Biochimica et Biophysica Acta, 1017 (1990) 235–241
Elsevier

BBABIO 43225

Changes in the organization of photosystem II following light-induced D_1-protein degradation

Torill Hundal, Ivar Virgin, Stenbjörn Styring and Bertil Andersson

Department of Biochemistry, Arrhenius Laboratories for Natural Sciences, University of Stockholm, Stockholm (Sweden)

(Received 28 December 1989)

Key words: D_1 protein; Manganese; Photoinhibition; Photosystem II; Thylakoid membrane dynamics

The composition and organization of photosystem II was studied in thylakoid membranes and subfractions which had been subjected to photoinhibitory light conditions. The results show that D_1-protein degradation can occur in vitro leading to a 50–60% loss of the protein. Apart from the D_2-protein, which shows a limited decline, there was no loss of any other photosystem II proteins. The D_1-protein degradation induced several changes in the organization of the photosystem II complex. Using inside-out thylakoid vesicles we demonstrate that concomitant with the D_1-protein degradation there is a release of the extrinsic 33, 23 and 16 kDa proteins from the inner thylakoid surface into the lumenal space. In addition, there is a release of four manganese ions per D_1-protein degraded. The correlation between the D_1-protein degradation and the release of manganese is also seen in inside-out thylakoid vesicles that have been $CaCl_2$-washed to remove the three extrinsic proteins prior to photoinhibitory illumination. Subfractionation of thylakoids subsequent to photoinhibitory treatment suggests a migration of certain photosystem II subunits from the appressed to the non-appressed thylakoid regions following D_1-protein degradation. The photosystem II subunits showed an individual migration behaviour, suggesting a disassembly of the photosystem II core. Our data suggest that repair of photodamaged photosystem II involves, apart from reinsertion of new D_1-proteins, reassembly of the photosystem II complex including lateral movement of proteins between the two thylakoid regions and religation of the manganese cluster.

Introduction

Photosystem II of higher plants possesses several unique properties with respect to function, organization and protein turn-over. It is a multisubunit protein complex which in plants is composed of at least 15 different polypeptides [1]. The two reaction centre polypeptides, designated D_1 and D_2, appear to carry all the redox components necessary for the primary photochemistry of photosystem II [2–4] and possibly also the manganese cluster [5–8]. The great majority of the photosystem II units are located in the appressed thylakoid regions in association with its chlorophyll *a/b* antenna [9].

Apart from the central catalytic role as a water-plastoquinone oxido-reductase, photosystem II plays a central role for the long and short term light acclimation of the photosynthetic apparatus [9]. Photosystem II is also the target for the photoinhibition process which leads to impaired electron transport capacity and subsequently to breakdown of the two reaction centre subunits, in particular the D_1-protein [10–13]. The loss of the D_1-protein raises several questions concerning the organization of photosystem II during photoinhibitory conditions. These relate to ligation of cofactors, assembly of intrinsic and extrinsic protein subunits, site and mechanism of repair. In a previous study we observed a close correlation between the release of manganese from the thylakoid membrane and D_1-protein degradation [7].

In the present work we have analyzed changes in the organization of photosystem II proteins accompanying D_1-protein degradation in isolated spinach thylakoids and thylakoid subfractions. The results reveal that degradation of the D_1-protein leads to loss of manganese, release of extrinsic proteins into the lumenal space, partial disassembly of the photosystem II core and lateral migration of proteins from the appressed to the non-appressed thylakoid regions.

Abbreviations: CP, chlorophyll binding protein; LHC, light harvesting complex; Mes, 2-[*N*-morpholino]ethanesulphonic acid; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

Correspondence: Department of Biochemistry, The Arrhenius Laboratories for Natural Sciences, University of Stockholm, S-106 91 Stockholm, Sweden.

