Comparison of *psbO* and *psbH* Deletion Mutants of *Synechocystis* PCC 6803 Indicates that Degradation of D1 Protein Is Regulated by the Q_B Site and Dependent on Protein Synthesis†

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ABSTRACT: Mutants of the cyanobacterium *Synechocystis* PCC 6803 lacking the *psbO* or *psbH* gene are more vulnerable to photoinhibition than the wild type (WT). In the case of the *psbO*-less mutant, the increased sensitivity to photodamage is also accompanied by accelerated turnover of the D1 protein and a rapid rate of recovery on transfer to non-photoinhibitory conditions. In contrast, in low light the *psbH*-less mutant has a poor ability to recover after photoinhibition and has a reduced rate of D1 turnover as compared with WT. Since the *psbO* gene encodes the 33 kDa manganese-stabilizing protein associated with the water-splitting reaction, the increased sensitivity to photoinduced damage is attributed to perturbation of electron transfer processes on the donor side of photosystem II (PSII). In contrast, the absence of H protein, encoded by the *psbH* gene, affects the acceptor side of PSII with preferential photoinhibitory damage occurring at the Q_B site. The apparent consequence of this is that the *psbH*-less mutant, unlike the *psbO*-less mutant, is not able to regulate the rate of turnover of the D1 protein. In all cases it was shown that chloramphenicol, which blocks protein synthesis, enhances the rate of photoinhibition as judged by a decrease in oxygen evolution but slows down the rate of degradation of D1 protein compared to that observed during normal turnover. We conclude either that a factor or enzyme that is rapidly turned over is required to allow the D1 degradation to occur at *in vivo* rates or that the degradation and removal of the D1 protein from damaged reaction centers is synchronized with the availability of newly synthesized D1 protein. We favor the latter on the basis of the relationship between turnover rates and message level. Our findings also support the concept that D1 turnover is in some way regulated by the state of the Q_B-binding pocket.

The D1 protein is encoded by the *psbA* gene of oxygenic photosynthetic organisms (Erickson & Rochaix, 1992). The D2 protein, together with the *psbD* gene product, forms the reaction center of photosystem II (PSII) (Nanba & Satoh, 1987; Barber et al., 1987). In many respects the D1 and D2 proteins share functional and structural similarities to the L and M subunits of the reaction centers of purple photosynthetic bacteria (Trebst, 1987; Barber, 1987; Michel & Deisenhofer, 1988). However, one very distinct difference is that the D1 protein, unlike its bacterial counterpart, the L subunit, turns over rapidly (Mattoo et al., 1984; Kyle et al., 1984). This property is believed to be a consequence of photoinhibitory damage of PSII and indicates the existence of a repair system (Ohad et al., 1984; Kyle et al., 1984; Prásil et al., 1992).

Studies with *in vitro* systems have identified two separate routes for the photochemical damage of the PSII reaction center [for recent review, see Barber and Andersson (1992), Prasil et al. (1992), Aro et al. (1993)]. An acceptor side mechanism occurs when the primary quinone acceptor Q_A undergoes an abnormal second reduction so that the radical pair state $P680^+Pheo^-$ has an increased probability of recombination (Vass et al., 1992a). Such a recombination involves a singlet and triplet mechanism. The yield of the P680 triplet is about 30% at room temperature (Takahashi et al., 1987; Durrant et al., 1990) and can, under aerobic conditions, bring about the generation of singlet oxygen as suggested by McTavish et al. (1989) and proven by direct detection (Macpherson et al., 1993; Telfer et al., 1994). According to studies with isolated reaction centers, the singlet oxygen formed initially attacks the chlorophylls that constitute P680 and thus destroys normal reaction center function (Telfer et al., 1990; Durrant et al., 1990). In the absence of oxygen, such damage is not sustained. The other photoinhibitory mechanism occurs when there is impairment of electron flow on the donor side such that the lifetime of $P680^+$ is lengthened. This species has an oxidizing potential of 1.12 V as estimated by Klimov et al. (1979) which is sufficiently high to extract electrons from water and, under conditions when this does not occur, from its surrounding environment. Indeed, experiments using isolated reaction centers of PSII clearly show that both β-carotene and accessory chlorophylls associated with this complex are irreversibly bleached as a consequence of their oxidation by $P680^+$ (Telfer et al., 1991; De Las Rivas et al., 1993b).

It is believed that the detrimental photochemical reactions described above specifically trigger the D1 protein for degradation, possibly by inducing conformational changes that activate proteolysis at defined cleavage sites (Aro et al.,

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1993). Using *in vitro* systems, it has been found that the acceptor side mechanism involves a primary cleavage of the D1 protein in the loop that joins its putative transmembrane segments IV and V (Salter et al., 1992; De Las Rivas et al., 1992, 1993a). This stromal located cleavage site, which had been previously identified in the pioneering work of Greenberg et al. (1987), contrasts with that observed for donor side photoinhibition. In this latter case, the primary cleavage is on the lumenal surface of the protein in a loop joining transmembrane segments I and II (Shipton & Barber, 1991; Barbato et al., 1991; De Las Rivas et al., 1992).

