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COMPARISON STUDIES ON THE EFFECTS OF ULTRAVIOLET IRRADIATION ON PHOTOSYNTHESIS*

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SUMMARY

1. A comparison of chloroplasts from which plastoquinone had been extracted with ultraviolet irradiation supports the conclusion that plastoquinone destruction is not the major cause of ultraviolet inhibition of photosynthesis. No photodestruction of chloroplast lipids, carotenoids or soluble proteins by ultraviolet irradiation was detected.

2. Phenazine methosulfate-mediated cyclic photophosphorylation and variable yield fluorescence were inhibited at the same rate as the Hill reaction. Examination of fluorescence emission spectra of chloroplasts and whole algal cells revealed decreases in both the 685-nm and long-wavelength emission peaks.

3. Digestion of chloroplasts with lipase decreased fluorescence in a manner similar to ultraviolet irradiation. Hill reaction activity was also inhibited by lipase digestion.

4. It is concluded that the inhibition of photosynthesis by ultraviolet irradiation is most likely due to a disruption of the structural integrity of the lamellar membranes which results in the loss of System II activity and associated reactions.

INTRODUCTION

Ultraviolet irradiation is a potent and specific inhibitor of photosynthesis and of a number of partial reactions of photosynthesis including the Hill reaction, photoreduction, and non-cyclic photophosphorylation[1]. Based on the finding that ultraviolet irradiation produced a proportional decrease in both Hill reaction activity and plastoquinone content of spinach chloroplasts, BISHOP[2] proposed that the destruction of plastoquinone might be the cause of ultraviolet inhibition. The apparent parallelism between plastoquinone destruction and loss of Hill reaction activity was confirmed and extended by SHAVIT AND AVRON[3] and also TREBST AND PISTORIUS[4]. However, these researchers noted that the addition of unirradiated plastoquinone

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; CCCP, *m*-chlorocyanocarbonylphenylhydrazone; PMS, phenazine methosulfate.

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to irradiated chloroplasts did not restore the Hill reaction. In a more detailed study on the photoinhibition of chloroplast reaction JONES AND KOK[5] also were unable to confirm the correlation suggested originally by BISHOP[2]. Additional studies by MANTAI AND BISHOP[1] on the effects produced by ultraviolet irradiation and light petroleum extraction of plastoquinone on the 520-nm light-induced absorbance change, and also on the ultraviolet-induced loss of photosynthetic activity and destruction of plastoquinone in whole cells, led them to conclude that plastoquinone destruction was not the single major cause of ultraviolet inactivation.

In this paper additional findings on the ultraviolet inhibition of electron transport, cyclic photophosphorylation, and on the variable-yield fluorescence of chloroplasts and algae will be presented. Also studies on the similar inhibitory action of various lipases on such reaction are given. The nearly identical effects of lipase treatment and ultraviolet irradiation suggest a mechanism for ultraviolet inhibition independent of plastoquinone.

MATERIALS AND METHODS

The green alga *Scenedesmus obliquus* and isolated spinach chloroplasts were employed for measurement of the 520-nm absorbance change and plastoquinone analysis. Spinach (*Spinacea oleracea*) chloroplasts were prepared according to the method of AVRON[6].

The 520-nm absorbance change was measured with the instrument previously described[1]. Fluorescence kinetic measurements were made with an apparatus similar to that described by DUYSENS AND SWEERS[7] and BUTLER AND BISHOP[8]. Fluorescence emission spectra were measured with a similar apparatus but with accessories necessary for the maintenance of low temperatures.

Galactolipase from scarlet runner beans was isolated and purified according to the procedure of SASTRY AND KATES[9]. Commercial pancreatin lipase was further purified by dissolving 400 mg of the material in 100 ml of 15 mM phosphate buffer (pH 6.5) and centrifuging at 25000 $\times$ *g* for 10 min to remove the insoluble components. The clear supernatant was brought to 50% saturation with $(NH_4)_2SO_4$ and centrifuged at 25000 $\times$ *g* for 10 min. The resulting pellet was redissolved in 10 ml of 15 mM phosphate buffer (pH 6.5), dialyzed against this buffer and employed as the enzyme source.

