In photoinhibited photosystem II particles pheophytin photoreduction remains unimpaired

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Oxygen-evolving photosystem II particles (DT 20) isolated from pea chloroplasts by digitonin-Triton X-100 fractionation were photoinhibited with 150 $W \cdot m^{-2}$ white light, at 20°C under three conditions: aerobic, anaerobic and strongly reducing (E_h poised to approx. −250 mV with dithionite). Hill reaction rate ($H_2O \rightarrow BQ$) and variable fluorescence (F_v) declined in parallel in all three cases with shortening half times: 30, 10 and 2.5 min, respectively. Light-induced absorbance changes at 685 nm characteristic of reversible photoaccumulation of reduced pheophytin ($E_h \approx$ −250 mV) remained essentially unchanged. We conclude that the three types of photoinhibitory treatment do not impair the separation of charges between chlorophyll P-680 and pheophytin in the photosystem II reaction center.

Photoinhibition; Charge separation; Pheophytin; Oxygen-evolving PS II particle; (Pea chloroplasts)

1. INTRODUCTION

The problem of photoinhibition attracts considerable interest particularly since it became clear that an inactivation of PS II occurs even at irradiances far below photosynthesis saturation [1–5]. Within the last few years two antagonistic views on the mechanism of the process became established: one emphasizing the central role of the socalled Q_B-protein [6–8] and the other concentrating on events taking place in the PS II reaction center [9–12]. If it becomes confirmed that the Q_B-

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Abbreviations: PS, photosystem; RC, reaction center; P-680, the primary electron donor; Pheo (pheophytin), the intermediary electron acceptor; Q_A, the primary (quinone) electron acceptor in PS II; F_o, F_v, F_m, constant, variable, maximum fluorescence; HRR, Hill reaction rate ($H_2O \rightarrow BQ$); BQ, *p*-benzoquinone; Mes, 2-(*N*-morpholino)ethanesulfonic acid

protein is a constituent part of the RC [13,14] the discrepancy may be largely reconciled. Anyway, there seems to be a fairly general consent at present that the primary step in PS II photoinactivation is a low quantum yield photochemical event affecting one of the actors of the primary charge separation and stabilization within the core of PS II [1,10,11,15,16]. In line with this view the rate of the PS II photoinactivation under aerobic conditions is a linear function of irradiance far beyond the level at which electron transfer in the thylakoids becomes light saturated [17]. Where exactly the primary damage occurs, remains unsolved. Melis and collaborators claim [11,12] that it is the primary separation of charges that becomes inactivated. Our results presented here are at variance with this conclusion. In profoundly photoinhibited PS II oxygen-evolving particles the charge separation between P-680 and pheophytin remains unaffected.

Kinetics and other characterics of photoinhibition differ markedly when brought about under aerobic, anaerobic and reducing conditions (e.g.

[9,15,18,19]. We have, therefore, performed the experiments under these three conditions.

2. MATERIALS AND METHODS

Oxygen-evolving PS II particles DT 20 were isolated from pea chloroplasts and their spectrophotometric properties were measured in the phosphoroscopic set-up [20]. Assuming the differential extinction coefficient for reduced pheophytin (Pheo$^{\cdot-}$-Pheo) to be 32 mM$^{-1}\cdot$cm^{-1}, the particles contained 1 pheophytin molecule per 80-100 chlorophyll molecules. Photoinhibition treatment was applied and electron transport through PS II was assessed as described earlier [5]. The exposure and measuring medium (medium A) had the following composition: 20 mM Mes (pH 6.5), 35 mM NaCl, 5 mM MgCl$_2$, 300 mM sucrose. Anaerobic conditions were established by oxidation of glucose (10 mM) catalysed by glucose oxidase (50 units$\cdot$ml^{-1}) in the presence of catalase (10^3 units$\cdot$ml^{-1}), referred to in the text as GGOC treatment (see also [21,22]). Reducing conditions were brought about by poising the E_h of the mixture to about -250 mV by the addition of solid dithionite to the final concentration of approx. 0.5 mg$\cdot$ml^{-1} (cf. [20]). Before measuring the values of HRR, F_v and F_o in the samples from the anaerobic and reducing conditions the particles were spun down and resuspended in a fresh medium containing oxygen.

