BBA 45516

STUDIES ON THE EFFECTS OF ULTRAVIOLET IRRADIATION ON PHOTOSYNTHESIS AND ON THE 520 nm LIGHT–DARK DIFFERENCE SPECTRA IN GREEN ALGAE AND ISOLATED CHLOROPLASTS*

K. E. MANTAI AND N. I. BISHOP

Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oreg. (U.S.A.)

(Received August 2nd, 1966)

SUMMARY

Ultraviolet irradiation inhibits photosynthesis, photoreduction, Hill reaction and the 520 nm light–dark absorbance change of algae and spinach chloroplasts. With total inactivation of these reactions the endogenous plastoquinone level dropped only about 40%. Ascorbate–2,6-dichlorophenolindophenol will at least partially restore a 520 nm signal in 3(3,4-dichlorophenyl)-1,1-dimethylurea- or ultraviolet-treated chloroplasts, but not in petroleum ether-extracted chloroplasts. It is concluded that total destruction of endogenous plastoquinone is not the exclusive cause of ultraviolet light inhibition of photosynthesis.

INTRODUCTION

Early experiments of ARNOLD[1] and HOLT, BROOKS AND ARNOLD[2] demonstrated that ultraviolet irradiation (253.7 nm) of whole cells of Chlorella and of isolated chloroplasts results in inhibition not only of photosynthesis but also of the Hill reaction. Since practically all partial reactions of photosynthesis, *e.g.*, Hill reaction, photoreduction, and non-cyclic photophosphorylation, are inhibited by ultraviolet irradiation, to a greater extent than endogenous respiration and activities of specific enzymes it has been assumed that its mode of action is comparable to that of 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and *o*-phenantroline. Following the finding that plastoquinone restores photochemical activity to petroleum ether-extracted chloroplasts, BISHOP[3] proposed that the inhibitory action of ultraviolet light on photosynthesis might result from the destruction *in vivo* of plastoquinone. Preliminary experimental evidence showed that irradiation of spinach chloroplasts produced a proportional decrease in Hill reaction activity and of plastoquinone concentration[4]. These findings have been extended by SHAVIT AND AVRON[5] and TREBST[6] and it is apparent that oxygen evolution and cyclic and non-cyclic photophosphorylation are inhibited by ultraviolet irradiation and further that an apparent parallel destruction of plastoquinone seems to occur.

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

* The data presented in this paper represent a portion of the Ph.D. dissertation of one of us (K.E.M.).

Through extensive studies on the 520 nm light-induced spectral shift in chloroplasts and in green algae WITT and colleagues[7] have concluded that this absorption change is related to the redox status *in vivo* of plastoquinone. This conclusion has been reached through detailed analysis on the kinetics of the absorption change occurring *in vivo* at 254 nm and by comparison of such kinetics to those of the absorption change around 520 nm. Studies on the effects of extraction of plastoquinone on the presence or absence of this spectral change, and the effects of the readdition of plastoquinone to extracted chloroplasts also have been used in support of this interpretation.

It is the purpose of this paper to present further evidence on the role of plastoquinone in the formation of the 520 nm light-induced absorption change and to show that ultraviolet irradiation produces a parallel inhibition of not only photosynthesis, photoreduction, and the Hill reaction but also of the 520 nm absorption change.

MATERIAL AND METHODS

The green algae *Scenedesmus obliquus*, strain D_3 and *Chlorella pyrenoidosa*, strain Emerson were employed for measurement of photosynthesis, photoreduction, and the quinone–Hill reaction. Chloroplasts were prepared from spinach (*Spinacea oleracea*) according to the method of AVRON[8].

Changes of absorbance at 520 nm were measured with an apparatus similar to that described by DE KOUCHKOVSKY AND FORK[9]. The desired monochromatic light was obtained with a combination of Jena-Schott and Baird-Atomic interference filters and Corning glass filters. The light-initiated absorbance changes were monitored either on an oscilloscope screen (Hewlett-Packard-140-A) or an oscillograph (BLH-Meterite).