Although there is considerable data about the molecular processes giving rise to photoinhibitory damage in isolated systems, the relevance of such information to the *in vivo* process has to be established. Some studies have indicated that there is a closely coordinated link between the degradation and resynthesis of the D1 protein (Adir et al., 1990; Tyysjärvi et al., 1992; Schnettger et al., 1992), but the mechanism and regulatory factors involved in the exchange of this protein *in vivo* are not understood.

Recently it has been shown that a mutant of the cyanobacterium *Synechocystis* PCC 6803, lacking the *psbO* gene encoding the 33 kDa manganese-stabilizing protein of PSII, is very sensitive to photoinhibition as compared to the wild type (Philbrick et al., 1991; Mayes et al., 1991; Vass et al., 1992b). This sensitivity can be attributed to perturbations in the functioning of its water-splitting system due to the absence of the 33 kDa protein. Another mutant of *Synechocystis* 6803 which is also sensitive to photoinhibition is that which has had its *psbH* gene deleted (Mayes et al., 1993). In this case, the absence of the protein perturbs electron flow between Q_A and Q_B on the acceptor side of PSII. In this paper we have investigated the relationship between photoinactivation of PSII and D1 protein turnover under various conditions in the *psbO*-less and *psbH*-less mutants as compared to the wild type with the view to gaining a better understanding of the relationship between degradation and resynthesis of this protein.

MATERIALS AND METHODS

Strains and Culture Conditions. The glucose tolerant strain *Synechocystis* PCC 6803-G (Williams, 1988), referred to as wild type (WT), and its *psbO* deletion mutant IC2 (Mayes et al., 1991) and *psbH* deletion mutant IC7 (Mayes et al., 1993) were grown in BG-11 medium supplemented with 10 mM TES/NaOH, pH 8.2, 5 mM glucose and, in the case of the IC2 and IC7 mutants, 25 μg/mL kanamycin. Plate medium had in addition 1.5% agar and 0.3% sodium thiosulfate (Pakrasi et al., 1988); 500 mL liquid cultures in conical flasks were gently stirred, bubbled with air, and irradiated with 100−150 μEm^{-2} s^{-1} [1] of white light at 30−32 °C.

Light Treatment. The cells in the late-exponential growth phase (A_{730nm} of 2−4) were harvested by centrifugation and resuspended in fresh BG-11 medium to a chlorophyll concentration of 25 μg/mL. They were stirred in flat glass dishes at 32 °C and subjected to heat-filtered white light having an intensity of approximately 100 (low light) or 1000 (high light) μEm^{-2} s^{-1}.

Pulse Chase Labeling of the Cyanobacterial Cells. The cells were washed and resuspended in the BG-11 medium in which $MgSO_4$, $ZnSO_4$, and $CuSO_4$ had been substituted with $MgCl_2$, $ZnCl_2$, and $Cu(NO_3)_2$ (BG-11-S). Chlorophyll concentration was adjusted to 25 μg/mL, and the cell suspension was exposed to white light (200 μEm^{-2} s^{-1}) for 1 h at 32 °C. Then [^{35}S]methionine was added (final activity 1 μCi ml^{-1}) and the light treatment continued for another 30 min. Labeled cells were spun down and resuspended in the same volume of fresh BG-11-S, and cold methionine was added to a final concentration of 2 mM. For characterization of light-induced D1 degradation (chase), cells were subjected to high- or low-light treatment and 10 mL aliquots were withdrawn at appropriate time intervals and used for the preparation of thylakoid membranes (see below). In some experiments, the protein synthesis inhibitor chloramphenicol (CAP) was added to the cell suspension (final concentration 50 μg/mL). The effect was checked by its ability to inhibit the incorporation of radiolabeled methionine into the thylakoid proteins during an 8 h pulse experiment (data not shown).

Preparation of Thylakoid Membranes. The cells (250 μg of chlorophyll) were spun down, washed in buffer A [50 mM MES/NaOH, pH 6.5, containing 5 mM EDTA, 1 mM phenylmethanesulfonyl fluoride (PMSF), 2 mM aminocaproic acid, and 1 mM benzamidine], and resuspended in 0.4 mL of buffer A. The same volume of glass beads was added and the cells were broken in an Eppendorf tube by vortexing twice for 90 s with 1 min interruption for cooling on ice. The unbroken cells, cell debris, and thylakoid membranes were decanted off by repeated washing of the glass beads with buffer A. The decanted material was then centrifuged at 9000*g* for 30 s to remove unbroken cells and debris. The supernatant was centrifuged at 15000*g* for 15 min to obtain a pellet of thylakoids which was finally resuspended in 50 mM Tris/HCl, pH 7.5, containing 1 M sucrose. The isolation procedure was carried out on ice in a cold room.

Protein Analysis. Proteins of isolated thylakoids were separated in a 10−17% resolving polyacrylamide gel containing 6 M urea and 0.1% SDS. The stacking gel contained 6% acrylamide. The gel and electrophoretic buffer system were according to Laemmli (1970); 5 μg of chlorophyll was loaded per lane, and the gels were stained with Coomassie Blue G and, after destaining, dried. Autoradiography was carried out by exposing Hyperfilm β-max at room temperature for 2−3 days. For quantification of the radiolabeled D1 protein, the autoradiographs were scanned with a Hirschmann Elsript 400 densitometer. Normalization was achieved by loading the same amount of chlorophyll with additional correction being made using the intensity of a 43 kDa band which was fully stable throughout the experiments. Correct identification of the radiolabeled D1 and D2 proteins was confirmed by western blotting using specific antibodies (Nixon et al., 1986).