3. RESULTS

Fig.1 serves to define the fluorescence (F_v, F_o) and absorption (ΔA_{685}) characteristics of the DT 20 particles that were monitored during the photoinhibition experiments. It also illustrates the indirect ('F_v suppression') method to determine the components of DT 20 fluorescence (see also [19,20]). Fig.2A shows a part of the difference absorption spectrum (light minus dark) of the accumulated anion radical of pheophytin in the DT 20 particles. The signal at 685 nm was used for the routine assay but the specificity of the absorption change for reduced pheophytin was checked by measuring simultaneously the ΔA signals at other wavelengths: at 675 nm and 650 nm as exemplified in fig.2B or at 545 nm as shown in fig.1B.

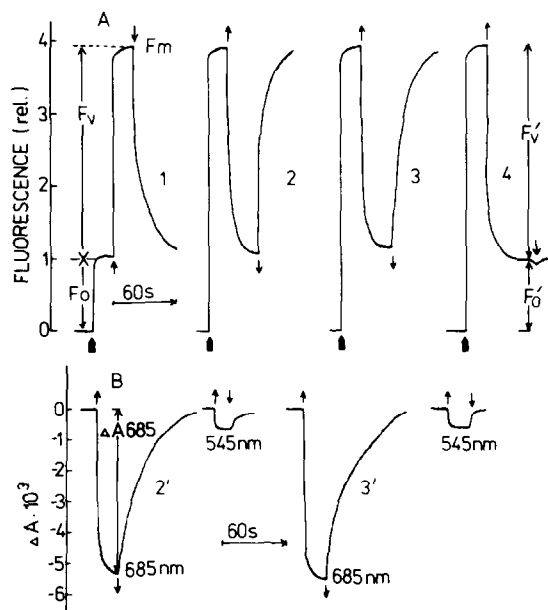Fig.3A illustrates the course of PS II photoinac-

Fig.1. Fluorescence behavior and absorbance changes of the DT 20 PS II particles suspended in medium A. (A) Under aerobic conditions (1) the measuring beam (0.1 W$\cdot$m^{-2}, $\lambda = 480$ nm; heavy arrow) elicits F_o and the actinic beam (120 W$\cdot$m^{-2}, white light, light arrow) rises fluorescence to F_m; $F_v = F_m - F_o$. Anaerobic conditions (2) were established by the GGOC reaction (see section 2) and reducing conditions (3) by bringing the anaerobic mixture to $E_h \approx -250$ mV with dithionite. In both conditions F_m is seen soon after switching on the measuring light and the actinic light brings fluorescence down to nearly F_o due to photoreduction of pheophytin. Thus, reducing conditions, also at $E_h \approx -450$ mV (4) offer an alternative possibility to determine F_v' and F_o'. (B) Kinetics of the photoinduced ΔA characteristic of the Pheo$^{\cdot-}$ accumulation under anaerobic (2') and reducing (3') conditions.

tivation in DT 20 particles exposed to 150 W$\cdot$m^{-2} white light under aerobic conditions. HRR declines in parallel with F_v while F_o and ΔA_{685} remain essentially constant. In samples taken from a suspension of DT 20 particles kept in the dark (fig.3B) none of the measured quantities shows any significant change during the experiment.