Ultraviolet irradiation was performed in a thermostated device similar to that described by ANDERSON[10]. A Westinghouse 15 W germicidal lamp was used as the source for ultraviolet light. Approx. 85 % of the energy of this lamp is emitted at 253.7 nm. Incident intensity during irradiation was approx. $3.8 \cdot 10^4$ ergs$\cdot$sec$^{-1}\cdot$cm^{-2}.

Plastoquinone was extracted from chloroplasts with acetone and from algae with warm methanol. Since these procedures not only remove plastoquinone but also chlorophylls, carotenoids, neutral lipids, *etc.*, further purification was necessary for quantitative estimation of plastoquinone. The pigments were subsequently partitioned into purified petroleum ether (45–60°), washed free of methanol (or acetone), dehydrated with anhydrous Na_2SO_4, and evaporated to dryness. Further purification of the plastoquinone was performed with column chromatography on silicic acid according to methods previously described[3]. The fraction removed with the solvent mixture of 75 % chloroform–25 % isooctane contained plastoquinone. The fraction was evaporated to dryness, redissolved in ethanol (absolute) and the concentration of plastoquinone determined by measuring the absorbance change caused upon the addition of $NaBH_4$. Further purification of plastoquinone was accomplished with thin-layer chromatography[11].

RESULTS AND DISCUSSION

In Fig. 1a the effects of ultraviolet irradiation on photosynthesis and photoreduction of Scenedesmus are presented; it is apparent that about equal inhibition is produced for both reactions. Similarly the Hill reaction activity of spinach chloro-

plasts, irrespective of the oxidant used, is suppressed by ultraviolet irradiation (Fig. 1b). The notable exception involves the reduction of $NADP^+$ by chloroplasts in the presence of the artificial electron-donor system DCIP–ascorbate (Fig. 1b). Also, the

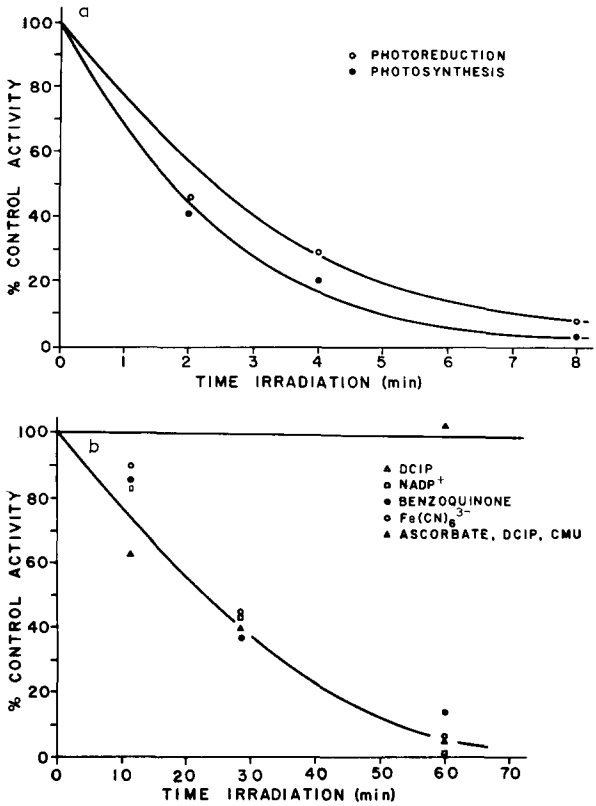

Fig. 1. a. Effect of ultraviolet irradiation on photosynthesis and photoreduction of Scenedesmus. Experiments performed on aliquots of the same suspension of cells b. The action of ultraviolet irradiation on various photochemical reactions of isolated spinach chloroplasts. Temperature, 25°; light intensity, 10^5 ergs·sec^{-1}·cm^{-2}.

photooxidation of reduced cytochrome *c*, as catalyzed by plastocyanin, is not affected. Both of these systems are thought to constitute part of Photosystem I of photosynthesis and from these data it appears that this portion of the photosynthetic mechanism is not damaged by ultraviolet irradiation.