Lipase experiments were performed by adding an appropriate amount of the enzyme to the chloroplast preparation and incubating the flasks at 26° in a water bath with continuous shaking. Samples were withdrawn periodically for assays of activity. A control sample without added enzyme was always run under identical conditions.

Ultraviolet irradiation of samples was carried out as previously described[1]. Hill reaction activity was determined either manometrically or spectrophotometrically. The reaction mixture for manometric measurements consisted of 24 μmoles $K_3Fe(CN)_6$ or 4.6 μmoles of *p*-benzoquinone, 100 μmoles phosphate buffer (pH 7.0), chloroplasts equivalent to 0.35 mg chlorophyll and sucrose–KCl solution to a final volume of 3.0 ml. Reactions were run at 20° in a gas phase of air. White light with an intensity of 10^6 ergs·cm^{-2}·sec^{-1} was used for illumination. For spectrophotometric analysis of $NADP^+$ or 2,6-dichlorophenolindophenol (DCIP) reduction assays were

performed on a Zeiss PMQ-II spectrophotometer adapted for continuous illumination of the reaction cell. Changes in absorbance at the appropriate wavelength were recorded directly. For DCIP assays the reaction mixture contained 0.1 μmole DCIP and chloroplasts containing 35 μg chlorophyll, 250 μmoles Tris buffer (pH 7.5) and sufficient sucrose–KCl solution to bring the final volume to 3.0 ml. Absorbance changes were monitored at 610 nm.

Cytochrome *c* and $NADP^+$ reduction were also measured spectrophotometrically at 550 and 340 nm, repectively. The reaction mixtures were nearly identically to that for DCIP reduction assay except for the addition of 2 mg cytochrome *c* or 0.8 μmole $NADP^+$ and saturating amounts of ferredoxin and $NADP^+$ reductase.

Ascorbate–DCIP-coupled reduction of $NADP^+$ was performed as indicated above except for the addition of 20 μmoles sodium ascorbate, 0.1 μmole DCIP and 1.0 μmole 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Actinic light was provided by a 1000-W projection lamp and the appropriate lense system to focus the light onto the top of a standard 1-cm cuvette. The light was first passed through a water bath and an infrared absorbing filter (Corning filter 1-69) and a glass cutoff filter (Corning filter 2-61) which transmitted wavelengths greater than 600 nm. The photomultiplier of the spectrophotometer was shielded from excessive light by placing a 610-nm interference filter over the entrance to the photomultiplier housing.

RESULTS AND DISCUSSION

Comparison of ultraviolet irradiation and light petroleum extraction on chloroplast reactions

Several earlier papers have related ultraviolet inhibition of photosynthetic reactions with destruction of plastoquinone; in general these experiments have yielded inconclusive results. From the results of experiments comparing the effects of light petroleum extraction *versus* ultraviolet irradiation on the 520-nm absorbance change, and also from the lack of correlation observed between plastoquinone destruction and the inhibition of photosynthesis in the green alga Chlorella, MANTAI AND BISHOP[1] concluded that plastoquinone destruction was not the major cause of ultraviolet inhibition of photosynthesis. Further evidence supporting this conclusion was obtained from experiments on the effects of light petroleum extraction and ultraviolet irradiation on various reactions of isolated chloroplasts. The reduction of cytochrome *c* (or $NADP^+$, not shown), a reaction which requires the participation of both photosystems, is inhibited strongly by the removal of plastoquinones; the amount of inhibition is directly related to the amount of plastoquinones removed. DCIP reduction is inhibited only about 50% when essentially all of the plastoquinones has been removed and, similarly, the reduction of $NADP^+$, with ascorbate–DCIP as electron donor, is only partially inhibited (Table I). HENNINGER AND CRANE[10] have reported similar results. The effects of ultraviolet irradiation on these reactions are shown in Fig. 1; regardless of the Hill oxidant employed practically identical inhibition is produced by ultraviolet irradiation. From these data it appears that the effects of ultraviolet irradiation and plastoquinone extraction on chloroplast reactions are quite different. We also observed that the addition of unirradiated plastoquinone or vitamin K to irradiated chloroplasts did not restore Hill reaction activity.

