Light-induced D1-protein degradation in isolated photosystem II core complexes

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Received 23 April 1990; revised version received 15 June 1990

Photoinhibitory illumination of isolated oxygen evolving photosystem II core complexes results in a substantial degradation of the D1-protein which is accompanied by the appearance of high amounts of at least 4 different degradation products. It is suggested that the degradation is due to a protease that is an integral part of the photosystem II complex.

D1-protein degradation; Endogenous protease; Photosystem II; Photoinhibition; Thylakoid membrane subfractionation

1. INTRODUCTION

Most or even all of the redox components of the photosystem II (PS II) activity are closely associated with the D1/D2-protein reaction centre heterodimer [1–4]. Therefore the specific and rapid turnover of the central D1-protein following photoinhibition of PS II electron transport is one of the most strange events associated with photosynthetic energy conversion. Our understanding of the organizational consequences to PS II, the molecular mechanisms and the physiological significance of this D1-protein turnover is rather limited. It has been postulated that a PEST-like sequence [5] in the D1-protein would be of importance for the degradation process [6]. The triggering of the D1-protein for degradation in high light has been suggested to involve effects of highly oxidative species like oxygen or hydroxyl radicals produced at the acceptor side [7,8], or radicals formed at the donor side of PS II [9,10]. The cleavage sites of the protein are not known, mainly due to the difficulties to identify and isolate peptide fragments.

The temperature dependence of the light induced D1-protein degradation [7,11,12] in combination with the possibility to degrade the triggered protein in total darkness [12] suggest that the reaction is of a proteolytic nature rather than a direct light-induced chemical peptide cleavage. The identification and isolation of such a protease or proteases responsible for the D1-protein turnover have so far been unsuccessful. However, several studies on isolated thylakoids and subfractions suggest that the protease is membrane bound [4,10,12,13–16].

In this study we show that light-induced D1-protein degradation can occur in isolated PS II core particles and that relatively high amounts of four digestion fragments can be obtained in vitro.

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Abbreviations: Mes, 2-(*N*-morpholino)ethanesulfonic acid; PVDF, polyvinylidene difluoride; SDS, sodium dodecylsulphate

2. MATERIALS AND METHODS

Spinach PS II core complexes were isolated from a PS II membrane preparation by the method described by Ghanotakis et al. [17]. The resulting fraction, containing the pure complexes, was suspended to a concentration of 100 μg chlorophyll/ml in 50 mM Mes, pH 6.0/10 mM NaCl/0.4 M sucrose and 0.05% dodecyl-β-D-maltoside. The suspension was subjected to photoinhibitory illumination by exposure to heat filtered white light (10000 uE$\cdot$m$^{-2}\cdot$s^{-1}) for 5 min under aerobic conditions at room temperature. After the illumination the PS II core complexes were collected in an Amincon ultrafiltration cell (YM-5) at 4°C and total darkness.

SDS-polyacrylamide gel electrophoresis was carried out as previously described [18]. Immunoblotting was performed essentially according to [19] using monospecific antisera against the D1 and D2 polypeptides. The antibodies used (Figs 1 and 2) were raised against the entire spinach D1- and D2-proteins respectively excised from the gel after SDS-PAGE of PS II reaction centre particles [20]. Another antibody used was raised against a fusion protein representing approximately 70% of the C-terminal side of the D1-protein of *Amaranthus hybridus* [21]. For identification ^{125}I-labelled protein A or antirabbit sera conjugated with phosphatase were used. Quantification of radiolabelled bands, excised from PVDF paper was performed in a gamma-counter.

3. RESULTS

In order to understand the mechanism of D1-protein degradation, the effect of photoinhibitory illumination on isolated PS II core complexes was investigated. These isolated complexes [17] are devoid of membrane structure, they virtually lack all lipids, and have a sim-

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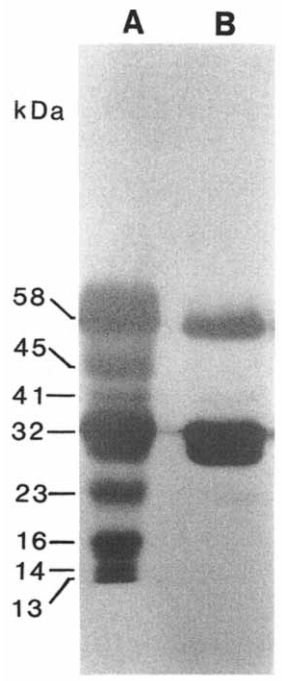

Fig. 1. D1-protein composition after illumination of isolated PS II core complexes. The analyses were performed by immunoblotting against ^{125}I-labelled protein A for detection. The antibody was raised against the entire D1-protein excised from SDS-PAGE [20]. (A) PS II core complexes illuminated for 5 min at 10000 $\mu E \cdot m^{-2} \cdot s^{-1}$. (B) Control PS II core complexes.

