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Reduced content of the quinone acceptor Q_A in photosystem II complexes isolated from thylakoid membranes after prolonged photoinhibition under anaerobic conditions

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The plastoquinone content of photosystem II complexes isolated from spinach photosystem II-enriched membranes subjected to strong photoinhibitory illumination under anaerobic conditions was determined by HPLC. A pronounced decrease of the plastoquinone content was found in the photoinactivated complexes. These results corroborate earlier models in which photoinhibitory illumination is suggested to eventually lead to release of doubly reduced and protonated Q_A from its site on the D2-protein.

Photoinhibition; Photosystem II; Plastoquinone

1. INTRODUCTION

Photosystem II (PSII) utilises light energy to reduce plastoquinone with electrons derived from water (for a review see [1]). At high light intensities PSII electron transport is impaired, due to a phenomenon generally denoted as photoinhibition [2–5]. This inactivation of the electron transport is followed by degradation of the PSII reaction centre proteins, in particular the D1 and to a minor extent also the D2 protein [2–4]. Several hypotheses exist for the reactions that lead to photoinhibition and there is experimental evidence to suggest that it may be induced by limitations on either the acceptor- or the donorside of PSII or a combination of both [2–5]. A detailed sequential mechanism for the acceptor-side induced inactivation has recently been proposed based upon studies of photoinhibition of spinach PSII under anaerobic conditions in vitro [5–8]. As judged by fluorescence and EPR spectroscopy, reversibly inhibited states containing singly reduced, long-lived Q_A-species are formed. Subsequently, the long-lived Q_A^- is proposed to be further stabilized through protonation [7,8] which, during the continued strong illumination, is followed by double reaction of Q_A. The available data indicate that the photoinactivation up to this stage is reversible [6–10] and it can be reversed simply by reoxidation of Q_A [7]. Finally, upon further photoinhibitory treatment under the anaerobic conditions an irreversibly inhibited state is formed. This state was proposed to represent centres where doubly reduced and protonated Q_A has left its binding site on the D2-protein [7]. Several of the inactivation intermediates can give rise to chlorophyll triplets in the light [7,8] which in the presence of oxygen can promote singlet oxygen formation which in turn has been proposed to damage the D1-protein thereby inducing its degradation [7,11].

The occurrence of empty Q_A-sites was initially suggested to take place after chemical reduction of Q_A [12] or during photoinactivation of PSII under aerobic conditions [13]. However, there exist no biochemical evidence that Q_A (in any oxidation state) might actually leave its binding site in the reaction centre of PSII. Therefore in an attempt to elucidate and corroborate the mechanistic model outlined in [7] by other than spectroscopic methods we have analysed the plastoquinone content in isolated PSII core complexes by HPLC after photoinhibitory illumination under anaerobic conditions. It was found that the quinone content in the PSII core complexes was reduced by up to 63% suggesting that Q_A by prolonged strong illumination can be lost from its binding site.

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Abbreviations: Chl, chlorophyll; EPR, electron paramagnetic resonance; HPLC, high pressure liquid chromatography; MES, 2-(*N*-morpholino)ethanesulphonic acid; PSII, photosystem II; PQ, plastoquinone; Q_A and Q_B, primary and secondary plastoquinone acceptors, respectively; P680, primary donor in photosystem II; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

2. MATERIALS AND METHODS

PSII enriched thylakoid membranes (BBY-particles) from spinach leaves were isolated according to [14] and suspended in 50 mM MES,

pH 6.0, 10 mM NaCl, 5 mM $CaCl_2$ and 400 mM sucrose to a concentration of 0.2 mg chlorophyll ml^{-1}.

PSII core complexes were isolated according to Ghanotakis et al. using extraction with *n*-octyl β-D-glucopyranoside [15]. The chlorophyll a/b ratio, measured as in [16] was about 12 indicative of very low contamination of accessory light-harvesting chlorophyll a/b proteins. All the preparation steps were carried out on ice and in darkness. The PSII core complexes were stored in liquid nitrogen until quinone extraction.

Photoinhibitory illumination was performed under anaerobic conditions. A sample of 30 ml of PSII enriched membranes (0.2 mg chlorophyll ml^{-1}) was photoinactivated at 20°C with white light at an intensity of 5500 μmol photons $m^{-2}·s^{-1}$ in anaerobic glass vials as in [6]. The non-photoinhibited control material was kept under the same incubation conditions but in complete darkness. Immediately after the photoinhibitory treatment the BBY-particles were chilled to 4°C and the PSII core complexes were prepared in complete darkness.

Quinones and lipids were extracted from the purified PSII core complexes with chloroform/methanol/water (3:2:1) as described in [17]. The lipid extract was applied on a silica gel column and eluted with diethyl ether (25%) in hexane. The eluate was dried under nitrogen and dissolved in a mixture of methanol/propanol/hexane (2:1:1).