Materials and Methods

Thylakoid membrane preparations

Thylakoid membranes were prepared from spinach leaves according to Ref. 14. Inside-out vesicles were isolated by aqueous polymer two-phase partition following Yeda press fragmentation of thylakoids as described in Ref. 15. Stroma lamellae vesicles were isolated by Yeda press fragmentation and differential centrifugation [16].

Removal of extrinsic proteins

Inside-out vesicles were treated in darkness with 2 M $CaCl_2$ containing 300 mM sorbitol, 10 mM NaCl and 40 mM Mes-NaOH (pH 6.5) on ice for 30 min [17] and then centrifuged at $50000 \times g$ for 10 min. This treatment was repeated and the final pellet was washed twice in 200 mM NaCl, 300 mM sorbitol, 50 mM $CaCl_2$ and 40 mM Mes-NaOH (pH 6.5) and finally suspended in the latter medium prior to photoinhibitory treatment.

Conditions for photoinhibition

Isolated thylakoid membranes were suspended to a concentration of 150 μg chlorophyll/ml in a buffer containing 10 mM Na-phosphate (pH 7.4), 5 mM NaCl, 5 mM $MgCl_2$ and 100 mM sucrose. Illumination was performed with heatfiltered white light of the indicated intensities at 20°C under aerobic conditions. After termination of the illumination, the membranes were subfractionated to yield inside-out vesicles or stroma lamellae vesicles.

In another set of experiments, inside-out thylakoid vesicles were first isolated and then subjected to photoinhibition treatment. Inside-out vesicles were illuminated under the same conditions but at a lower chlorophyll concentration of 100 μg/ml. $CaCl_2$-washed inside-out vesicles were suspended to a concentration of 70 μg chlorophyll/ml in 200 mM NaCl, 300 mM sorbitol, 50 mM $CaCl_2$ and 40 mM Mes-NaOH (pH 6.5) prior to illumination.

Protein and manganese analysis

SDS-PAGE was run according to Refs. 18 and 19. Western blotting using antibodies against different individual photosystem II polypeptides and CF_0 subunit II was performed essentially according to [20] using I-125 labelled protein A for detection. For quantification, the autoradiographs were scanned by a laser densitometer. The relative amount of chlorophyll-proteins were determined and quantified by mild SDS-PAGE according to [21]. Chlorophyll *a/b* was determined according to Arnon [22].

For the manganese analysis, the thylakoids were collected by centrifugation and the manganese content was determined by atomic absorption at 279.5 nm. The sample solutions were made up to 1% HNO_3, dried at 200°C for 25 s, ashed at 800°C for 40 s and atomized at 2100°C for 3 s.

Results

In an earlier report [7] we demonstrated a close correlation between D_1-protein degradation and release of manganese from the thylakoid membrane. In this subfraction study we have looked for possible additional changes in the organization of photosystem II following degradation of the D_1-protein.

Inside-out vesicles from both control and photoinhibited thylakoids were isolated and analyzed with respect to protein content. As revealed from immunoblotting analysis the inside-out vesicles isolated from the control thylakoids kept in the dark contained normal levels of all photosystem II proteins (Fig. 1). The vesicles derived from the photoinhibited samples showed a marked reduction of the D_1-protein (Figs. 1 and 2). In addition, they were highly depleted in the extrinsic 33, 23 and 16 kDa proteins (Fig. 1). This observation was somewhat surprising, since the levels of all three extrinsic proteins were normal in the photoinhibited thylakoids prior to subfractionation (Fig. 1, [7]). This phenomenon was further investigated in another experiment where inside-out thylakoid vesicles were isolated and then subjected to photoinhibitory treatment. Subsequently, the vesicles were pelleted and analyzed for