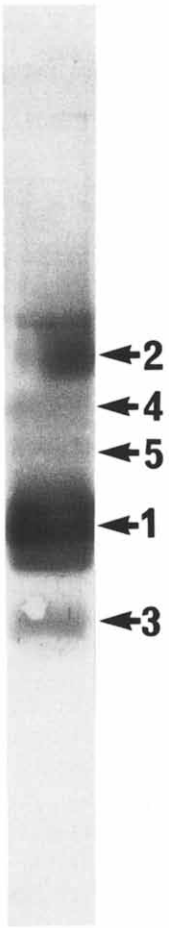

Fig. 2. D2-protein composition after illumination of isolated PS II core complexes showing: (1) monomer form, (2) D1/D2 heterodimer, (3) 19 kDa fragment, (4–5) peptide bands crossreactive to both D1 and D2 antisera (cf. Fig. 1). The analysis was performed by immunoblotting using anti-rabbit sera conjugated with phosphatase for detection. The antibody was raised against the entire D2-protein excised from SDS-PAGE [20].

ple protein subunit composition. They possess a high rate of oxygen evolving activity when supplied with Ca^{2+} and Cl^- ions [22].

The immunoblot of Fig. 1 shows the effect on the D1-protein composition after photoinhibitory illumination of the isolated PS II core complexes. In the control sample there is a double band in the 32 kDa region representing the monomeric form of the D1-protein (Fig. 1, lane B). The lower component is probably a conformer of the D1-protein [6]. The band in the 58 kDa region represents the D1/D2-protein heterodimer since it cross-reacts with antibodies against both the D1- and D2-proteins (Figs 1 and 2). The relative immunoresponse with respect to the D1-protein for the monomer and heterodimer form is 79% and 21%, respectively (Table I). In the illuminated PS II core complexes, which showed no PS II activity after 1–2 min of light treatment, a substantial loss of the D1-protein can be seen (Fig. 1, lane A). There is a 64% decrease in the monomer form and a 24% decrease in the heterodimer form (Table I). The decrease in the level of D1-protein was highly temperature dependent and was not seen at all after illumination at 2°C although the same degree of electron transport inhibition occurred (not shown).

Moreover, several fragments of the D1-protein could easily be detected (Fig. 1, lane A). Below the 32 kDa monomeric form four polypeptide fragments with a size of 23, 16, 14 and 13 kDa were cross-reacting with the D1-protein antibody. In contrast to previous studies [4,6,13,14,23] fragments are present in relatively high amounts. In relation to the total immunoresponse in the control complexes the relative abundance of the four fragments is 7.2%, 12.2%, 3.3% and 2.0%, respectively (Table I). Moreover, above the 32 kDa monomeric form two bands cross-reacting with the D1-protein antibody appeared at 45 and 41 kDa (Fig. 1, lane A). These bands probably represent D1-protein fragments still associated with the D2-protein, since polypeptides in the 40–50 kDa region of the photoinhibited sample showed cross-reactivity also with the antiserum against the D2-protein (Fig. 2). The D1-protein fragments at 16, 14 and 13 kDa could all be candidates for such an association with the D2-protein.

