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Molecular mechanisms behind light-induced inhibition of Photosystem II electron transport and degradation of reaction centre polypeptides

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Introduction

Excessively high light intensities are damaging to the photosynthetic apparatus. Photosynthetic organisms are therefore faced with the problem of maintaining sufficient excitation power under limiting light conditions whilst avoiding photodamage under high light conditions. The primary target for this photodamage is Photosystem II (PSII), which undergoes inhibition of its electron transport followed by irreversible damage to and degradation of the D_1 and D_2 reaction centre polypeptides. This degradation is the initial event in a repair cycle of photodamaged complexes and is followed by protein synthesis and reassembly of functional PSII.

This report will deal with recent information relevant to the molecular mechanisms of PSII electron transport impairment and of protein degradation, particularly the D_1 protein, and the chemical link between these two events. Data will be presented in support of the following sequence of reactions (Fig. 1), which take place at PSII under high-light stress: (i) overreduction of the acceptor side, leading to the formation of stably reduced Q_A species; (ii) these events facilitate the formation of chlorophyll triplets which react with molecular oxygen to form singlet oxygen; (iii) this highly reactive and damaging species will oxidize pigments and/or amino-acid residues leading to irreversible damage to the reaction centre, mainly to the D_1 protein; (iv) the oxidative damage induces a conformational change in the D_1 protein which triggers it for degradation; (v) the degradation is catalyzed by a protease which is an integral part of the PSII protein complex.

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Light-induced inhibition of Photosystem II

A central question is at what electron-transfer step photoinhibition is initiated. This problem is not easy to address since the PSII chemistry involves an array of complicated redox reactions [3,4]. Most previous studies on photoinhibition focused on events associated with the secondary quinone (Q_B) binding site [1]. However, more recent experiments in vitro suggest that the impairment is due to overreduction of the primary quinone acceptor Q_A [4]. In such a study, we used fluorescence and EPR analyses of photoinhibited thylakoids under anaerobic conditions [5]. The advantage with the anaerobic approach is that D_1 protein degradation does not normally occur in the absence of oxygen [6] and it is possible to trap reversible intermediates during the inhibition process [7]. By fluorescence measurements, four kinetically different sequential intermediates could be resolved [5]. Mainly based upon EPR spectroscopy data, these intermediates could be characterized in the following way. (1) The plastoquinone pool becomes overreduced and leaves the Q_B site empty, thereby allowing the primary tightly bound quinone (Q_A) to accumulate in an unusually long-lived singly reduced state. (2) This long-lived Q_A^- is further stabilized via protonation, yielding Q_A^--H^+. (3) The primary charge separation is still operational and this Q_A species receives a second electron, whereupon the abnormal state Q_AH_2 is formed. (4) This Q_AH_2 species finally leaves its binding site in the reaction centre. Apart from spectroscopic data for this final event, we have recent evidence based upon HPLC analyses for

