Restoration of light induced photosystem II inhibition without de novo protein synthesis

Torill Hundal[1], Eva-Mari Aro[1,2], Inger Carlberg[1] and Bertil Andersson[1]

[1]*Department of Biochemistry, Arrhenius Laboratories, Stockholm University, S-106 91 Stockholm, Sweden* and [2]*Department of Biology, University of Turku, SF-20500 Turku, Finland*

Received 9 May 1990

Illumination of isolated spinach thylakoid membranes under anaerobic conditions gave rise to severe inhibition of photosystem II electron transport but did not result in D_1-protein degradation. When these photoinhibited thylakoids were incubated in total darkness the photosystem II activity could be fully restored in vitro in a process that required 1–2 h for completion.

Anaerobiosis; D_1-protein degradation; Light stress; Photosystem II; Recovery from photoinhibition

1. INTRODUCTION

Photoinhibition is characterized by inactivation of photosystem II (PSII) electron transport by high light intensities [1]. Several mechanisms for the light-induced impairment of PSII activity have been proposed. These include effects on the acceptor side of PSII [2] such as double reduction of the primary quinone acceptor Q_A [3] or damages on the level of the second quinone acceptor, Q_B [4]. Also a direct impairment of the primary charge separation [5,6] or involvement of the donor side reactions [7–9] have been suggested.

A subsequent event to the photoinhibition is a proteolytic degradation of the reaction centre protein, D_1 [2,10,11] and disassembly of PSII [12]. The induction of this specific breakdown of the D_1-protein has been suggested to include radicals formed directly in the reaction centre or indirectly through PSII mediated formation of oxygen or hydroxyl radicals [2,3,9,13,14].

Recovery from photoinhibition is generally thought to require de novo synthesis of the D_1-protein and reassembly to create functional PSII centres [15–17].

However, degradation of the D_1-protein does not always occur in response to light-induced inhibition of PSII. It has recently been reported that the D_1-protein is not degraded at low temperatures although photoinhibition of PSII electron transport readily occurs [18]. In addition, photoinhibited but D_1-protein containing PSII centres are also created by strong light under anaerobic conditions [10,19,20].

In this study we show that subsequent to strong illumination of thylakoids under anaerobic conditions, photoinhibited but D_1-protein containing PSII centres are able to regain their electron transport capacity in vitro without de novo protein synthesis.

2. MATERIALS AND METHODS

Spinach thylakoids were isolated according to [21]. Photoinhibitory illumination of the thylakoid membranes was performed in a medium composed of 50 mM Tricine, pH 7.6, 100 mM sorbitol, 5 mM $MgCl_2$ and 20 mM NaCl at a chlorophyll concentration of 0.2 mg/ml using white light of 4500 μmol photons $m^{-2}s^{-1}$. Anaerobic experiments were performed in rubber stoppered vials that had been repeatedly evacuated and flushed at least 10 times with catalytically purified argon.

Subsequent to illumination the thylakoids were incubated for different time periods at indicated temperatures in complete darkness, low intensity white light (10 μmol photons $m^{-2}s^{-1}$) or far red light (710 nm) either in the presence or absence of oxygen.

PSII electron transport was measured in a Hansatech oxygraph at saturating light with phenyl-*p*-benzoquinone as electron acceptor. SDS-polyacrylamide gel electrophoresis and Western blotting were performed principally according to Laemmli [22] and Towbin et al. [23], respectively. Chlorophyll was measured according to Arnon [24].

3. RESULTS

When isolated thylakoid membranes were subjected to strong illumination under anaerobic conditions the inhibition of PSII electron transport was quite severe (Fig. 1). Only 15–20 min of illumination was required to obtain 90% inhibition. The rate and degree of inhibition at anaerobic conditions were more pronounced than at aerobic conditions (Fig. 1) in agreement with

Correspondence address: T. Hundal, Department of Biochemistry, Arrhenius Laboratories, Stockholm University, S-106 91 Stockholm, Sweden

Abbreviations: SDS, sodium dodecylsulphate; Q_A, first quinone acceptor; Q_B, second quinone acceptor