Previously, we have shown that the DCMU-insensitive portion of the 520-nm

absorbance change in whole cells required 2–3 times the period of irradiation as photosynthesis to show complete inhibition[1]. It appeared feasible that a correlation might exist between plastoquinone destruction and loss of this portion of the 520-nm signal. However, irradiation experiments with Chlorella and Scenedesmus did not show a significant correlation between signal magnitude and plastoquinone content. Also, chloroplasts isolated from ultraviolet-irradiated Scenedesmus cells, wherein the 520-nm signal had been decreased by varying amounts, still possessed an active Photo-

TABLE I

THE EFFECTS OF LIGHT PETROLEUM EXTRACTION ON VARIOUS PARTIAL REACTIONS OF PHOTOSYNTHESIS IN LYOPHILIZED SPINACH CHLOROPLASTS

Control values were as follows (μmoles reduced per mg chlorophyll per h): cytochrome *c* = 28.4, DCIP = 43.2, and $NADP^+$ = 7.4.

Number of extractions	*Plastoquinone remaining** (%)	*Readditions*	*% Control activity*		
			Cytochrome c	*DCIP*	*$NADP^+$ reduction (ascorbate–DCIP)*
0	100	None	100	100	100
1	31	None	21	47	52
4	10	None	4	48	35
1	31	Total extract	63	73	58
1	31	Plastoquinone	78.5	102	68
4	10	Total extract	10	52	39
4	10	Plastoquinone	56	83	48

* Ratio of plastoquinone A:B:C + D remained constant.

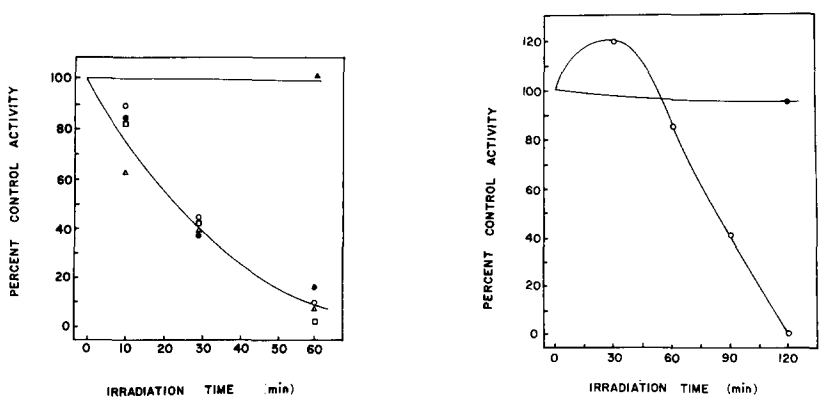


Fig. 1. Effects of ultraviolet irradiation on various Hill reactions and ascorbate–DCIP-mediated reduction of $NADP^+$; △—△, DCIP; □—□, $NADP^+$; ●—●, *p*-benzoquinone; ○—○, $Fe(CN)_6^{3-}$; ▲—▲, ascorbate–DCIP (DCMU). Control rates (expressed as μmoles substrate reduced per mg chlorophyll per h) are as follows: DCIP = 64, $NADP^+$ = 130, $Fe(CN)_6^{3-}$ = 40, *p*-benzoquinone = 38, ascorbate–DCIP-mediated reduction of $NADP^+$ = 54. For experimental conditions see MATERIALS AND METHODS.

Fig. 2. Comparison of the effect of ultraviolet irradiation on the system I 520-nm change (○—○) and on the ascorbate–DCIP-mediated reduction of $NADP^+$ (●—●) in Scenedesmus. Control value for $NADP^+$ reduction = 98 μmoles/mg chlorophyll per h. See MATERIALS AND METHODS for additional details on reaction mixtures and chloroplast isolation.

system I as measured by $NADP^+$ photoreduction (with ascorbate – DCIP as electron donor). These results are presented in Fig. 2.