When the same irradiance is applied under strictly anaerobic conditions (fig.4A) the photoinhibition of both HRR and F_v occurs noticeably faster (half-time less than 10 min). Also, as observed earlier [18,23], F_o increases taking a course nearly
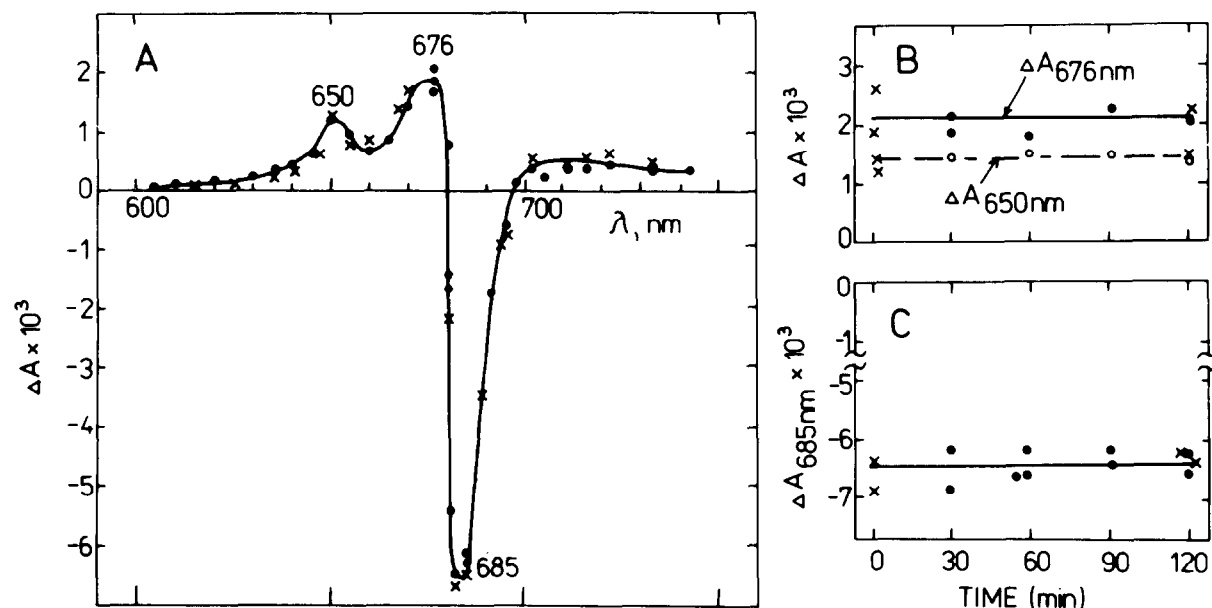
Fig.2. (A) Difference absorption spectrum of the reduced pheophytin in DT 20 particles suspended ($10\ \mu g$ Chl·ml^{-1}) in medium A, with added dithionite ($E_h \approx -250$ mV). Optical path length 10 mm. The ΔA elicited by white actinic light, 120 W·m^{-2}. Results of two measurements are shown ($\times$, ●) (B,C) During the photoinhibition experiment under reducing conditions (see fig.5) the ΔA was measured at the three wavelengths indicated; the conservation of proportionality shows that no photobleaching of other pigments is observed.
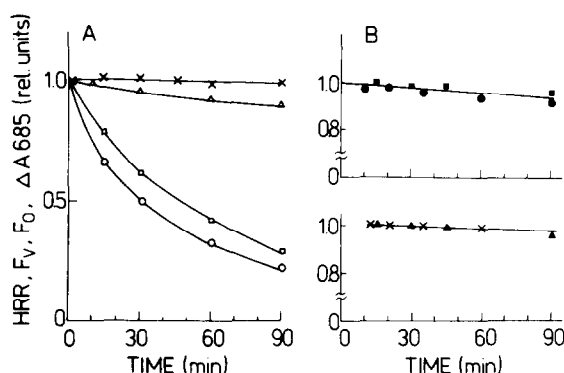

Fig.3. Photoinactivation of PS II in DT 20 particles exposed under aerobic conditions to 150 W·m^{-2} white light at 20°C. Particle concentration corresponds to 10 μg (Chl·ml^{-1}, optical path length 20 mm. (A) Samples from photoinhibition treatment. (B) Samples from the control kept in the dark. All quantities are represented as fractions of the initial value. This was 120 μmol O$_2$·mg^{-1} for HRR and -6.5×10^{-3} for ΔA_{685} in the cuvette with 10 mm optical path length. Fluorescence yields were not quantitated, but their relative magnitude can be compared reliably. Symbols: (○) F_v; (□) HRR; (×) F_o; (△) ΔA_{685}.

antiparallel to F_v. ΔA_{685} does not show any change within the period sufficient for 90% suppression of electron transport and variable fluorescence.

If the anaerobic reaction mixture is poised to approx. -250 mV by the addition of dithionite (fig.5A), the rate of photoinhibition further increases (half-times approx. 2.5 min). Nevertheless, the value of ΔA_{685} remains nearly constant even after light treatment of 120 min (see fig.2B). The
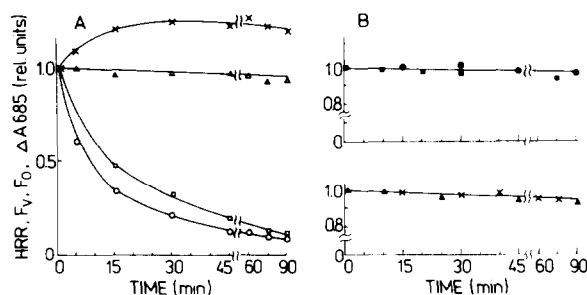