The HPLC analyses were performed as in [17] on a Schimadzu-LC4A system fitted with a C18 reverse phase column (Hewlett-Packard, ODS Hypersil, 3 μm). A combination of linear gradients from the initial methanol/water (9:1) in pump A to methanol/propanol/hexane (2:1:1) in pump B was operated for 35 min at a flow rate of 1.5 $ml·min^{-1}$. The absorbance of the eluate was monitored at 255 nm with a Flo-one beta detector (Radiomatic). The data were processed using the Flo-one A500 program. The amount of plastoquinone in the samples was estimated from a standard curve. The recovery reached in the chromatographic procedure was > 80% as determined from the recovery of plastoquinone-6, which was used as an internal standard, since it did not comigrate with plastoquinone-9 (not shown).

Proteins were separated on a 12–22,5% gradient SDS-PAGE containing 4 M urea [18] and then transferred electrophoretically [19] to PVDF membrane (Millipore) with a semidry electrophoresis unit (Pharmacia LKB, Multiphor 2117). Quantitative analysis was performed using antibodies against the D1- and D2-proteins (a generous gift from Dr. W. Vermaas) using ^{125}I-labelled protein A for detection. The exposed X-ray films were scanned with a laser densitometer (Molecular Dynamics) and analysed by the Image Quant program.

The PSII-mediated oxygen evolution was assayed in Clark type oxygen electrode (Hansa-Tech) in 50 mM MES, pH 6.0, 10 mM NaCl, 5 mM $CaCl_2$ and 400 mM sucrose using 0.4 mM phenyl-*p*-benzoquinone as electron acceptor.

3. RESULTS

Strong light under anaerobic conditions leads to inactivation of PSII electron transport, but unlike in the presence of oxygen, there is no degradation of the two reaction centre subunits D1 and D2 [6,7,20,21]. This is an experimental advantage since compositional and organizational changes to PSII induced by the light stress can be analysed prior to the biodegradation of the damaged reaction centres. Typically, in our experiments, when PSII-enriched membranes were subjected to strong illumination under anaerobic conditions, the inactivation half-time of electron transport was 27 min (Fig. 1). After 80 min only 15% of the activity remained (Fig. 1) and the majority of the PSII centres were found to be trapped in the irreversibly inhibited state previously suggested to lack Q_A [7]. Despite the pronounced inactivation of the PSII electron transport in this experiment there was virtually no degradation of the D1- or D2-proteins (not shown).

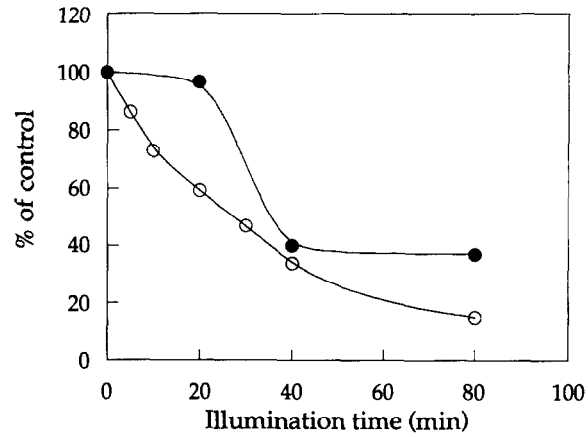

Fig. 1. Inactivation of oxygen evolution (-○-) and loss of plastoquinone (Q_A) (-●-) from PSII induced by strong illumination (5500 μmol photons $m^{-2}·s^{-1}$) under anaerobic conditions. The oxygen evolving activity in the control PSII enriched membranes was 424 μmol O_2 $h^{-1}·mg\ chl^{-1}$. PSII core complexes isolated from dark control BBY-membranes contained 1 plastoquinone per 35 chlorophyll.

At different time points, samples were taken to analyse the plastoquinone content of PSII. Prior to the quinone analysis, PSII core complexes were isolated from the photoinactivated BBY-particles. This was done in order to eliminate any contribution from the bulk pool of plastoquinone in the membrane thereby allowing a direct estimation of the amount of quinones actually bound to the PSII reaction centres. Moreover, since Q_B is lost from PSII during isolation of the core complexes [15] any variations in plastoquinone content between dark control complexes and photoinactivated complexes should be related to the amount of Q_A still in the site.

HPLC analysis of the quinones after the extraction of the isolated PSII core complexes revealed one peak which comigrated with purified plastoquinone. Quantification of the extracted plastoquinone showed that in control PSII core complexes isolated from BBY-membranes kept in the dark there was approximately one plastoquinone molecule per 35 molecules of chlorophyll, which is similar to what has previously been observed in this kind of PSII core preparations [22,23]. Photoinhibitory illumination for only 20 min did not give rise to any reduced content of Q_A in the isolated PSII complexes despite a 40% inactivation of the oxygen evolving activity (Fig. 1). This is consistent with previous observations that photoinactivation of PSII under anaerobic conditions in its early stages is reversible [6,7,9]. However, after photoinhibitory illumination for 40 min a pronounced reduction in the content of plastoquinone could be seen. After 80 min of photoinhibitory treatment there was only 1 plastoquinone per 94 chlorophyll molecules. This means that the PSII core complexes isolated after 80 min strong illumination of

the BBY-membranes contained only 37% plastoquinone as compared to those obtained from the dark control material (Fig. 1).