Examination of the red light-induced absorbance change at 520 nm revealed a parallel decrease in the signal's magnitude and the rate of photosynthesis or Hill reaction (Fig. 2). This was seen both in Chlorella and in Scenedesmus with the latter being much less sensitive to irradiation. The biphasic nature of this particular light-induced absorption change is well known from earlier studies (Fig. 3). It is clear, at least for Scenedesmus, that ultraviolet irradiation initially destroys the second portion of the signal. This 'overshoot' has been attributed to reactions of Photosystem II of photosynthesis[12,13]. Comparison of Figs. 2 and 3 shows that inhibition of photosynthesis coincides with the disappearance of this portion of the overall signal.

Examination of the plastoquinone levels of irradiated algal cells or of chloro-

plasts indicate that only about a 40 % loss of plastoquinone content had occurred when maximal inhibition of photosynthesis or of the Hill reaction was attained (Table I). Attempts to reverse the effects of ultraviolet damage to chloroplasts by the addition

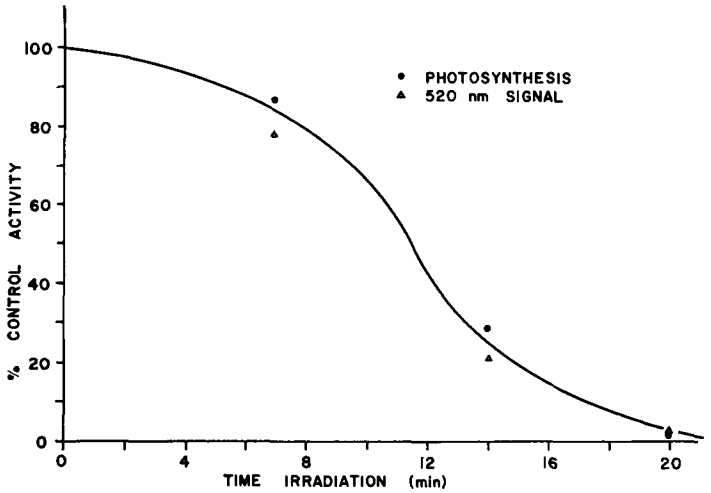

Fig. 2. Comparison of the effects of ultraviolet irradiation on photosynthesis and on the 520 nm signal (overshoot portion) in Scenedesmus. Experimental conditions as indicated under METHODS and in Fig. 1.

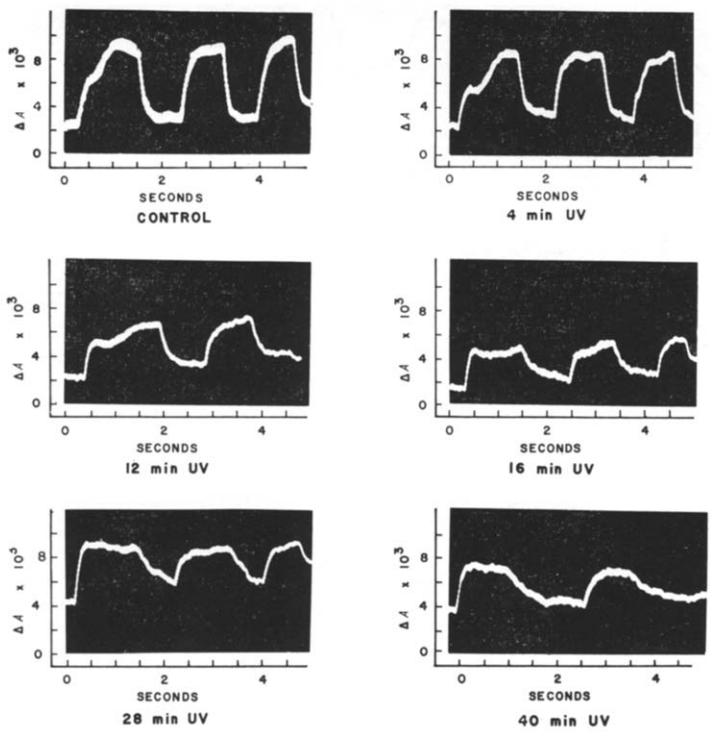

Fig. 3. Oscilloscope tracings demonstrating the effect of ultraviolet (UV) light on the 520 nm signal of Scenedesmus. Experimental conditions as indicated under METHODS.