Fig.4. Photoinactivation of PS II in DT 20 particles under anaerobic conditions established by the GGOC reaction (see section 2). Other conditions and symbols as in fig.3.
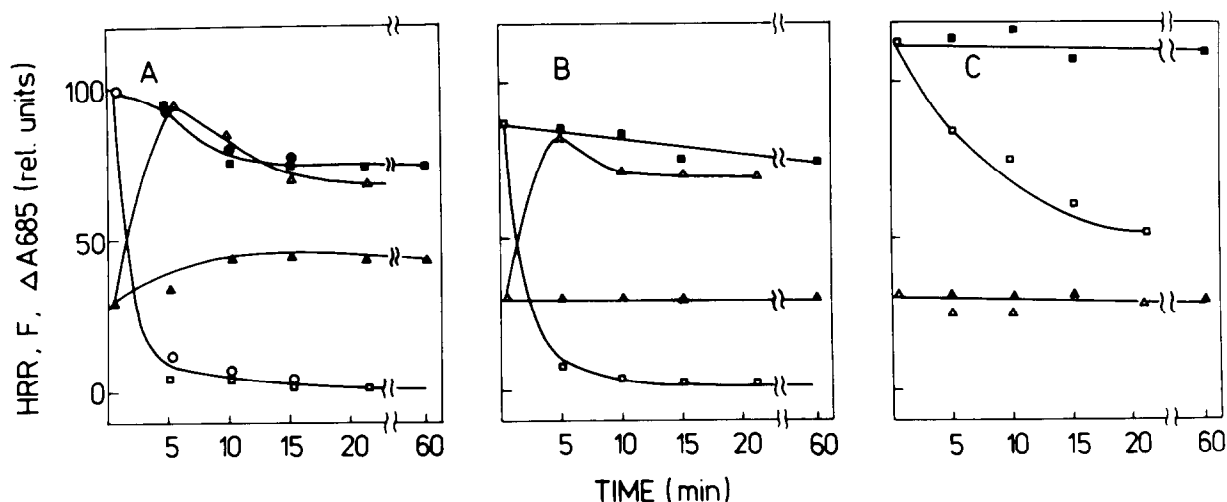
Fig.5. Photoinactivation of PS II in DT 20 particles under reducing conditions (E_h poised to -250 mV by dithionite). Fluorescence values in all three parts of the figure are referred to the initial value of F_v represented in part A as 100 units. The initial value of HRR was 130 μmol $O_2 \cdot mg^{-1}$ Chl$\cdot h^{-1}$. (A) Values measured in the samples cleared of dithionite by one centrifugation and resuspension in medium A. (B) Values measured upon addition of ferricyanide (final concentration approx. 1 μmol) to the samples measured in A. (C) Values measured upon reducing E_h to approx. -450 mV by addition of dithionite to the samples represented in B. Symbols: (O,●) HRR; (△,▲) F_o; (□,■) F_v; (O,△,□) photoinhibitory treatment; (●,▲,■) control kept in the dark. ΔA_{685} values illustrated in fig.2B.

behavior of F_o is, however, somewhat complex in this case. If measured in the usual way (i.e., upon removal of dithionite from the exposed samples by centrifugation) the values of F_o increase conspicuously in the first 5 min of photoinhibition and decline slowly afterwards (fig.5A). A slow increase of F_o is observed also in DT 20 particles incubated with dithionite in the dark but this increase can be suppressed by addition of ferricyanide (1 μM final concentration) to the washed samples before measuring the fluorescence (fig.5B). Such treatment is, however, without effect in photoinhibited samples (fig.5B). In contrast to these results are the values estimated in parallel samples by the 'F_v suppression method' (as defined in fig.1A, curve 4). F_o' remains stable throughout the experiment (fig.5C). The course of F_v' also differs from that of F_v; its decline is slower and it does not approach zero values. Experiments are continued to explain this behavior.

4. DISCUSSION

The primary motive for performing the experiments reported here was to check the possibili-ty that the first step in PS II photoinactivation is trapping of the PS II RC in the state with reduced pheophytin, i.e., the state P-680$\cdot$Pheo$\cdot^- Q_A^-$. Photoaccumulation of this state results in both quenching of fluorescence and loss of photochemical activity of PS II measured after removal of dithionite [19,20]. These are the characteristics of photoinhibited PS II. Our present results show clearly that PS II particles which were nearly completely photoinactivated, judging by the electron transport and fluorescence criteria, are still fully functional in the primary separation of charges between P-680 and Pheo. This fact falsifies not only our hypothesis but any other proposal situating the primary photoinhibitory event within the P-680–Pheo couple.