Sensitivity of cyclic and non-cyclic photophosphorylation to ultraviolet irradiation

A number of recent studies on the effect of ultraviolet on chloroplast reactions have indicated that cyclic photophosphorylation, with phenazine methosulfate (PMS) as the added cofactor, is much less sensitive to ultraviolet irradiation than is the Hill reaction or non-cyclic photophosphorylation. In prior publications it has been documented that ultraviolet irradiation causes an approximately equal inhibition of both photosynthesis and photoreduction in the alga *Scenedesmus obliquus*[1,11]. It is well established that photoreduction, in hydrogen-adapted algae, is a strict Photosystem I. From chloroplast studies, however, it is equally clear that only Photosystem II reactions are inhibited by ultraviolet irradiation. Consequently, in order to explain the ultraviolet inhibition of photoreduction it is our interpretation that the only common site for inhibition would be in the mechanism of cyclic photophosphorylation. Our experiments on PMS-catalyzed photophosphorylation support the hypothesis that ultraviolet irradiation influences not only System II activity but also photophosphorylation activity as well. Our findings (Fig. 3) show that the degree of inhibition of PMS-mediated photophosphorylation by spinach chloroplasts closely parallels that of DCIP photoreduction. The disparity between our results and those published by SHAVIT AND AVRON[3] and TREBST AND PISTORIUS[4] may be due to the lower light intensities employed in our experiments.

Further support for the above interpretation comes from the findings of JONES AND KOK[13] who observed that although $NADP^+$ reduction mediated by ascorbate–DCIP is not inhibited by ultraviolet irradiation, phosphorylation associated with this mode of electron transport is. They suggest that either ultraviolet is acting as an uncoupler of phosphorylation without affecting electron transport or that DCIP bypasses the site of ultraviolet damage without a loss in the rate of electron transport occurring. YAMISHITA AND BUTLER[14] have demonstrated that the photoreduction of $NADP^+$ in ultraviolet-irradiated chloroplasts can be restored by a number of electron donors, such as hydroquinone–ascorbate, which apparently substitutes for water. This observation, although strongly indicating that some component on the

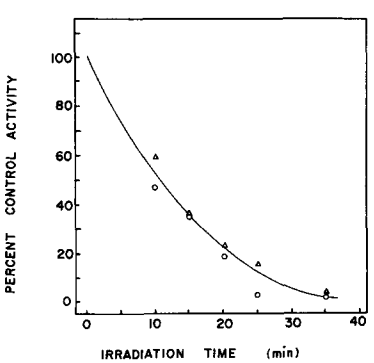


Fig. 3. Comparison of the influence of ultraviolet irradiation on Hill reaction and on cyclic photophosphorylation. O—O, Hill reaction; △—△, cyclic photophosphorylation. Control rates are as follows: Hill reaction = 169 μmoles DCIP reduced per mg chlorophyll per h. Photophosphorylation = 33 μmoles ADP esterified per mg chlorophyll per h.

oxidizing side of System II is affected by ultraviolet irradiation, can be interpreted also as supporting the hypothesis that an equally sensitive system would be the site of photophosphorylation, a site perhaps common to both cyclic and non-cyclic photophosphorylation.

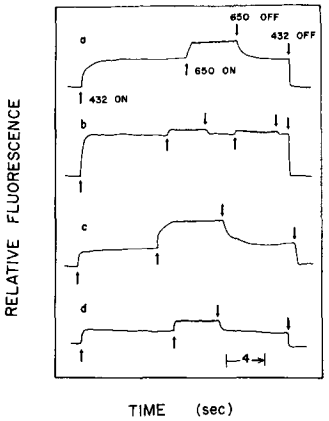
Action of ultraviolet irradiation on the variable-yield fluorescence of isolated chloroplasts and algae