of a petroleum ether extract of unirradiated cells or of authentic plastoquinone have not been successful. However, in ultraviolet-irradiated chloroplasts, a 520 nm signal is restored upon the addition of DCIP–ascorbate (Fig. 4c and d). This signal, unlike the normal signal of chloroplasts, is DCMU insensitive and apparently arises only through Photosystem I.

It is well authenticated that extraction of plastoquinone from lyophilized chloroplasts results in not only inactivation of the Hill reaction and photophosphory-

TABLE I

PLASTOQUINONE ANALYSIS OF ULTRAVIOLET-IRRADIATED CHLORELLA CELLS SHOWING COMPLETE INHIBITION OF 520 nm SHIFT

Per cent destruction of plastoquinone calculated by employing the $E^{1\%}_{1\,cm}$ (ox.–red.) = 19.6. Plastoquinone was extracted and purified as indicated in METHODS. The signal magnitude is given in in absorbance unit. N.S. signifies no detectable signal.

Expt. No.	$\Delta A_{255\ nm}$		*% Destroyed*	$\Delta A_{520\ nm}$	
	Control	*Irradiated*		*Control*	*Irradiated*
1	0.180	0.106	41	$8 \cdot 10^{-3}$	N.S.
2	0.080	0.055	31	$5 \cdot 10^{-3}$	N.S.
3	0.145	0.074	49	$5 \cdot 10^{-3}$	N.S.

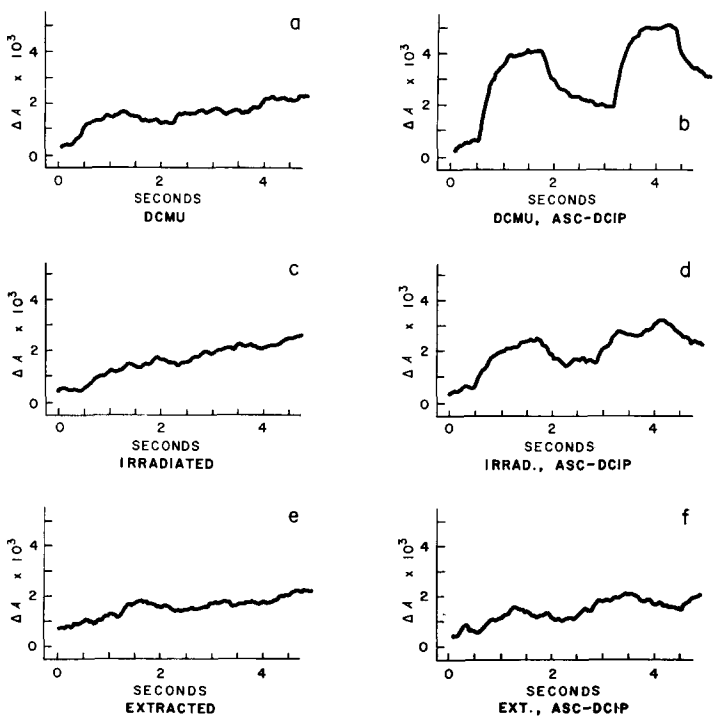

Fig. 4. Comparison of the influence of DCMU (a and b), ultraviolet irradiation (c and d), and petroleum ether extraction (e and f), on the ascorbate(ASC)–DCIP-catalyzed 520 nm absorbance change in lyophilized spinach chloroplasts. 16 mg chloroplasts resuspended in 1.5 ml 0.5 M sucrose–0.03 M KCl. DCMU, 0.015 μmole; ascorbate, 20 μmoles; DCIP, 0.2 μmole.