Oxygen Evolution Measurement. Light-saturated steady state rates of oxygen evolution in cell suspensions (10 μg of chl/mL) were measured at 30 °C using a Hansatech DW2 oxygen electrode at light intensity 3000 μEm^{-2} s^{-1} in the presence of an artificial electron acceptor system of 1 mM 2,5-dimethyl-*p*-benzoquinone (DMBQ) or 1 mM 2,6-dichloro-*p*-benzoquinone (DCBQ) and 3 mM potassium ferricyanide.

Measurement of Chlorophyll Concentration. For the measurement of chlorophyll, the cells were sedimented by

[1] Abbreviations: DMBQ, 2,5-dimethyl-*p*-benzoquinone; DCBQ, 2,5-dichloro-*p*-benzoquinone; E (einstein), mol quanta; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate.

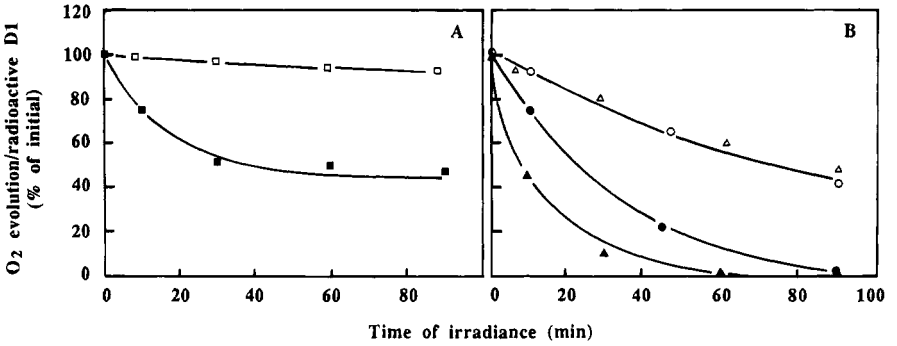

FIGURE 1: Effect of high-light treatment on the rates of oxygen evolution and D1 turnover in *Synechocystis* PCC 6803 WT (open symbols) and IC2 mutant (closed symbols). (A) Comparison of susceptibility to photoinhibition of WT (□) and IC2 mutant (■) in the absence of CAP. (B) Comparison of susceptibility to photoinhibition of WT (△) and IC2 mutant (▲) in the presence of CAP. Circles indicate the rate of D1 turnover measured in the absence of CAP for WT (open) and mutant (closed). The cells were treated with high light (1000 μEm^{-2} s^{-1}), and oxygen evolution was assayed during the treatment as described in Materials and Methods using 1 mM DMBQ plus 3 mM potassium ferricyanide. The D1 turnover was determined using pulse chase labeling with [^{35}S]methionine as also described in Materials and Methods.

centrifugation and extracted with 100% methanol. This extract was centrifuged and the clear supernatant used for absorption measurement. Concentration of chlorophyll was calculated from absorbance of the extract at 666 and 750 nm according to Wellburn and Lichtenthaler (1984).

RESULTS

Photoinactivation of PSII and Turnover of D1 Protein Is Much Faster in the psbO-Less Mutant Compared to Wild Type. Figure 1 shows how the rates of PSII activity and D1 turnover change as a consequence of increasing times of exposure to 1000 μEm^{-2} s^{-1} of white light in the cells of the cyanobacterium *Synechocystis* 6803 wild type (WT) and the *psb*O-less mutant (IC2). Loss of PSII activity was characterized by a decline in the rate of light-saturated oxygen evolution measured in whole cells in the presence of artificial electron acceptors DMBQ and ferricyanide. D1 turnover under exactly the same light conditions was determined as the rate of disappearance of the radioactive D1 band after pulse labeling using [^{35}S]methionine (for example, see Figure 3). As can be seen in Figure 1A, irradiation of nontreated wild type cells with high light showed only a negligible decrease in PSII activity. However, when CAP was present, the rate of oxygen evolution from wild type cells declined with a half time of ca. 100 min (see Figure 1B) which was comparable with the half time for the turnover of the D1 protein as judged from [^{35}S]methionine pulse chase experiments carried out in the absence of the protein synthesis inhibitor (Figure 1B). These results, therefore, clearly show that the synthesis of D1 protein is needed in order to avoid loss of photosynthetic activity in the wild type cells. In the case of the *psbO* deletion mutant IC2 a significant decline in PSII activity was observed even in the absence of CAP (see Figure 1A) confirming an increased sensitivity of this mutant to photoinhibition (Philbrick et al., 1991; Mayes et al., 1991; Vass et al., 1992b). This increased sensitivity became even more obvious when CAP was present (Figure 1B). In fact, in the presence of CAP, the rate of decline in oxygen-evolving activity was ca. 10 times faster in IC2 compared with WT. Pulse chase experiments using [^{35}S]methionine (Figure 1B) demonstrate that under the same light conditions the turnover of D1 protein is about 5 times faster in the IC2 mutant compared with the WT. However, despite this enhanced rate of D1