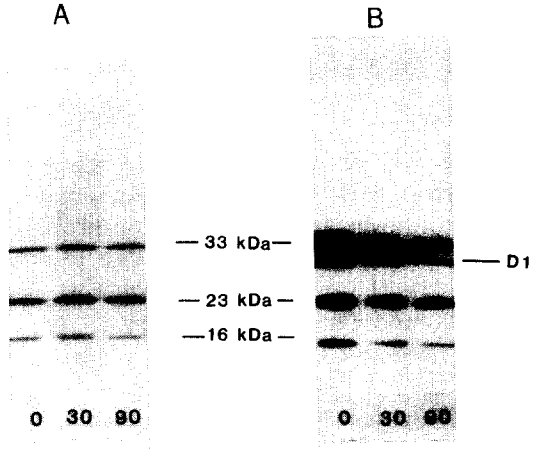

Fig. 1(A). Levels of the three extrinsic 33, 23, and 16 kDa proteins in isolated spinach thylakoids subjected to photoinhibitory illumination (5000 $\mu E \cdot m^{-2} \cdot s^{-1}$) for the indicated periods of time. The immunoblot shows that the levels of the three extrinsic proteins is unaffected by the illumination. The immunological detection was obtained by a mixture of antibodies against the D_1-, 33 kDa-, 23 kDa and 16 kDa proteins. The D_1-protein levels were 76% (30 min) and 42% (90 min) of the relative amount of the protein in control thylakoids. (B) Levels of the D_1-protein and the three extrinsic proteins in inside-out vesicles isolated from the illuminated thylakoids described in Fig. 1A. The level of D_1-protein is lowered. In addition, the amounts of the 33, 23 and 16 kDa protein is reduced. Relative D_1-protein levels were 67% (30 min) and 34% (90 min).

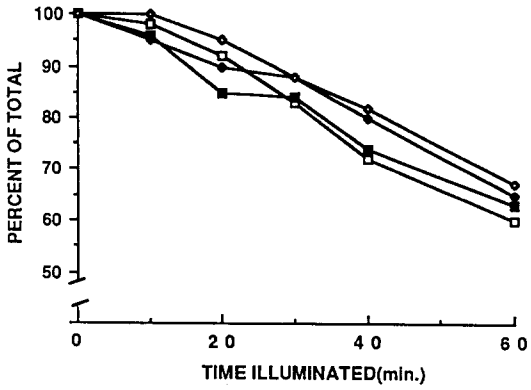

Fig. 2. Correlation between D_1-protein degradation (■) and the release of the extrinsic 33 (◇) 23 (◆) and 16 kDa (□) proteins in isolated inside-out vesicles subjected to strong illumination (5000 $\mu E \cdot m^{-2} \cdot s^{-1}$). Protein content was measured by immunoblotting using the same mixture of antibodies as in Fig. 1.

their protein content. Also in this experiment the vesicles showed a marked reduction of the 33, 23 and 16 kDa proteins. This release correlated closely with the D_1-protein degradation (Fig. 2). Taken together these results demonstrate that the 33, 23 and 16 kDa proteins upon D_1-protein degradation are released from the membrane surface to become retained in the thylakoid lumen. In addition, the observation that the D_1-protein can be degraded in the inside-out vesicles shows that the degradation system is present and functional in the appressed regions of the grana stacks.

A combination of the results of Figs. 1 and 2 and Ref. 7 suggests a release from the membrane of both the extrinsic proteins and manganese following D_1-protein degradation. The three proteins, particularly the 33 kDa subunit, are known to stabilize the manganese cluster of photosynthetic water oxidation [1,17]. The question therefore arises whether the manganese is lost from the thylakoid membrane due to D_1-protein degradation or as a consequence of the release of the 33 kDa protein. To address this question, inside-out thylakoid vesicles were washed with 2 M $CaCl_2$, prior to the photoinhibition treatment. This $CaCl_2$ treatment removed 85–90% of the 33 kDa protein and nearly all of the 23 and 16 kDa proteins but left all the manganese membrane bound in accordance with Ref. 17. These $CaCl_2$-washed inside-out vesicles retained about 20% of their oxygen evolving activity in the presence of 200 mM NaCl. This low activity is due to a slowing down of the S-state cycle in all photosystem II centres and not to a complete inhibition in 80% of the centres [23]. Therefore, these $CaCl_2$-washed vesicles can be used to compare the manganese release and D_1-protein degradation. As shown in Fig. 3, upon strong illumination of the polypeptide-depleted vesicles there was a release of four manganese ions for each copy of D_1-protein degraded. This experiment shows that the reduction of membrane-bound manganese during photoinhibition is not caused by the release of the 33, 23 and 16 kDa proteins.