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

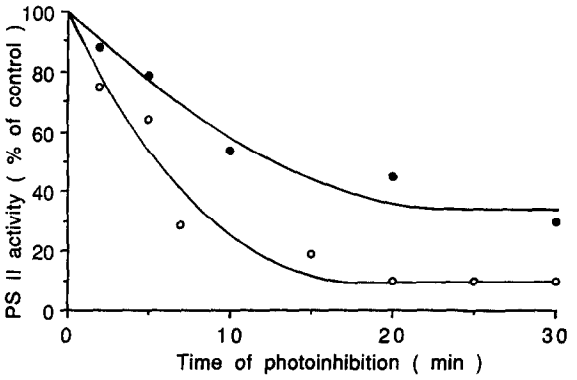

Fig. 1. Inhibition of PS II electron transport during strong illumination of isolated thylakoid membranes under aerobic (—●—) and anaerobic (—○—) conditions at 20°C. PSII oxygen evolution was measured at 20°C using phenyl-*p*-benzoquinone as acceptor.

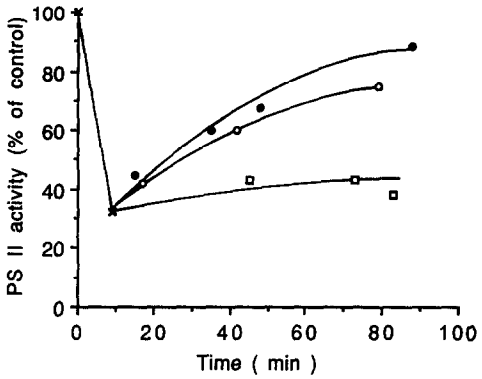

Fig. 2. Recovery of PS II activity after anaerobic photoinhibition. Isolated thylakoids were illuminated at 20°C for 9 min and subsequently transferred to darkness for reactivation in the presence (—●—) or absence (—○—) of oxygen or to low light (10 μE) in the presence of oxygen (—□—).

[10,25,26]. Moreover, under anaerobic conditions no degradation of the D_1-protein could be seen as judged by immunoblotting (Table I).

The D_1-protein remained stable in the photoinhibited thylakoids even after transfer to aerobic conditions and subsequent incubation in darkness for 90 min. The lack of D_1-protein degradation under anaerobic conditions therefore appears to have a different molecular basis to that seen at low temperatures in the presence of oxygen. In the latter case D_1-protein degradation is induced when the photoinhibited sample is transferred to higher temperatures and complete darkness (Table I).

Most strikingly, however, when the photoinhibited but D_1-protein containing thylakoids from the anaerobic illumination were kept in darkness the impairment of PSII electron transport was reversed (Figs. 2 and 3). The activity could be completely restored even after very severe photoinhibition. The recovery of the PSII activity occurred to about the same extent both in the presence and absence of oxygen (Fig. 2). However, the recovery was strongly prevented by light. White light with an intensity as low as 10 μmol photons $m^{-2}s^{-1}$ largely abolished the restoration process. Moreover,

Table I

D_1-protein content in thylakoids after illumination at different temperatures in the presence or absence of oxygen

Illumination conditions	D_1-protein content (% of control)	
	After illumination + dark incubation	
− oxygen, 20°C	100	100
− oxygen, 2°C	100	100
+ oxygen, 20°C	78	63
+ oxygen, 2°C	100	70

Isolated thylakoid membranes were illuminated for 30 min with strong light at 20°C and 2°C in the presence or absence of oxygen. D_1-protein content was quantified by immunoblotting directly after illumination and after an additional 90 min of incubation in darkness under aerobic conditions

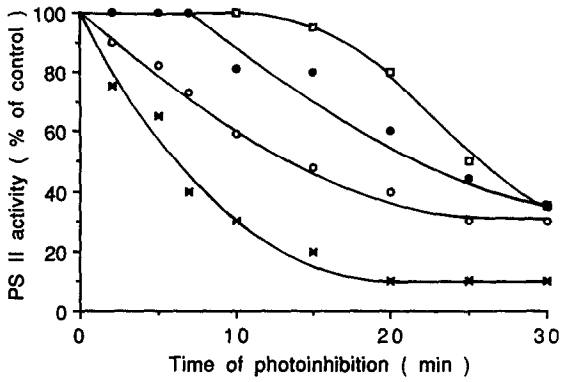

Fig. 3. Time course of the recovery process after different degrees of photoinhibition. At specified timepoints during anaerobic photoinhibition (—×—) samples were withdrawn and incubated aerobically in darkness at 20°C. The photosystem II activity was measured after 35 (—○—), 80 (—●—) and 120 (—□—) min of recovery.

only partial reactivation (30%) was seen when far red light (710 nm) was applied during the recovery process.