With our methodological approach it it not possible to deduce the kinetics for the quinone release with high precision but our data show that Q_A is released slower than the inactivation of oxygen evolution particularly during the initial phase of the light stress (Fig. 1).

It should be noted that neither in the control nor in the photoinactivated samples any peaks corresponding to plastoquinol or degradation products of plastoquinone were detected in the HPLC elution profile. In addition, the quinone extraction protocol used in this study was highly quantitative since neither a second extraction nor sonication of the protein pellet increased the yield of plastoquinone (not shown).

4. DISCUSSION

According to our recently presented mechanistic model for anaerobic photoinhibition, based upon EPR-spectroscopic evidence and fluorescence measurements in vitro [5,7,8], the primary quinone acceptor Q_A is reduced and protonated in a sequential manner leading ultimately to release of Q_A in the form of Q_AH_2. A release of Q_A, when it is fully reduced and protonated was originally suggested based upon analogy with the purple bacterial system [12,13]. However, there has been no direct analysis to support this hypothesis. In the present study we have therefore used biochemical methods to follow the fate of the tightly bound Q_A during strong illumination under anaerobic conditions. The plastoquinone analysis was performed by HPLC technique using isolated PSII core complexes where consequences of photoinhibitory treatment on the Q_A content can be studied without interference of Q_B or exogenous plastoquinone. After short photoinhibitory illumination there is no reduction in Q_A content of the PSII complexes consistent with the fact that photoinactivation under anaerobic conditions initially is reversible [6,7,9]. Notably, after more prolonged photoinhibitory illumination, there was a pronounced reduction in the Q_A content of the PSII core complexes isolated from the photoinactivated membranes (Fig. 1).

At present the mechanism behind this reduction in the Q_A content cannot be judged with certainty. It could be argued based upon the suggestions in [12,13], that the double reduction and protonation of Q_A in itself lead to a release of this species from the reaction centre in the same way as Q_BH_2 is released from the D1-protein during normal electron transport. Alternatively, the double reduction of Q_A or some other light-induced damage may induce a conformational change in the D2-protein that lowers the binding affinity of Q_A. Finally, at present it cannot be excluded that the photoinactivation destabilizes the Q_A-binding making the plastoquinone extractable by the detergent during the isolation of the

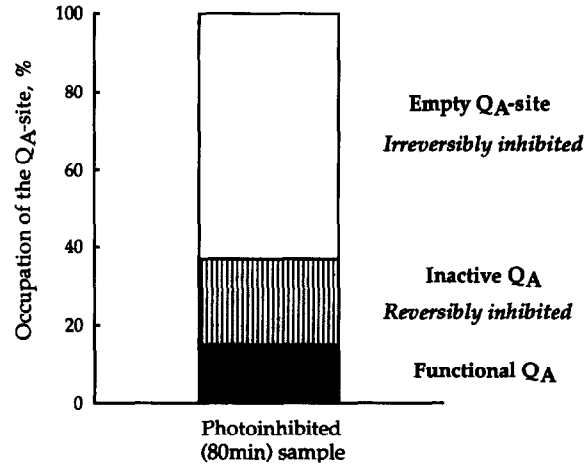

Fig. 2. Effect of prolonged (80 min) anaerobic photoinhibition of PSII enriched membranes on the Q_A content in isolated PSII core complexes. The various inhibited fractions are calculated with the assumption that all plastoquinone present in PSII complexes isolated from the dark control membranes represent Q_A and that all Q_A is active from the start. In total, approximately 37% of the initial plastoquinone remained bound to PSII after 80 min photoinhibitory illumination (hatched fractions).

PSII core complexes. However, the last alternative does not easily explain the transition from reversible to irreversible inactivation seen during prolonged strong illumination of the PSII-enriched membrane preparation [6,7]. As illustrated in Fig. 2, after 80 min of strong illumination under anaerobic conditions, the amount of Q_A lost correlates well with the proportion of PSII centres trapped in the irreversible inactivation state. The remaining centres were either still active (15%) or contained reversibly inhibited states (22%) containing stably reduced Q_A [7] still bound to the D_2-protein.

In conclusion this study lends further support to the model for acceptor side-induced photoinhibition previously presented [7] in that the ultimate consequence of anaerobic photoinhibition is an inoperational or empty Q_A-site. In the presence of oxygen, however, it is likely that an ampty Q_A-site is not reached very frequently. This is because several of the preceding intermediates, in particular the Q_AH_2 state will produce reaction centre chlorophyll triplets in the light and thus mediate singlet oxygen formation resulting in irreversibly damaged reaction centres [5,7,8].

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