For the survival of the plant after photoinhibition and D_1-protein degradation, a rapid repair of photosystem II is necessary. Crucial steps in this process are D_1-protein synthesis and reassembly into photosystem II [24]. For such a repair the lateral heterogeneity of the stacked thylakoid membrane possesses an obvious spatial problem. Photosystem II units undergoing photoinhibition are located in the appressed thylakoid regions [9,25], while the insertion of new D_1-proteins into the membrane takes place in the non-appressed thylakoid regions [24,26]. It is therefore important to know if the photosystem II complex, after D_1-protein degradation, remains in the appressed thylakoid regions or migrate to the non-appressed regions in order to be repaired. In an effort to study the effect of D_1-protein degradation on the lateral location of photosystem II polypeptides, thylakoids were subjected to strong light followed by Yeda press fragmentation and isolation of stroma lamellae vesicles. For this set of experiments, an illumination was chosen that resulted in a degradation of 20% of the D_1-protein. The isolated stroma lamellae vesicles were analyzed for changes in the levels of photosystem II proteins by immunoblotting. Antibodies against the D_1- and D_2-proteins, CP43, the 33 kDa protein, cytochrome b_{559}, and the 22 and 10 kDa-proteins [1] were used. Fig. 4 illustrates an experiment demonstrating the relative content of the 10 kDa protein and cytochrome b_{559} in stroma vesicles isolated from control and photoinhibited thylakoids. For both these proteins there is a pronounced increase in the

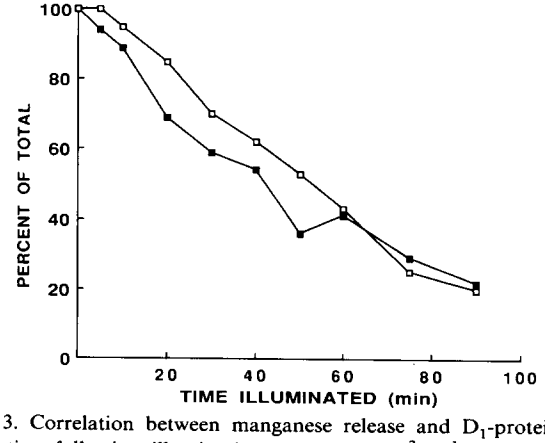

Fig. 3. Correlation between manganese release and D_1-protein degradation following illumination (5000 $\mu E \cdot m^{-2} \cdot s^{-1}$) of inside-out vesicles depleted of the extrinsic 33, 23 and 16 kDa proteins by $CaCl_2$-washing. Manganese content was measured by atomic absorption and protein content was measured by immunoblotting. (■) Mn; (□) D_1-protein.

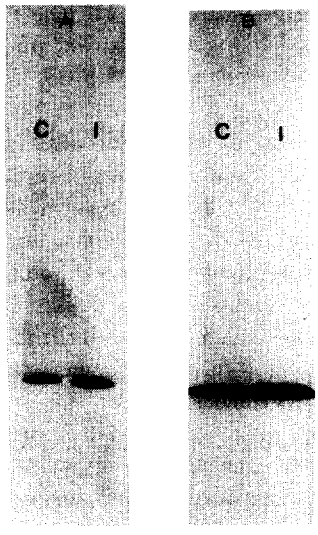

Fig. 4. Demonstration by immunoblotting of the increase in the levels of (A) the 10 kDa protein and (B) cytochrome b_{559} (9 kDa subunit) in stroma lamellae vesicles isolated from photoinhibited thylakoids. The left lanes represent control stroma thylakoids and the right lanes represent stroma thylakoids prepared from thylakoids illuminated for 60 min at 3000 $\mu Em^{-2}s^{-1}$.

stroma thylakoids isolated from the photoinhibited samples. Immunoblot analyses also revealed increased levels of the D_2-protein, the 33 kDa protein and CP43 in the stroma thylakoids of the photoinhibited samples.