The six identified fragments represent together approximately 32% of the total immunoresponse to the

Table I

Relative amounts of D1-protein and fragments in illuminated PS II core complexes

D1-protein fragment (kDa)	PS II core complex	
	Control cpm (%)	Illuminated cpm (%)
58*	8250 (21)	6310 (16)
45*	–	2230 (5.7)
41*	–	660 (1.7)
32	30670 (79)	11050 (28)
23	–	2800 (7.2)
16	–	4730 (12)
14	–	1280 (3.3)
13	–	780 (2.0)

Each of the ^{125}I-radiolabelled bands from the immunoblot analysis of Fig. 1 were excised and counted in a gamma-counter. The values within parentheses are the percentage values of the total immunoresponse in the control PS II core complexes. Polypeptide bands marked with a star also cross-react with antibodies against the D2-protein

D1-protein in the control PS II complexes (Table I). When the remaining levels of the intact D1-protein in the illuminated sample are added, the immunoresponses account for as much as 77% of total D1-protein in the control sample. The protein aggregation is quite limited but approximately 10% of the immunoresponse is found as a background in the 32–85 kDa region. The loss of total immunoresponse was approximately 13% between the control and illuminated sample. Even though the balance of immunoresponses described above is quite satisfactory, the analyses suffer somewhat from the possibility that the cross-reactivity of a certain antibody preparation may change after protein degradation. However, similar results were obtained using an antibody preparation [21] against the *Amaranthus* D1-protein.

As shown in the immunoblot of Fig. 2, there is also a limited degradation of the D2-protein in agreement with [23] giving rise to a fragment at 19 kDa. None of the other subunits in the core particles showed any change in their relative content after illumination as judged by immunoblot analyses of cytochrome b_{559}, the 10, 22 and 33 kDa proteins, and SDS-PAGE of the CP47 and CP43 proteins (not shown).

4. DISCUSSION

In this study we show that degradation of the D1-protein can occur in isolated PS II core particles. Significantly, the degradation is accompanied by the appearance of high amounts of D1-protein fragments. These results raise several questions concerning the nature of light-induced D1-protein degradation since it has been suggested that the system for degradation of the D1-protein is not active in vitro [24]. It could therefore be argued that the present fragmentation of the D1-protein in the relatively simple PS II core complexes would be the result of a non-enzymatic photocleavage event. However, the very pronounced temperature dependence of the D1-protein degradation in the core particles speaks against such an explanation. Moreover, it has recently been shown [12] that thylakoids photoinhibited at low temperatures show no loss of the D1-protein but the degradation starts in complete darkness once the inhibited sample is transferred to room temperature. We therefore favor the interpretation that D1-protein degradation is of proteolytic nature and that the protease(s) is an integral part of the PS II complex. If so, the substantial degradation of the D1-protein (Table I) seen after illumination of the isolated particles even under highly diluted conditions suggests at least one protease per PS II complex. Possibly, the same protease is also responsible for the limited degradation seen for the D2-protein.

The identification of such a protease is a challenging task. At present, all identified protein subunits present in the isolated PS II core complexes must be considered. These include, apart from the reaction centre subunits D1 and D2, the chlorophyll *a* proteins CP47 and CP43, cytochrome b_{559}, the 10 and 22 kDa proteins, the extrinsic 33 kDa protein, the chlorophyll *a/b* protein CP29 and several small molecular weight proteins [17]. One tempting speculation is that photoinhibition of electron transport may induce an autoproteolytic activity in the D1-protein.

So far it has been difficult to obtain fragments after light-induced D1-protein degradation [4,6,13,14,23]. The most likely explanation is that the proteolytic fragments are degraded at a much higher rate than their formation from the primary cleavage of the D1-protein. However, in the isolated PS II core complexes quite high amounts of several proteolytic fragments can be identified. A possible explanation is that the initial proteolytic steps are fully operational but the terminal part of the proteolytic process is partially or completely inhibited.

A 23 kDa D1-protein fragment has been detected in previous photoinhibition studies in vivo and suggested to be the primary degradation product of the D1-protein [6]. Such a 23 kDa fragment was also seen in the present study which gives support that the proteolytic process seen in the isolated PS II core particles is related to the stepwise degradation suggested to occur in intact photosynthetic systems [6]. Moreover, in maize and *Solanum nigrum* there are reports on 8–14 kDa polypeptide fragments reacting with D1-protein antisera [6]. The relationship between the present D1-protein degradation fragments and the soluble breakdown products in the 10–23 kDa molecular mass range seen in illuminated ^{35}S-labeled thylakoids [25] remains to be established.

Acknowledgements: This study was supported by the Swedish Natural Science Research Council, the European Molecular Biology Organization and the Erna and Victor Hasselblad Foundation. The antibodies were a generous gift from Drs Wim Vermaas, Arizona State University and Lee McIntosh, Michigan State University. We are grateful for scientific discussions with Drs Eva-Mari Aro, Torill Hundal and Stenbjörn Styring.

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