Our results do not help to specify the nature of the primary event in PS II photoinactivation. They serve, however, to localize its site on the reducing side of the PS II reaction center. The reasoning is as follows: it is generally accepted that the primary damage in photoinhibition does not involve the oxygen-evolving complex, and that, on the oxidation side of PS II RC, only the primary oxidant Z and/or electron donation to P-680$^+$ might be af-

fected [7,10,24,25]. We have confirmed that in photoinhibited DT 20 particles the spectrum and the kinetics of the EPR signal II_f (g = 2.0042; H = 18 G) remain unchanged (not shown), thus demonstrating that the functioning of Z is not impaired. Since the primary separation of charges is not affected either, the target of the PS II photoinactivation must lie beyond the pheophytin. The increase of F_o under reducing and anaerobic conditions is also consistent with an impairment of electron transport in the Q_A-Q_B region. Our results also show that the disappearance of the Q_B-protein (which is probably a component of the PS II RC) from the thylakoid membrane is a consequence of PS II photoinactivation and not a part of its mechanism.

REFERENCES

[1] Powles, S. (1983) Annu. Rev. Plant Physiol. 35, 15–44.

[2] Satoh, K. (1970) Plant Cell Physiol. 11, 15–27.

[3] Šetlík, I., Šetlíková, E., Masojídek, J., Kalina, T. and Mader, P. (1981) in: Photosynthesis (Akoyunoglou, G. ed.) vol. 3, pp. 481–490, Balaban Int. Sci. Services, Philadelphia.

[4] Šetlíková, E., Masojídek, J., Nedbal, L. and Šetlík, I. (1984) in: Advances in Photosynthesis Research (Sybesma, C. ed.) vol. 3, pp. 255–258, Nijhoff/Junk, Dordrecht, The Netherlands.

[5] Nedbal, L., Šetlíková, E., Masojídek, J. and Šetlík, I. (1986) Biochim. Biophys. Acta 848, 108–119.

[6] Kyle, D.J. (1985) Photochem. Photobiol. 41, 107–116.

[7] Arntzen, C.J., Kyle, D.J., Wettern, M. and Ohad, I. (1984) in: Biosynthesis of the Photosynthetic Apparatus (Thornber, J.P. et al. eds) UCLA Symposium Series, no. 14, pp. 313–324, Alan Liss, New York.

[8] Reisman, S. and Ohad, I. (1986) Biochim. Biophys. Acta 849, 51–61.

[9] Satoh, K. and Fork, D.C. (1982) Plant Physiol. 70, 1004–1008.

[10] Cleland, R.E. and Critchley, C. (1985) Photobiochem. Photobiophys. 10, 83–92.

[11] Cleland, R.E., Melis, A. and Neale, P.J. (1986) Photosynth. Res. 9, 79–88.

[12] Demeter, S., Neale, P.J. and Melis, A. (1987) FEBS Lett. 214, 370–374.

[13] Trebst, A., and Depka, B. (1985) in: Antennas and Reaction Centers of Photosynthetic Bacteria – Structure Interaction and Dynamics (Michel-Beyerle, M.E. ed.) pp. 216–224, Springer, Berlin.

[14] Nanba, N. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–111.

[15] Arntz, B. and Trebst, A. (1986) FEBS Lett. 194, 43–49.

[16] Barényi, B. and Krause, G.H. (1985) Planta 163, 218–226.

[17] Šetlík, I., Nedbal, L., Šetlíková, E., Sofrová, D. and Masojídek, J. (1987) in: Progress in Photosynthesis Research (Biggins, J. ed.) vol. 4, pp. 55–58, Nijhoff, Dordrecht, The Netherlands.

[18] Krause, G.H., Köster, S. and Wong, S.C. (1985) Planta 165, 430–438.

[19] Karapetjan, N.V. and Klimov, V.V. (1973) Fiziologija Rastenij 20, 545–553.

[20] Klimov, V.V., Allakhverdiev, S.I. and Pashchenko, V.Z. (1978) Dokl. Akad. Nauk SSSR 242, 1204–1207.

[21] Klimov, V.V., Shuvalov, V.A. and Heber, U. (1985) Biochim. Biophys. Acta 809, 345–350.

[22] Klimov, V.V., Allakhverdiev, S.I. and Ladygin, V.G. (1986) Photos. Res. 10, 355–361.

[23] Satoh, K. (1971) Plant Cell Physiol. 12, 13–27.

[24] Callahan, F.E., Becker, D.W. and Cheniae, G.M. (1986) Plant Physiol. 82, 261–269.

[25] Tytler, E.M., Whitelam, G.C., Hipkins, M.F. and Codd, G.A. (1984) Planta 160, 229–234.