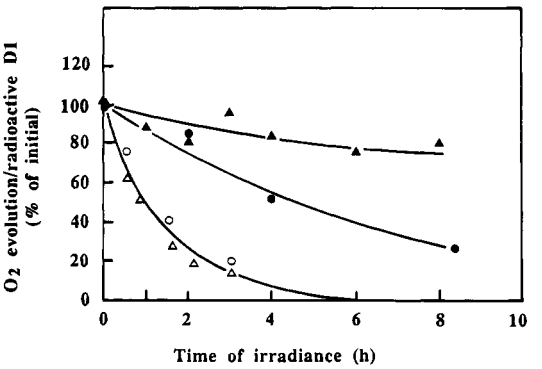

FIGURE 2: Effect of low-light treatment (100 μEm^{-2} s^{-1}) on the rate of oxygen evolution measured in the presence of 50 μg mL CAP (triangles) and on the rate of D1 turnover (circles) in WT (closed symbols) and IC2 mutant (open symbols). For details of the measurement of oxygen evolution and D1 turnover, see Materials and Methods and Figure 1.

turnover in IC2, it seems that the repair process is not sufficient to match the rate of PSII damage in this mutant under the conditions employed. Consequently photoinhibition is observed even in the absence of CAP (see Figure 1A). Figure 1A also shows that the oxygen evolution rate from IC2 comes to a steady state level after the initial photoinhibitory decline which would be consistent with the situation when the rate of D1 turnover can sustain a certain level of PSII activity. In contrast, the rates of photodamage and D1 turnover in WT are about equal (Figure 1B) which would account for little or no observed photoinhibition in the WT (Figure 1A).

The relationship between PSII inactivation and D1 turnover was also studied for cells exposed to low light (100 μEm^{-2} s^{-1}) (Figure 2). Here no decline of oxygen-evolving activity occurred in the absence of CAP in either strains. In the presence of CAP, PSII activity again fell much faster in the mutant than in the WT. In both strains the rate of D1 turnover was slowed down under low compared to high irradiance, a result consistent with the proposed light intensity dependence of D1 turnover (Mattoo et al., 1984). As clearly shown in Figure 2, D1 turnover in IC2 closely matched inactivation of oxygen evolution from this mutant when measured in the presence of CAP. This correlation was also observed above for WT exposed to high light (Figure 1B) and is consistent with an efficient repair of PSII so as to fully counter photoinduced damage. This correlation, how-

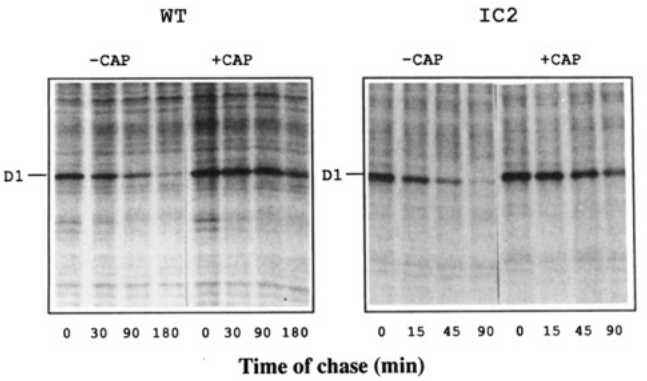

FIGURE 3: Effect of CAP on the rate of degradation of the D1 protein in WT and IC2 mutant. Autoradiograms are shown for thylakoid proteins from radiolabeled cells subjected to high irradiance (1000 μEm^{-2} s^{-1}) during the chase in the absence or presence of CAP (50 μg/mL). During the chase, aliquots of cells were taken at the times indicated, and thylakoid membranes isolated. They were then analyzed by SDS gel electrophoresis and dried for autoradiography as described in Materials and Methods.

ever, was less clear with the WT exposed to low light, indicating that some D1 turnover may occur independently of PSII inactivation.

Inhibition of Protein Synthesis Retards D1 Degradation in WT and IC2. The autoradiograms shown in Figure 3 not only demonstrate the increased rate of turnover of the D1 protein in IC2 compared with WT but also shows an unexpected finding that the rate of loss of D1 protein, due to illumination of the WT or the IC2 mutant, is significantly decreased in the presence of CAP. Similar results were obtained using lincomycin, which is also an inhibitor of protein synthesis. When protein synthesis is stopped, the slow loss of radioactive D1 protein observed in Figure 3 represents a reduction in the steady state level of this protein in the membrane. This was confirmed by monitoring the level of D1 protein using densitometer assays of Coomassie blue-stained gels or Western blotting (data not shown).

The above findings are surprising given that the rate of photoinhibition is increased on the addition of CAP (Figures 1 and 2). This lack of correlation between the rate of light-induced inhibition of electron transport and degradation of the D1 protein in the presence of CAP is similar to that seen in experiments using *in vitro* systems where no protein synthesis is occurring (Virgin et al., 1988; Aro et al., 1990). Our results seem to indicate therefore that active protein synthesis is necessary for fast and selective degradation of the D1 protein.