lation (both cyclic and non-cyclic) but also causes the loss of the 520 nm absorbance change. WITT and colleagues[7] have used this latter observation to implicate plastoquinone as being part of the chromophore responsible for this signal. Experiments comparable to those summarized in Fig. 4c and d, and Table I were made on chloroplasts extracted with cold petroleum ether; the absorbance change at 520 nm was almost completely abolished. These data substantiate the observations of WITT and colleagues on this particular signal for chloroplasts as to the effect of extraction. We have not been able, however, to restore the entire signal by readdition of either the petroleum ether extract or of purified plastoquinone. A DCMU-insensitive signal can be partially restored with DCIP–ascorbate (Fig. 4e and f). The data of Fig. 4 indicate that a partial restitution of a DCMU-insensitive signal occurs either with petroleum ether-extracted or ultraviolet-irradiated chloroplasts. The degree of recovery follows the Hill reaction activity of such chloroplasts even though in DCMU-treated cells, where little or no Hill reaction activity is present, a much larger signal is obtained with DCIP–ascorbate.

From data presented in Fig. 2 it is apparent that ultraviolet irradiation of chloroplasts results in a complete loss of the 520 nm absorbance change and various photochemical reactions associated with both Photosystem I and II. However, total destruction of plastoquinone was never observed. Despite the inactivation of Photosystem II by irradiation, the absorbance change at 520 nm is restored by the addition of alternate electron-donor systems. Since removal of the plastoquinone by extraction also results in the loss of this signal, which is not reversed by DCIP–ascorbate, it seems logical to conclude that the major cause of ultraviolet inactivation of photosynthesis is not due to a total destruction of plastoquinone. Because of the high concentration of plastoquinone A relative to other constituents of the photosynthetic electron-transport system the possibility cannot be dismissed that ultraviolet irradiation destroys a pool of plastoquinone, perhaps plastoquinone A or some of the other quinones such as plastoquinone B or C, or vitamin K, which occupies a key role in the electron-transport sequence. Since the effects of petroleum extraction on the 520 nm absorption change are not reversed with either the DCIP–ascorbate, or diaminodurol electron-donor system it appears that plastoquinone is required for the generation of this absorbance change even though the responsible light reaction occurs *via* Photosystem I. WITT's proposal that a portion of the 520 nm change can be attributed to a cyclic flow of electrons involving plastoquinone appears reasonable.

ACKNOWLEDGEMENT

This research was supported by a grant from the U.S. Atomic Energy Commission AT (45–1)-1783.

REFERENCES

1 W. A. ARNOLD, *J. Gen. Physiol.*, 17 (1933) 135.
2 A. S. HOLT, I. A. BROOKS AND W. A. ARNOLD, *J. Gen. Physiol.*, 34 (1951) 627.
3 N. I. BISHOP, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1696.
4 N. I. BISHOP, *Ciba Symposium on Quinones in Electron Transport*, Churchill, London, 1961, p. 385.
5 N. SHAVIT AND M. AVRON, *Biochim. Biophys. Acta*, 66 (1963) 187.
6 A. TREBST AND E. PISTORIUM, *Z. Naturforsch.*, 20b (1965) 885.

7 J. WEIKAR, A. MÜLLER AND H. T. WITT, *Z. Naturforsch.*, 18b (1963) 139.
8 M. AVRON, *Biochim. Biophys. Acta*, 40 (1960) 257.
9 Y. DE KOUCHKOVSKY AND D. C. FORK, *Proc. Natl. Acad. Sci. U.S.*, 52 (1964) 232.
10 D. R. ANDERSON, *Effects of Radiation on the Hill Reaction*, Ph.D. Dissertation, University of Utah, 1956.
11 N. I. BISHOP, *Record Chem. Progr.*, 25 (1964) 181.
12 D. RUBINSTEIN, *Biochim. Biophys. Acta*, 109 (1965) 41.
13 W. URBACH AND D. C. FORK, *Carnegie Inst. Yearbook*, 64 (1965) 390.