The relative increase of the various photosystem II proteins in the stroma thylakoids following photoinhibition was quantified after normalization to the relative amount of the CF_o subunit II (Table I). This subunit of the hydrophobic portion of the ATP synthase was chosen as an internal standard for stroma thylakoids, since it is known to have an exclusive localization to the non-appressed thylakoids [9,27] and its lateral distribution is not affected during photoinhibition (not shown). The relative amount of cytochrome b_{559} is raised by 50% while the increase of the D_2-protein and the 10 kDa protein is limited to 20% (Table I). There is also an increase (60%) of the extrinsic 33 kDa protein in the photoinhibited stroma thylakoids. The most pronounced increase was seen for the CP43 protein. Photoinhibited stroma thylakoid vesicles contained the double amount of this protein as compared to control stroma thylakoids. This suggests a quantitative dissociation and lateral migration of this subunit from all damaged photosystem II centres deprived of the D_1-protein (Fig. 5). This calculation is based upon the observation that 20% of the total D_1-protein is lost and that 80% of the photosystem II centres are located in the appressed thylakoid regions. The remaining 20% are

TABLE I

Changes in the relative levels of photosystem II polypeptides in stroma lamellae vesicles after D_1-protein degradation

Thylakoids were illuminated at 20°C. The light intensity was 3000 $\mu E \cdot m^{-2} \cdot s^{-1}$. After 60 min, when 20% of the D_1-protein had been degraded, thylakoids were fragmented by Yeda press treatment and subfractionated to yield stroma lamellae vesicles. Quantitative western blotting, using antibodies raised against different photosystem II polypeptides was applied to determine the changes of photosystem II proteins in stroma thylakoids after illumination. The changes were normalized to the relative amount of the CF_o subunit II.

Protein	Increase (+)/decrease (−)
D_1-protein	−10%
D_2-protein	+20%
cyt b_{559}	+50%
33 kD protein	+60%
22 kD protein	−20%
10 kD protein	+20%
CP43	+100%

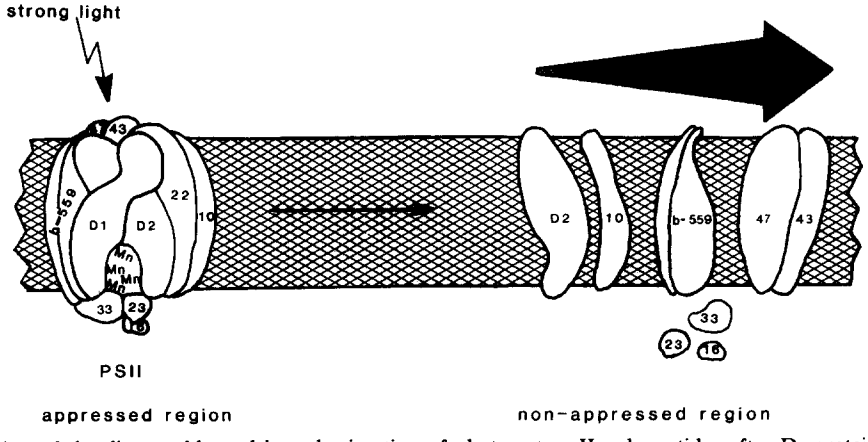

Fig. 5. Schematic illustration of the disassembly and lateral migration of photosystem II polypeptides after D_1-protein degradation. The model suggests a differential migration of photosystem II proteins from the appressed to the non-appressed thylakoid regions. The most pronounced-mobility is seen for CP43 and CP47, while a relatively limited migration is seen for the 10 kDa protein and the D_2-protein. The three extrinsic membrane proteins are thought to randomize in the thylakoid lumen.

assumed to be located in the stroma exposed thylakoids and considered to be insensitive to photoinhibition [25]. Consequently, for the extrinsic 33 kDa protein, cytochrome b_{559}, the D_2-protein and the 10 kDa protein there is only a partial lateral migration, since their increase in the photoinhibited stroma thylakoids is limited to 20%–60% (Fig. 5).

