature of the sensitizer in UV light will be discussed in a subsequent paper (14).

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