Photoinactivation of the PSII Q_B Site Is Faster in the psbH-Less Mutant than in the Wild Type while Recovery from Photoinhibition Is Slower. It has been shown previously that a *Synechocystis* mutant with its *psbH* gene deleted (IC7) exhibits an increase in sensitivity to photoinhibition as compared with WT (Mayes et al., 1993). In Figure 4 it is shown that the damage incurred by IC7 is site specific since, as shown in Figure 4B, it is more readily observed when monitored using DMBQ rather than DCBQ. This difference in the ability of DCBQ and DMBQ to act as an electron acceptor after preillumination with 1000 μEm^{-2} s^{-1} white light was not observed with the WT (Figure 4A) or IC2. It is believed that DMBQ mainly accepts electrons from the Q_B site, while DCBQ is able to directly interact with Q_A as well as Q_B (Graan & Ort, 1986). Therefore, the results

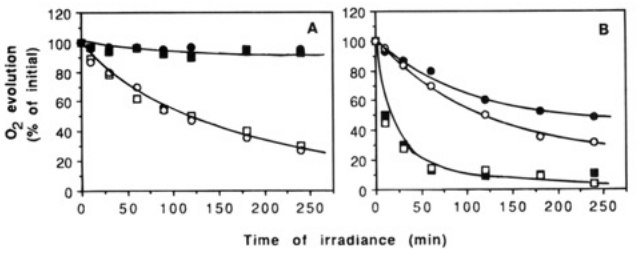

FIGURE 4: Oxygen evolution rates measured with WT (A) or IC7 (B) using either 1 mM DCBQ (circles) or 1 mM DMBQ (squares) plus 3 mM potassium ferricyanide as electron acceptor after irradiation with 1000 μEm^{-2} s^{-1} white light for the times indicated in the absence (closed symbols) or presence (open symbols) of 50 μg/mL CAP.

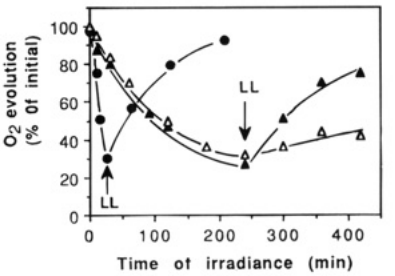

FIGURE 5: Comparison of recovery rates after photoinhibition of WT (▲), IC7 (△), and IC2 (●). Oxygen evolution rates were measured using 1 mM DCBQ and 3 mM ferricyanide. Photoinhibitory irradiation was 1000 μEm^{-2} s^{-1} in the presence of 50 μg/mL CAP. Recovery was followed in low light (LL) (100 μEm^{-2} s^{-1}) after cells had been centrifuged, washed, and resuspended to remove CAP.

presented in Figure 4 indicate that in the IC7 mutant the Q_B site has undergone more photoinduced modifications than the Q_A site.

The results presented in Figure 4 also reveal another striking feature of IC7 that, unlike the WT and IC2, the rate of photoinhibition (measured with either DCBQ or DMBQ) is not significantly stimulated by the presence of CAP (compare panels A and B in Figure 4). This would indicate that the repair process in the IC7 mutant is inhibited compared to the WT. To check this further, the rate of recovery after a photoinhibitory period was measured (see Figure 5). Mutant (both IC2 and IC7) and WT cells were exposed to 1000 μEm^{-2} s^{-1} light in the presence of CAP. After an appropriate period of time when the oxygen evolution rate measured with DCBQ had dropped by 70%, the cells were rapidly washed by centrifugation to remove CAP. As can be seen in Figure 5, the IC2 mutant was rapidly photoinhibited compared to the WT, in agreement with the data of Figure 1. The WT and IC7 showed a similar rate of photoinhibition when DCBQ was used to monitor activity, which is consistent with the data in Figure 4. The new observation in Figure 5 is that relative to the WT the IC7 mutant had a decreased rate of recovery when the washed cells were illuminated with low light (100 μEm^{-2} s^{-1}). Also of note is that the IC2 mutant recovered faster than the WT which is consistent with its higher rate of D1 synthesis.

Inhibition of Protein Synthesis also Retards D1 Degradation in IC7. The difference in rate of recovery between WT and IC7 (Figure 5) and the lack of effect of CAP on the susceptibility of IC7 to photoinhibition (Figure 4) suggest that the D1 turnover rate in IC7 is slower than in the WT. Pulse chase experiments using [^{35}S]methionine support this

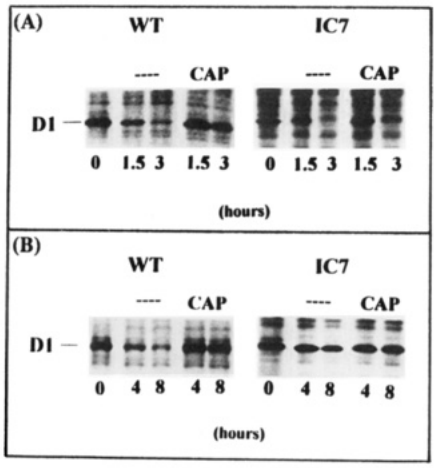

FIGURE 6: Effect of (A) high (1000 μEm^{-2} s^{-1}) and (B) low (100 μEm^{-2} s^{-1}) white light irradiation on the degradation of the D1 protein monitored by pulse chasing using [^{35}S]methionine for the initial labeling and autoradiography (see Materials and Methods). The results compare WT and IC7 with and without 50 μg/mL CAP present during the irradiation period (see legend of Figure 3).

conclusion (see Figure 6). The slower rate of turnover of D1 protein in IC7 compared with WT is most clearly seen at low light intensities (Figure 6B). The data of Figure 6 also clearly shows that when CAP is present the turnover of the D1 protein is blocked in the IC7 mutant as well as in the WT.