The changes in the lateral location of chlorophyll-proteins following D_1-protein degradation was also analyzed by mild SDS-PAGE of the two stroma thylakoid samples (not shown). For the CP43 and CP47 proteins, more than a doubling was seen in the photoinhibited stroma thylakoids after normalization to CF_o. The resolution of the SDS-PAGE did not allow an accurate quantification of the relative amount for each of the two chlorophyll *a* binding proteins. However, the shape of the partially resolved chlorophyll *a* peaks after scanning the gel did not change after photoinhibition, suggesting an equally high migration of both CP43 and CP47. In contrast, the mild SDS-PAGE did not reveal any increase of LHCII in stroma thylakoids following photoinhibition. This observation is supported by the very small decrease in the chlorophyll *a/b* ratio from 6.8 to 6.4 in stroma thylakoids after photoinhibition.

The yield of stroma lamellae vesicles from control and photoinhibited thylakoids was virtually the same, indicating that there was no significant destacking induced by the photoinhibition and the D_1-protein degradation.

Discussion

Most studies on D_1-protein turnover under high-light conditions have been performed in various in vivo systems [28]. In the present study we show that D_1-protein degradation does not only occur in isolated thylakoid membranes [7,11] but also in the highly photosystem II-enriched inside-out vesicles (Fig. 2). The possibility for D_1-protein degradation in vitro is in accordance with Refs. 7 and 11, but in variance to results suggesting that strong illumination in vitro leads to aggregation of photosystem II proteins [29]. Our present observations suggest that the D_1-protein degradation system is located in the appressed thylakoid regions although a location also in the non-appressed regions can not be excluded.

Considering the central functional and structural role of the D_1-protein, its degradation should influence the overall organization of the photosystem II complex. The present results demonstrate that this is the case, since degradation of the D_1-protein leads to release of manganese, disassembly of extrinsic and intrinsic protein subunits and lateral migration of proteins between the appressed and non-appressed thylakoid regions.

There is a release of the extrinsic 33, 23 and 16 kDa proteins from the inner thylakoid surface and the three proteins are maintained in the lumenal space. This may be of physiological significance, since the three proteins can easily be available and reused when a new D_1-protein is inserted into the membrane and photosystem II is reassembled.

The release pattern (Figs. 1 and 2) suggests a close association between the 33, 23 and 16 kDa proteins and the photosystem II reaction centre. However, the release of the extrinsic membrane proteins from the thylakoid membrane does not explain the loss of membrane bound manganese following photoinhibition [7]. In inside-out vesicles which were stripped of the three proteins by $CaCl_2$-washings prior to the strong illumination, the close correlation between the manganese release and the D_1-protein degradation still persisted. This suggests that the manganese is released as a direct consequence of the D_1-protein degradation or a general disassembly of the photosystem II core. The discrimination between these two possibilities is under current experimental investigation.