The fluorescence of algae and of isolated chloroplasts is believed to consist of two components; the first is a fluorescence of constant quantum yield, probably emanating from the bulk absorbing chlorophyll, which is not affected by changes in the efficiency of the photochemical reactions and the second is a fluorescence of variable quantum yield which directly relates to the photosynthetic capacity and presumably emanates from the trapping centers of System II. KAUTSKY *et al.*[15] and DUYSENS AND SWEERS[7] proposed a mechanism of the variable-yield fluorescence based upon the oxidation and reduction of an electron transport system between the two photosystems wherein the fluorescence of chlorophyll a_{II} is quenched by the oxidized state of a hypothetical compound but not by its reduced form. The currently accepted terminology for the hypothetical compound is Substance Q (ref. 7). More involved kinetic considerations have predicted that an additional compound, Substance A, is involved in the reversible quenching phenomenon; various lines of evidence indicate that it is probably plastoquinone. A more thorough documentation of the recent studies and interpretation on fluorescence has been prepared by BUTLER[16].

Because of the association between electron transport and the variable-yield fluorescence and the possibility that one of the major quenchers of fluorescence is plastoquinone, the influence of ultraviolet irradiation on fluorescence was investigated. It is well documented that factors which alter the pattern of electron flow in photosynthesis influence the variable-yield fluorescence. DCMU, a specific inhibitor of System II, causes a maximum yield of fluorescence presumably because it blocks reoxidation of the reduced Substance Q; Hill reaction oxidants decrease the fluorescence yield because of the increased oxidation of the electron transport chain; specific wavelengths of light which are preferentially absorbed by either Photosystem I or II decrease or increase the variable-yield fluorescence respectively because of their influence on the equilibrium established between reduced and oxidized states of the various redox pools in the electron transport system. In Fig. 4 the influence of DCMU, *m*-chlorocyanocarbonylphenylhydrazone (CCCP), and $Fe(CN)_6^{3-}$ on the fluorescence of isolated chloroplasts is presented. These treatments elicit the response anticipated from the previously discussed interpretation of the mechanism underlying the variable-yield fluorescence, *i.e.*, DCMU increases the variable-yield fluorescence and diminishes the response to System II light, the phosphorylation uncoupler CCCP lowers the fluorescence yield caused by excitation with 436-nm light, and a Hill reaction oxidant, $K_3Fe(CN)_4$, similarly lowers the fluorescence yield by causing increased electron flow. The variations in the kinetics of the 650-nm light stimulation of the fluorescence yield of chloroplasts treated with CCCP requires a more thorough discussion than can be given here.

From such information it is possible to predict that ultraviolet irradiation should increase the variable-yield fluorescence proportional to the decrease in Hill

reaction activity. The contrary is observed; the fluorescence of irradiated chloroplasts (with and without DCMU) decreases and at a rate parallel to the loss of Hill reaction activity (Fig. 5). Measurements in the presence of DCMU should give a relative measurement of the concentration of the fluorescence emitter. Similar experiments with



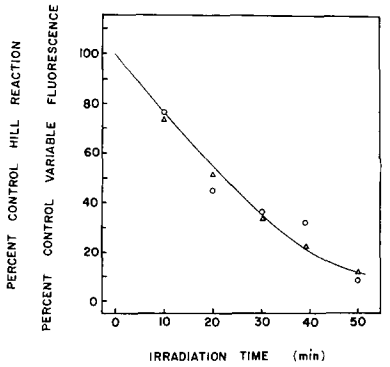


Fig. 4. The influence of various factors on the variable-yield fluorescence (686 nm) of spinach chloroplasts. Chloroplasts containing 150 μg of chlorophyll were suspended in 1.0 ml of sucrose–KCl solution. All additions were made to give a total volume of 1.0 ml. Additions were as follows: (a) no additions; (b) DCMU (1.5 μmoles); (c) CCCP (2.0 μmoles); (d) $Fe(CN)_6^{3-}$ (4 μmoles). Exciting wavelength = 436 nm. ↑ 650-nm light on, ↓ off. Intensities were 500 and 3000 ergs·cm^{-2}·sec^{-1}, respectively. Time constant < 10 msec.