DISCUSSION

Both the *psbO*-less (IC2) and *psbH*-less (IC7) deletion mutants of *Synechocystis* PCC 6803 are more vulnerable to photoinhibition than the WT. However the reason for this is different for the two mutants. The IC2 mutant is more sensitive to photoinduced damage than the WT as indicated by the much faster rate of loss of PSII activity after exposure to 1000 μEm^{-2} s^{-1} in the presence of CAP. This feature of IC2 is probably due to the absence of the *psbO* gene product (33 kDa protein) which results in the impairment of activity on the donor side of PSII. This protein is believed to stabilize the manganese cluster involved in water oxidation (Babcock et al., 1989). Previous studies have detected perturbations in donor side activity in *Synechocystis* mutants lacking the *psbO* gene (Mayes et al., 1991; Vass et al., 1992b) which have been partly linked to changes in the calcium requirement for the PSII water-splitting reactions (Philbrick et al., 1991). The increased rate of photochemical damage occurring in the PSII reaction center of the *psbO*-less mutant is signaled to the repair system in some way since the organism increases its rate of D1 turnover as a consequence. This ability of the IC2 mutant to intensify its repair mechanism in response to increased susceptibility to photoinduced damage means that when placed in relatively weak light after being photoinhibited, it efficiently recovers normal activity. In fact, under the conditions used in our experiments, its recovery rate was faster than that of the wild type. When the white light intensity was lowered to 100 μEm^{-2} s^{-1}, no photoinhibition was observed with IC2 even though the rate of damage (measured in the presence of CAP) was very much higher than that occurring in the WT (see Figure 2). The reason for the lack of observable photoinhibition can be attributed to the equally increased rate of D1 turnover. These results support the model that the degree of photoinhibition observable as a net drop in photosynthetic rate is determined by the balance between the rate of photoinduced damage of PSII (measured as a loss of activity in the presence of CAP) and the rate of repair (measured as the rate of turnover of the D1 protein using the pulse chase technique). This general concept seemed not to hold for low-light treated WT cells as indicated in Figure 2. However, the long duration of this experiment (over 8 h) introduces greater errors, and other physiological factors such as cell growth and division come into play.

In contrast to the findings with the *psbO*-less mutant, the IC7 mutant sustained photochemical damage at about the same rate as the WT when illuminated in the presence of CAP and when its activity was assayed with DCBQ. However, when DMBQ was used to assay damage, the increased sensitivity of this mutant to photoinhibition was revealed whether CAP was present or not. The IC7 mutant has its *psbH* gene deleted and has been shown to be perturbed on the acceptor side of PSII (Mayes et al., 1993). The perturbation is such as to effect the electron exchange between Q_A and Q_B with the equilibrium shifted toward Q_A (Mayes et al., 1993). The results presented here indicate that this modification has two effects. It increases the susceptibility of the Q_B site to photoinduced damage as indicated by the differential effect of DMBQ and DCBQ (Figure 4), and it interferes with the "signaling" process that regulates the turnover of D1 protein. Apparently it is the combination of these two effects which gives rise to the increased susceptibility of the IC7 mutant to photoinhibition.

The above comparisons of the IC2 and IC7 mutants indicate that the signal controlling turnover of the D1 protein is in some way associated with the Q_B-binding site on the D1 protein, as suggested previously (Prásil et al., 1992; Bracht & Trebst, 1994). If photoinduced damage is occurring but does not involve the Q_B site, as in the case of the IC2 mutant, the signal controlling D1 protein turnover remains operative and indeed will respond in such a way as to increase the rate of the compensatory repair process. If, however, the Q_B site is damaged, then the repair process seems to be inhibited.

The other important conclusion of this work is the finding that although blocking protein synthesis increases the rate of photoinhibition (as would be expected), it significantly slows down the degradation of D1 protein. The observation held true for IC2 and IC7 as well as for WT and therefore seems to be a general feature. This finding contrasts with those of Kanervo et al. (1994) who observed no difference between degradation rates of D1 protein in *Synechocystis* 6803 in the presence or absence of protein synthesis inhibitors. This inconsistency can be explained by the light intensity used by Kanervo et al. which was sufficient to induce strong photoinhibitory damage even in the absence of protein synthesis inhibitors. Indeed, in our work the difference in the degradation rates in the presence and absence of CAP became more significant at lower light intensities. More recently, using *Lemna gibba*, Gong (1994) also noted that D1 degradation was slower in the presence of chloroplast protein synthesis inhibitors than in their absence indicating that this phenomenon occurs also in eukaryotes.