The restoration of the PSII activity was highly time dependent (Fig. 3). Maximal recovery required as long as 80–120 min for completion, depending on the degree of inhibition. The electron transport could be fully restored even when the photoinhibition was as high as 70%. However, when the maximal level of inhibition (85–90%) was reached the reversibility gradually decreased. This indicates that prolonged illumination induces an irreversible impairment of the PSII activity. Under aerobic conditions the photoinhibition was always irreversible even after very short periods of illumination.

4. DISCUSSION

In this work, we have for the first time been able to demonstrate in vitro restoration of steady state PSII activity lost during a photoinhibitory process. The

recovery, which was seen in photoinhibited but D_1-protein containing PSII complexes, occurred without de novo protein synthesis. It was strictly dependent on anaerobic conditions during the illumination and on complete darkness during the subsequent recovery. The restoration was not dependent on anaerobiosis but proceeded quite independently of the relative oxygen content. These novel observations raise several questions related to light impairment of PSII electron transport, D_1-protein turnover and repair of photoinhibition.

At present, there is very little experimental basis to understand the molecular mechanism behind the present reactivation of PSII activity. Moreover, theoretical considerations are hampered by the lack of consensus among the models trying to explain the events leading to photoinhibition of PSII electron transport [3–9]. Still another complication is that different mechanisms may apply to photoinhibition under aerobic and anaerobic conditions [13,27].

Most studies on photoinhibition point to effects on the acceptor side of PSII, mainly impairments of the Q_A or Q_B functions. These could involve double reduction of Q_A [28] as has been suggested for aerobic high light photoinhibition [3] or the formation of stable Q_A^- [27] connected to a rapid deactivation phase seen under anaerobic photoinhibition. These impairments would require reoxidation of the quinones in order to reestablish functional PSII. Such reoxidations can not be excluded in the present restoration. However, illumination by far red light in order to oxidize PSII through PSI electron transfer did not enhance but rather reduced the rate of recovery. The impairment could also result from the lack of quinone binding in photoinhibited reaction centres and consequently a reoccupation of empty quinone sites would be a prerequisite for recovery of the PSII activity. The reversibility could also be attributed to the D_1-protein itself. Conformational changes connected to the inhibition process could reverse provided the degradation of the D_1-protein does not occur. Both reoccupation of empty quinone sites and/or protein conformational changes may proceed quite slowly as is the case for the present restoration process (Fig. 3).

Prolonged illumination of the thylakoids under anaerobic conditions resulted in an irreversible photoinhibition (Fig. 3). This points to two inhibition phases during anaerobic light stress; the first phase being reversible and the later irreversible. Recent in vitro photoinhibition studies [20,27] under anaerobic and aerobic conditions resolved the impairment of PSII activity into one fast and two slow phases. The fast phase was observed only under anaerobiosis, while the slower phases were seen both under anaerobic and aerobic conditions. This fast photoinhibition seen by Setlik et al. [27] may resemble the reversible inhibition described in the present study.

Ohad and coworkers [29] have recently proposed a protein synthesis independent partial recovery of aerobic photoinhibition in vivo. If the recovery seen in the present study has any common properties with this aerobic in vivo recovery can not be judged at present. The physiological significance of recovery from photoinhibition without a repair cycle involving D_1-protein turnover should be an interesting subject for future investigations.

Finally, our results show that there is a mechanistic difference between the photoinhibited but D_1-protein containing PSII centres obtained at low temperatures [18] or anaerobic conditions (Table I). In the latter case the D_1-protein is not triggered for degradation which is the case for the D_1-protein after aerobic low temperature photoinhibition (Table I). These observations strongly corroborate that oxygen radicals formed during illumination are involved in triggering the D_1-protein for degradation.