As revealed from the subfractionation experiment of photoinhibited thylakoids, the D_1-protein degradation was accompanied by increased amounts of various photosystem II proteins in isolated stroma thylakoid vesicles. We therefore conclude that the D_1-protein degradation induces lateral migration of photosystem II units into the stroma-exposed thylakoids (Fig. 5) in a manner related to what has been shown to occur in thylakoids at elevated temperatures [9]. However, in contrast to the situation after heat treatment of thylakoids, the increase of the various photosystem II subunits is not uniform. This suggests that a photodamaged photosystem II does not leave the appressed thylakoid regions as one entity. The individual migration of polypeptides suggests a partial disassembly of the photosystem II core. The most pronounced change is seen for the two chlorophyll *a* binding proteins, since their relative amount is doubled in the stroma thylakoids isolated from illuminated thylakoids. In the case of the extrinsic 33 kDa protein the increase is probably due to a randomization in the lumenal space since this protein is released from the membrane due to D_1-protein degradation (Figs. 1 and 2). For the integral membrane proteins we envisage a lateral migration within the membrane bilayer as has previously been shown in connection with protein phosphorylation and elevated temperatures [9]. The driving force for the membrane traffic in connection to D_1-protein degradation is not obvious. It should be noted that the illuminations were performed in the absence of added ATP, thereby excluding a connection between phosphorylation and protein migration in the present experiments.

In contrast to changes in the organization of photosystem II occurring after protein phosphorylation or elevated temperatures, strong illumination in vitro does not induce migration of LHCII into the stroma-exposed thylakoids. This suggests a complete disconnection be-

tween LHCII and the photosystem II core under conditions of D_1-protein degradation.

The changes in the lateral distribution of photosystem II subunits at this moderate level of D_1-protein degradation cannot be attributed to a destacking event, since the yield of vesicles derived from non-appressed regions did not change after strong illumination. Moreover, destacking would lead to the same relative increase of all photosystem II subunits in the non-appressed thylakoids in contrast to what has been observed (Table I). We suggest that the lateral migration of photosystem II units from the appressed to the non-appressed thylakoid regions could be part of a repair cycle following photoinhibition.

For two of the photosystem II subunits, the D_1-protein and the integral 22 kDa protein, there is a decrease in their relative amount in stroma-exposed regions following strong illumination. It could be argued that the reduction of the D_1-protein is due to photodamage of photosystem II centres with a normal location in non-appressed thylakoids. However, this explanation is less likely, since these photosystem II centres are not considered to undergo photoinhibition [24]. Maybe these undamaged photosystem II centres can move into the appressed regions as a first aid before synthesis of new D_1-protein can occur? At present, the function of the 22 kDa is not known [1] and its reverse migration behaviour is not easily understood. Possibly, the 22 kDa protein could move into the appressions to act as a nucleation protein in the reassembly of photosystem II after photoinhibition.

The partial disassembly of photosystem II following light-induced D_1-protein degradation is consistent with site directed mutagenesis work on cyanobacteria showing that deletion of the D_1-protein effects the assembly of other photosystem II proteins [30,31]. Moreover, the green algae *Chlamydomonas Reinhardii* mutants, lacking the D_1-protein, possess a destabilized photosystem II complex [32].

In conclusion, our present study demonstrates several organization changes of photosystem II under strong illumination and that the repair after photoinhibition does not only require biosynthesis and assembly of new D_1-proteins into photosystem II but it also involves reassembly of several integral and extrinsic photosystem II subunits, including lateral movement between the two thylakoid regions. Furthermore, it involves religation of the manganese cluster and the other cofactors which are bound to the D1/D2 reaction centre heterodimer.

Acknowledgements

This work was supported by grants from the Swedish Natural Science Research Council and the Knut and Alice Wallenberg Foundation. Ivar Virgin is the recipient of a fellowship from the Sven and Lilly Lawski Foundation and Stenbjörn Styring is a fellow of the Knut and Alice Wallenberg Foundation. Antibodies against the D_1- and D_2-proteins were generous gifts from Drs. Lee McIntosh and Wim Veermaas and the antibody against the CF_o was a gift from Dr. Richard Berzborn. The excellent technical assistance of Ms. Sophie Bingsmark is highly appreciated.