The surprising effect of CAP seems to suggest that during rapid D1 turnover there is a synchronization between the degradation of D1 protein in the damaged reaction center

and the synthesis of a new D1 molecule. It seems reasonable that the D1 degradation and replacement is normally synchronized in order to avoid complete disassembly of the PSII complex. Recently it has been suggested that in the granal thylakoids of higher plants, where this synchronization may be prevented, photoinactivated PSII complexes are protected from more extensive damage and disassembly by N-terminal phosphorylation (Rintamäki et al., 1995).

Alternatively, the inhibition of D1 degradation in the presence of CAP could be due to the existence of another rapidly synthesized protein required to enhance the degradation process. The latter conclusion would imply the requirement of a specific enzyme or factor involved in selective cleavage of the D1 protein. Such a suggestion has some support from the recent findings of Bracht and Trebst (1994) and Gong (1994). Bracht and Trebst found that degradation of the D1 protein in *Chlamydomonas* was inhibited by the presence of a cycloheximide especially under conditions of phosphate deficiency. They concluded, therefore, that a rapidly turning over nuclear-encoded factor was involved in the D1 replacement process. Since *Synechocystis* is a prokaryotic organism, it has only 70S ribosomes, and chloramphenicol inhibits all protein synthesis. It is therefore possible that the factor identified by Bracht and Trebst is required for D1 turnover in *Synechocystis* and that its synthesis is blocked by CAP. The work of Gong (1994), however, suggested that an additional chloroplast-encoded protein may be needed for D1 protein replacement, and this protein should also rapidly turnover.

Despite the existence of several possibilities to explain the slowing down of D1 protein degradation in the presence of CAP, it seems likely that it is the availability of newly synthesized D1 protein which regulates the degradation rate. This conclusion is based on correlation between the decrease in the rate of D1 turnover and the lower D1 message levels in the *Synechocystis* 6803 strain TC31 (Nixon et al., 1992; Dalla Chiesa, 1994).

It should be noted that even in the presence of CAP there is a gradual degradation of the D1 protein in continuous light. This is reminiscent of the light-induced D1 degradation observed with *in vitro* systems ranging from isolated thylakoids to reaction centers (Virgin et al., 1990; Aro et al., 1990, 1993; Shipton & Barber, 1991; De Las Rivas et al., 1993) where no protein synthesis is occurring. With *in vitro* systems, D1 degradation is often accompanied by D2 degradation, and this tendency was also observed in CAP-treated cells, while in nontreated *Synechocystis* the D2 did not turnover (data not shown). It therefore seems that in the absence of rapid D1 replacement a more extensive damage and loss of cofactors can occur leading to a disassembly of the PSII complex as shown by Hundal et al. (1990) and Barbato et al. (1992).

Understanding the nature of the signal which regulates the turnover of the D1 protein in response to changes in environmental conditions is and will continue to be a major task in PSII research for some years. The studies reported here open up new thoughts and avenues for further experiments. Clearly mutants with modified donor and acceptor side activities provide an excellent experimental system to further investigate these intriguing features of PSII and will help us to understand how they can regulate the exchange of a single protein of key functional importance in a multiprotein structure in response to environmental factors.