Fig. 5. The effect of ultraviolet irradiation on the maximal variable-yield fluorescence (△—△) and on the Hill reaction (○—○) of spinach chloroplasts. Hill reaction control rate = 92 μmoles DCIP reduced per mg chlorophyll per h. Maximum values of fluorescence determined in the presence of DCMU as in Fig. 4.

whole cells of Scenedesmus gave results comparable to those obtained with spinach chloroplasts but with more complex kinetics. The fluorescence data might be explained by one of three possible interpretations: an increased efficiency of drainage of energy occurs from the trapping centers of System II (assumed to be the variable-yield fluorescence emitter), even though electron flow through the normal pathway is blocked; actual destruction of the fluorescence emitter results; or perhaps increased internal quenching of fluorescence caused by a collapse of chloroplast membranes occurs.

Examination of the low-temperature (–196°) and room-temperature fluorescence emission spectra of ultraviolet-irradiated chloroplasts and algal cells reveal a progressive decrease in fluorescence at all wavelengths. This pattern is particularly apparent in the low-temperature measurements with Scenedesmus cells where it was observed that the emission peaks at 715 and 685 nm drop equally, *i.e.*, the ratio of the relative intensities of the two peaks (F715/F685) remained essentially constant (Fig. 6a). After irradiation times of longer than 30 min no fluorescence emission was detected. A similar pattern was also observed for spinach chloroplasts in that a steady decrease in fluorescence occurred at both 685 and at 727 nm (Fig. 6b). However, the ratio of the two peaks did not remain constant since the 727-nm fluorescence appears to be more drastically altered by irradiation.

Inhibitory action of lipases on Hill reaction and variable-yield fluorescence

OKAYAMA[17] demonstrated that the Hill reaction and fluorescence of chloroplasts treated with lipase isolated from pig pancreas were inhibited. This finding suggested a similarity of action between ultraviolet irradiation and the action of certain hydrolytic enzymes on chloroplast reactions. Since his measurements were made on total fluorescence and did not distinguish between the constant-yield and variable-yield components of fluorescence, certain aspects of these experiments were repeated. The results showed that treatment of chloroplasts with a partially purified lipase of pancreatin (see MATERIALS AND METHODS) caused an equal inhibition of the variable-yield fluorescence and of the Hill reaction (Fig. 7). These data, coupled with the observations of OKAYAMA[17] that System I reactions are unaffected by this type of treatment (which we have also confirmed), are very similar to those obtained by ultraviolet irradiation.

The low-temperature fluorescence emission spectra of spinach chloroplasts treated with either galactolipase or pancreatin lipase closely resemble those of ultraviolet-treated chloroplasts. Low concentrations of pancreatin lipase cause an approximately equal lowering of the two fluorescent peaks but at higher concentrations (see Fig. 8) the 735-nm peak disappears and only the 686-nm peak remains. Unpublished data from this laboratory on mutants of Scenedesmus incapable of forming normal chloroplast lamellae also show only one peak (686 nm) in the low-temperature fluorescence emission spectrum.

The pancreatin lipase primarily hydrolyzes phospholipids of the chloroplast and the lipase of scarlet runner beans specifically hydrolyzes galactolipids. However, the fluorescence and various reactions of chloroplasts treated with either lipase are altered in a similar fashion; both treatments yield results identical to those caused by ultraviolet irradiation.

The possibility that ultraviolet might act by destroying some portion of the lipid material of the chloroplasts was checked by isolating the lipids from non-irradiated and irradiated chloroplasts and separating them by thin-layer chromatography; no significant differences were detected. Similar studies on the carotenoid and soluble-protein fractions of irradiated chloroplasts also yielded negative findings. The most consistent observation with irradiated chloroplasts was the loss of plastoquinones A and C and α-tocopherol.

The observed coincidence of the loss of plastoquinone (A and C) and Hill reaction in ultraviolet-irradiated chloroplasts and the parallel of the absorption spectrum of plastoquinone and the action spectrum for ultraviolet inhibition of photosynthesis have provided evidence which suggests that the mechanism of ultraviolet inhibition of photosynthesis is through destruction of plastoquinone. However, the findings reported here, and also in other publications, demonstrate that such a simple correlation is not apparently correct.