Acknowledgements: This work was financed by the Swedish Natural Science Research Council and the Academy of Finland. We are grateful to Dr Stefan Nordlund for supplying facilities for anaerobic experimentation. We thank Drs. Stenbjörn Styring and Imre Vass for useful discussions.

REFERENCES

[1] Powles, S.B. (1984) Annu. Rev. Plant Physiol. 35, 15–44.
[2] Kyle, D.J. (1987) in: Topics in Photosynthesis, Vol. 9 (Kyle, D.J., Osmond, C.B. and Arntzen, C.J. eds.) Elsevier, Amsterdam, pp. 197–226.
[3] Styring, S., Virgin, I., Ehrenberg, A. and Andersson, B. (1990) Biochim. Biophys. Acta, 1015, 269–278.
[4] Kyle, D.J., Ohad, I. and Arntzen, C.J. (1984) Proc. Natl. Acad. Sci. USA 81, 4070–4074.
[5] Cleland, R.E., Melis, A. and Neale, P.J. (1986) Photosynth. Res. 9, 79–88.
[6] Demeter, S., Neale, P.J. and Melis, A. (1987) FEBS Lett. 214, 370–374.
[7] Theg, S.M., Filar, L.J. and Dilley, R.A. (1986) Biochim. Biophys. Acta 849, 104–111.
[8] Callahan, F.E., Becker, D.W. and Cheniae, G.M. (1986) Plant Physiol. 82, 261–269.
[9] Jegerschöld, C., Virgin, I. and Styring, S. (1990) Biochemistry, in press.
[10] Arntz, B. and Trebst, A. (1986) FEBS Lett. 194, 43–48.
[11] Virgin, I., Styring, S. and Andersson, B. (1988) FEBS Lett. 233, 408–412.
[12] Hundal, T., Virgin, I., Styring, S. and Andersson, B. (1990) Biochim. Biophys. Acta, in press.
[13] Krause, G.H. and Cornic, G. (1987) in: Topics in Photosynthesis, Vol. 9, (Kyle, D.J., Osmond, C.B. and Arntzen, C.J. eds), Elsevier, Amsterdam, pp. 169–196.
[14] Greenberg, B.M., Sopory, S., Gaba, V., Mattoo, A.K. and Edelman, M. (1990) in: Current Research in Photosynthesis, vol. 1 (Baltscheffsky, M. ed) Kluwer, Dordrecht, pp. 209–216.
[15] Kyle, D.J. and Ohad, I. (1986) in: Encyclopedia of Plant Physiology, Vol. 19, (Staehlin, L.A. and Arntzen, C.J., eds.) Springer, Berlin, pp. 468–475.
[16] Greer, D.H., Berry, J.A. and Björkman, O. (1986) Planta 168, 253–260.

[17] Samuelsson, G., Lönneborg, A., Gustafsson, P. and Öquist, G. (1987) Plant Physiol. 83, 438–441.
[18] Aro, E.-M., Hundal, T., Carlberg, I. and Andersson, B. (1990) Biochim. Biophys. Acta, in press.
[19] Kuhn, M. and Böger, P. (1990) Photosynth. Res. 23, 291–296.
[20] Nedbal, I., Masojidek, J., Komenda, J., Prasil, O. and Setlik, I. (1990) Photosynth. Res., in press.
[21] Andersson, B., Åkerlund, H.-E. and Albertsson, P.-Å. (1976) Biochim. Biophys. Acta 423, 122–132.
[22] Laemmli, U.K. (1970) Nature 227, 680–685.
[23] Towbin, H., Staehlin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[24] Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
[25] Krause, G.H., Köster, S. and Wong, S.C. (1985) Planta 165, 430–438.
[26] Allakhverdiev, S.I., Setliková, E., Klimov, V.V. and Setlik, I. (1987) FEBS Lett. 226, 186–190.
[27] Setlik, I., Allakhverdiev, S.I., Nedbal, L., Setliková, E. and Klimov, V. (1990) Photosynth. Res. 23, 39–48.
[28] Rutherford, A.W. and Zimmermann, J.L. (1984) Biochim. Biophys. Acta 767, 168–175.
[29] Ohad, I., Adir, N., Koike, H., Kyle, D.J. and Inoue, Y. (1990) J. Biol. Chem. 265, 1972–1979.