References

1 Andersson, B. and Herrmann, R.G. (1988) in Plant Membrane Structure, Assembly and Function (Harwood, J. and Walton, T.J., eds.), pp. 33–45, The Biochemical Society, London.
2 Michel, H. and Deisenhofer, J. (1986) in Encyclopedia of Plant Physiology (Staehelin, L.A. and Arntzen, C.J., eds.), Vol. 18, pp 371–381, Springer Verlag, Heidelberg.
3 Trebst, A. (1986) Z. Naturforsch. 41, 240–245.
4 Namba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
5 Coleman, W.J. and Govindjee (1987) Photosynth. Res. 13, 199–223.
6 Dismukes, G.C. (1988) Chemica Scripta 28, 99–104.
7 Virgin, I., Styring, S. and Andersson, B. (1988) FEBS Lett. 233, 408–412.
8 Seibert, M., Tamura, N. and Inoue, Y. (1989) Biochim. Biophys. Acta 974, 185–191.
9 Anderson, J.M. and Andersson, B. (1988) Trends Biochem. Sci. 13, 351–355.
10 Powles, S.B. (1984) Annu. Rev. Plant Physiol. 35, 15–44.
11 Ohad, I., Kyle, D.J. and Hirschberg, J. (1985) EMBO J. 4, 1655–1659.
12 Arntz, B. and Trebst, A. (1986) FEBS Lett. 194, 43–49.
13 Styring, S., Virgin, I., Ehrenberg, A. and Andersson, B. (1990) Biochim. Biophys. Acta, 1015, 269–278.
14 Andersson, B., Åkerlund, H.-E. and Albertsson, P.-Å. (1976) Biochim. Biophys. Acta 423, 122–132.
15 Åkerlund, H.-E. and Andersson, B. (1983) Biochim. Biophys. Acta 725, 34–40.
16 Andersson, B. and Anderson, J.M. (1980) Biochim. Biophys. Acta 593, 427–440.
17 Ono, T.-A. and Inoue, Y. (1983) FEBS Lett. 164, 255–260.
18 Laemmli, U.K. (1970) Nature (Lond.) 227, 680–685.
19 Ljungberg, U., Åkerlund, H.-E. and Andersson, B. (1986) Eur. J. Biochem. 158, 477–482.
20 Towbin, H., Staehelin, T. and Cordon, J. (1979) Proc. Natl. Acad. Sci. USA, 76, 4350–4353.
21 Camm, E.L. and Green, B.R. (1983) Biochim. Biophys. Acta 724, 291–293.
22 Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
23 Miyao, M., Murata, N., Lavorel, J., Maison-Petri, B., Boussac, A. and Etienne, A.-L. (1987) Biochim. Biophys. Acta 890, 151–159.
24 Mattoo, A.K. and Edelman, M. (1987) Proc. Natl. Acad. Sci. USA, 84, 1497–1501.
25 Mäenpää, P., Andersson, B. and Sundby, C. (1987) FEBS Lett. 215, 31–36.
26 Wettern, M. (1986) Plant Sci. 43, 173–177.
27 Berzborn, R.J., Müller, D., Roos, P. and Andersson, B. (1981) in Photosynthesis III. Structure and Molecular Organization of the Photosynthetic Apparatus (Akoyunoglou G., ed.), pp. 107–119, Balaban International, Philadelphia.
28 Mattoo, A.K., Marder, J.B. and Edelman, M. (1989) Cell 56, 241–246.

29 Schuster, G., Shochat, S., Adir, N. and Ohad, I. (1989) in Techniques and New Developments in Photosynthesis Research (Barber, J. and Malkin, R., eds.), pp. 499–510, Plenum Press, New York.
30 Jansson, C., Debus, R.J., Osiewacs, H.D., Gurevitz, M. and McIntosh, L. (1987) Plant Physiol. 85, 1021–1025.
31 Nilsson, F., Andersson, B. and Jansson, C. (1990) Plant Mol. Biol., in press.
32 Bennoun, P., Spierer-Herz, M., Erickson, J., Girard-Bascou, J., Pierre, Y., Delosmoe, M. and Rochaix, J.-D. (1986) Plant Mol. Biol. 6, 151–160.