The noted decrease in the variable-yield fluorescence and in the low-temperature fluorescence emission spectrum as caused by either lipase treatment (pancreatin or galactolipase) or by ultraviolet irradiation suggests an alternative explanation for the nature of ultraviolet inhibition. It is a general observation that ultraviolet-irradiated chloroplasts, as well as lipase-treated, possess a normal amount of chlorophyll; furthermore, measurements of the absorption spectra of such material are only slightly altered in certain peak absorptions[18]. It appears that the observed decrease

in fluorescence cannot be due to photodestruction of the primary emitter of fluorescence in general. However, the possibility is not ruled out that the complex of the chloroplast responsible for the variable-yield fluorescence is preferentially destroyed by irradiation or by lipase treatment.

YAMISHITA AND BUTLER[14] have shown that ultraviolet-irradiated chloroplasts lose System II activity and variable-yield fluorescence but it was possible to restore a DCMU-sensitive $NADP^+$ photoreduction (with ascorbate–hydroquinone as the

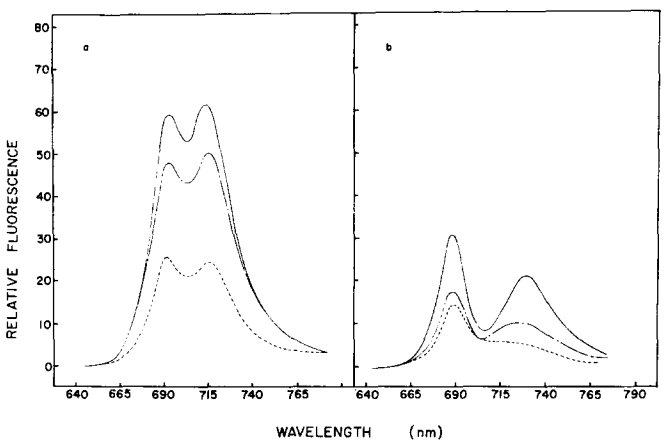


Fig. 6. a. Low-temperature emission spectra of Scenedesmus cells irradiated with ultraviolet for varying lengths of time. 10-μl cells were suspended in 1 ml growth medium and frozen in liquid N_2. Excitation wavelength = 436 nm. Excitation intensity = 150 ergs·cm^{-2}·sec^{-1}. b. Low-temperature fluorescence emission spectra of ultraviolet-irradiated spinach chloroplasts. Chloroplasts containing 25 μg chlorophyll were suspended in 1.0 ml of 0.5 M sucrose–0.01 M KCl solution and frozen in liquid N_2. ———, control; —·—, 15 min; -------, 30 min.

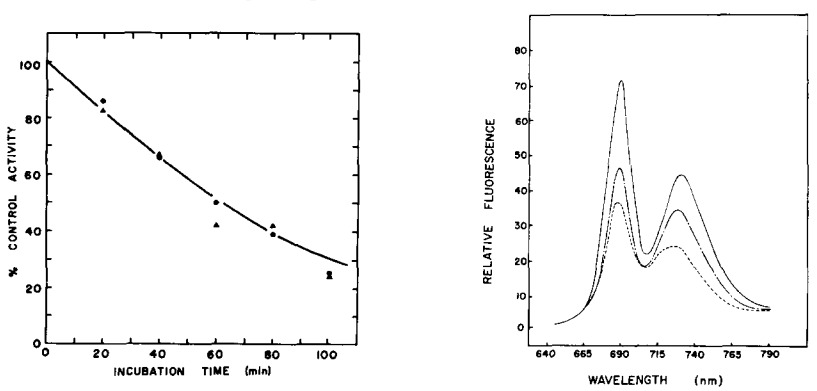


Fig. 7. Comparison of the action of lipase treatment on the Hill reaction activity (●—●) and the variable-yield fluorescence (▲—▲) of spinach chloroplasts. Chloroplasts containing 50 μg chlorophyll per ml were incubated in the presence of partially purified pancreatin lipase for the times indicated. Enzyme extract equivalent to 200 μg protein was added to 25 ml of chloroplast suspension and incubated at 25°. Suspending medium, 0.5 M sucrose – 0.02 M KCl. Hill reaction control rate = 155 μmoles DCIP reduced per mg chlorophyll per h. Fluorescence measurements made under the conditions described in MATERIALS AND METHODS and in the legend of Fig. 5.