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REFERENCES

Adir, N., Shochat, S., & Ohad, I. (1990) *J. Biol. Chem. 265*, 12563−12568.
Aro, E.-M., Hundal, T., Carlberg, I., & Andersson, B. (1990) *Biochim. Biophys. Acta 1019*, 269−275.
Aro, E.-M., Virgin, I., & Andersson, B. (1993) *Biochim. Biophys. Acta 1143*, 113−134.
Babcock, G. T., Barry, B. A., Debus, R. J., Hoganson, C. W., Atamian, M., McIntosh, L., Sithole, I., & Yocum, C. F. (1989) *Biochemistry 28*, 9558−9565.
Barbato, R., Shipton, C. A., Giacometti, G. M., & Barber, J. (1991) *FEBS Lett. 290*, 162−166.
Barbato, R., Friso, G., Rigoni, F., Vecchia, F. D., & Giacometti, G. M. (1992) *J. Cell Biol. 119*, 325−335.
Barber, J. (1987) *Trends Biochem. Sci. 12*, 123−124.
Barber, J., & Andersson, B. (1992) *Trends Biochem. Sci. 17*, 61−66.
Barber, J., Chapman, D. J., & Telfer, A. (1987) *FEBS Lett. 220*, 67−73.
Bracht, E., & Trebst, A. (1994) *Z. Naturforsch. 49c*, 439−446.
Dalla Chiesa, M. (1994) Ph. D. Thesis, University of London.
De Las Rivas, J., Andersson, B., & Barber, J. (1992) *FEBS Lett. 301*, 246−252.
De Las Rivas, J., Shipton, C. A., Ponticos, M., & Barber, J. (1993a) *Biochemistry 32*, 6944−6950.
De Las Rivas, J., Telfer, A., & Barber, J. (1993b) *Biochim. Biophys. Acta 1142*, 155−164.
Durrant, J. R., Giorgi, L. B., Barber, J., Klug, D. R., & Porter, G. (1990) *Biochim. Biophys. Acta 1017*, 167−175.
Erickson, J. M., & Rochaix, J. D. (1992) *The Photosystems: Structure, Function and Molecular Biology* (Barber, J., Ed.) Vol. 11, pp 101−177, Elsevier, Amsterdam.
Gong, H. S. (1994) *Biochim. Biophys. Acta 1188*, 422−426.
Graan, T., & Ort, D. R. (1986) *Biochim. Biophys. Acta 852*, 320−330.
Greenberg, B. M., Gaba, V., Mattoo, A. K., & Edelman, M. (1987) *EMBO J. 6*, 2865−2869.
Hundal, T., Virgin, I., Styring, S., & Andersson, B. (1990) *Biochim. Biophys. Acta 1017*, 235−241.
Kanervo, E., Mäenpää, P., & Aro, E.-M. (1994) *J. Plant Physiol. 142*, 669−675.
Klimov, V. V., Allakhverdiev, S. I., Demeter, S., & Krasnovskii, A. A. (1979) *Dokl. Akad. Nauk. SSSR 249*, 227−230.
Kyle, D. J., Ohad, I., & Arntzen, C. J. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4070−4074.
Laemmli, U. K. (1970) *Nature 227*, 680−685.
Macpherson, A. N., Telfer, A., Barber, J., & Truscott, T. G. (1993) *Biochim. Biophys. Acta 1143*, 301−309.
Mattoo, A. K., Hoffman-Falk, H., Marder, J. B., & Edelman, M. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1380−1384.
Mattoo, A. K., Marder, J. B., & Edelman, M. (1989) *Cell 56*, 241−246.
Mayes, S. R., Cook, K. M., Self, S. J., Zhang, Z.-H., & Barber, J. (1991) *Biochim. Biophys. Acta 1060*, 1−12.
Mayes, S. R., Dubbs, J. M., Vass, I., Hideg, E., Nagy, L., & Barber, J. (1993) *Biochemistry 32*, 1454−1465
McTavish, H., Picorel, R., & Seibert, M. (1989) *Plant Physiol. 89*, 452−456.
Michel, H., & Deisenhofer, J. (1988) *Biochemistry 27*, 1−7.
Nanba, O., & Satoh, K. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 109−112.
Nixon, P. J., Dyer, T. A., Barber, J., & Hunter, C. N. (1986) *FEBS Lett. 209*, 83−86.
Nixon, P. J., Trost, J. T., & Diner, B. A. (1992) *Biochemistry 31*, 10859−10871.

Ohad, I., Kyle, D. J., & Arntzen, C. J. (1984) *J. Cell Biol. 99*, 481−485.
Pakrasi, H. B., Williams, J. G. K., & Arntzen, C. J. (1988) *EMBO J. 7*, 325−332.
Philbrick, J. B., Diner, B. A., & Zilinskas, B. A. (1991) *J. Biol. Chem. 266*, 13370−13376.
Prásil, O., Adir, N., & Ohad, I. (1992) *The Photosystems: Structure, Function and Molecular Biology* (Barber, J., Ed.) Vol. 11, pp 295−348. Elsevier, Amsterdam.
Rintamäki, E., Kettunen, R., Tyysjärvi, E., & Aro, E.-M. (1995) *Physiol. Plant. 93*, 191−195.
Salter, A. H., Virgin, I., Hagman, A., & Andersson, B. (1992) *Biochemistry 31*, 3990−3998.
Schnettger, B., Leitsch, J., & Krause, G. H. (1992) *Photosynthetica 27*, 261−265.
Shipton, C. A., & Barber, J. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 6691−6695.
Takahashi, Y., Hansson, Ö., Mathis, P., & Satoh, K. (1987) *Biochim. Biophys. Acta 893*, 49−59.
Telfer, A., He, W.-Z., & Barber, J. (1990) *Biochim. Biophys. Acta 1017*, 143−151.
Telfer, A., De Las Rivas, J., & Barber, J. (1991) *Biochim. Biophys. Acta 1060*, 106−114.
Telfer, A., Bishop, S. M., Phillips, D., & Barber, J. (1994) *J. Biol. Chem. 269*, 13244−13253.
Trebst, A. (1987) *Z. Naturforsch. 42c*, 742−750.
Tyysjärvi, E., Aliyrkko, K., Kettunen, R., & Aro, E.-M. (1992) *Plant Physiol. 100*, 1310−1317.
Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, A., & Andersson, B. (1992a) *Proc. Natl. Acad. Sci. U.S.A. 89*, 1408−1412.
Vass, I., Cook, K. M., Deak, Zs., Mayes, S. R., & Barber, J. (1992b) *Biochim. Biophys. Acta 1102*, 195−201.
Virgin, I., Styring, S., & Andersson, B. (1988) *FEBS Lett. 233*, 408−412.
Virgin, I., Ghanotakis, D. F., & Andersson, B. (1990) *FEBS Lett. 269*, 45−48.
Wellburn, A. R., & Lichtenthaler, H. (1984) in *Advances in Photosynthesis Research* (Sybesma, C., Ed.) Vol. II, pp 9−12, Nijhoff/Junk, Hague.
Williams, J. G. K. (1988) *Methods Enzymol. 167*, 766−778.

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