Fig. 8. Low-temperature fluorescence emission spectra of spinach chloroplasts treated with pancreatin lipase. Conditions for measurement were the same as described in the legend of Fig. 7. ———, control; –·—·–, 15 min; – – – –, 30 min.

electron donor system in place of water); however, restoration of a variable-yield fluorescence was not possible. The decreased yield of fluorescence was also not reversed by the addition of dithionite. These findings suggest that the reducing side of System II remains intact after ultraviolet irradiation; this portion of the mechanism must also include the pigments of System II since YAMISHITA AND BUTLER[14] demonstrated that in their system a typical red-drop in the quantum efficiency of the photoreduction of $NADP^+$ occurred when ascorbate–phenylenediamine (or hydroquinone) was employed as the electron donor system. This apparent anomalous behavior between the capacity to restore electron transport, but not the variable-yield fluorescence, we believe can be interpreted only by the alteration of the lamellar structure of the chloroplasts which results in the loss of O_2-evolving capacity and also the loss of variable-yield fluorescence. The latter effect might be due to a collapse of the lamellar structure which results in an increased internal quenching of the fluorescence. We assume that the chlorophylls are attached through a matrix of lipoprotein and that alteration of this framework by a variety of agents may lead to internal quenching of fluorescence. Preliminary experiments on the possible alteration of chloroplast structure have indicated a change in the integrity of the lamellae as caused by ultraviolet irradiation. More extensive work is required to correlate such changes with the observed loss of photosynthetic activity.

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REFERENCES

1 K. E. MANTAI AND N. I. BISHOP, *Biochim. Biophys. Acta*, 131 (1967) 350.
2 N. I. BISHOP, in G. E. W. WOLSTENHOLME AND D. M. CONNORS, *Ciba Found. Symp. Quinones Electron Transport*, (1961) 385.
3 N. SHAVIT AND M. AVRON, *Biochim. Biophys. Acta*, 66 (1963) 187.
4 A. TREBST AND E. PISTORIUS, *Z. Naturforsch.*, 20b (1965) 885.
5 L. W. JONES AND B. KOK, *Plant Physiol.*, 41 (1966) 1037.
6 M. AVRON, *Biochim. Biophys. Acta*, 40 (1960) 257.
7 L. N. M. DUYSENS AND H. E. SWEERS, in *Microalgae and Photosynthetic Bacteria*, Japanese Society of Plant Physiology, University of Tokyo Press, Tokyo, 1963.
8 W. L. BUTLER AND N. I. BISHOP, in B. KOK AND A. T. JAGENDORF *Photosynthetic Mechanisms of Green Plants, Natl. Acad. Sci. – Natl. Res. Council Publ.*, 1145 (1963), 91.
9 P. S. SASTRY AND M. KATES, *Biochemistry*, 3 (1964) 1280.
10 M. D. HENNIGER AND F. L. CRANE, *Biochemistry*, 2 (1963) 1168.
11 A. S. HOLT, I. A. BROOKS AND W. A. ARNOLD, *J. Gen. Physiol.*, 34 (1951) 627.
12 A. T. JAGENDORF AND M. AVRON, *J. Biol. Chem.*, 231 (1958) 277.
13 B. KOK AND G. M. CHENIAE, in D. R. SANADI, *Current Topics in Bioenergetics*, Vol. 1, Academic Press, New York, 1966, p. 147.
14 T. YAMISHITA AND W. L. BUTLER, *Plant Physiol.*, 43 (1968) 2037.
15 H. KAUTSKY, W. APPEL AND H. AMANN, *Biochem. Z.*, 332 (1960) 277.
16 W. BUTLER, in D. R. SANADI, *Current Topics in Bioenergetics*, Vol. 1, Academic Press, New York, 1966.
17 S. OKAYAMA, *Plant Cell Physiol.*, 5 (1964) 145.
18 M. MICHEL-WOLWERTZ, *Carnegie Inst. Year Book*, 67 (1968) 505.