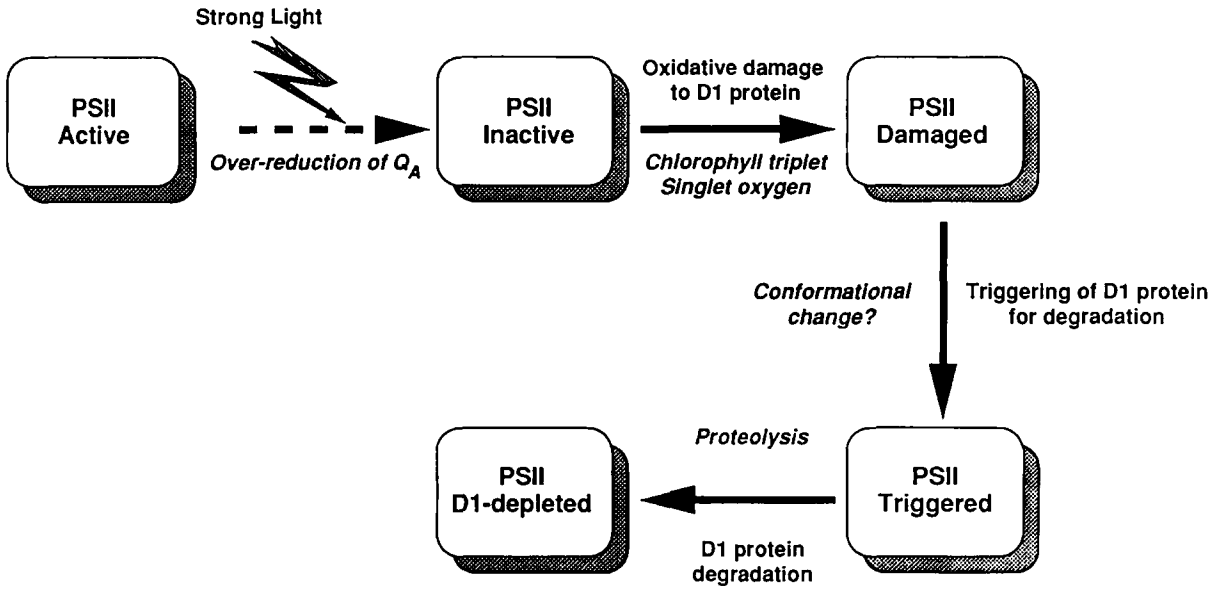

Fig. 1. Schematic diagram of the sequential events leading to light-induced photoinhibition of PSII electron transport and D_1 protein degradation.

loss of Q_A in photoinhibited PSII complexes (Koivuniemi et al., unpublished). The intermediate stages 1–3 are reversible in the dark via re-establishment of Q_A to Q_B electron transfer [5].

Irreversible damage of the D_1 protein

Degradation of the D_1 protein is an event subsequent to the photoinhibition of PSII electron transfer [1,2]. It is generally considered that photoinhibition leads to irreversible damage of the D_1 protein and that restoration of PSII function requires replacement of the damaged protein. Below we will provide experimental evidence for the nature of this damage and show how it is linked to the process of light-induced impairment of the PSII acceptor side as discussed above.

In the absence of functional Q_A, the primary charge separation can give rise to a chlorophyll triplet (^{3}P680). Indeed, we have been able to detect such a chlorophyll triplet by EPR spectroscopy in three of the photoinhibitory intermediates (stages 2–4) under anaerobic conditions [5]. Chlorophyll triplets themselves are not dangerous, but they readily react with oxygen to produce highly toxic singlet oxygen. Notably, when oxygen was introduced into the anaerobically photoinhibited thylakoid sample the chlorophyll triplet was quenched and the D_1 protein started to degrade with the same kinetics.

We therefore conclude that singlet oxygen is produced as a consequence of the impairment of the PSII acceptor side, which gives rise to oxidative damage of the D_1 protein. Studies on isolated reaction centres have shown that singlet oxygen preferentially leads to destruction of P680 [8] but oxidative damage to certain amino acids cannot be excluded at present.

Triggering damaged D_1 protein for degradation

The damaged D_1 protein has to be degraded to allow for a new copy to be inserted into the PSII complex. As will be discussed below, this degradation is catalyzed by proteolytic reactions. Thus the protease(s) must be able to discriminate between a functional D_1 protein and a damaged one. We propose that the oxidative damage results in a gross conformational change in the D_1 protein, thus turning it into a substrate for proteolysis. How this conformational triggering is manifested at the molecular level is not yet known and will require further experiments.

Interestingly, it is possible to trap photoinhibited PSII centres in a triggered state at low temperatures [9]. When high-light stress is applied at 2°C there is no degradation of the D_1 protein, despite a pronounced inhibition of electron transport. Notably, when such inhibited samples are transferred to room temperature in absolute darkness the D_1 protein-degradation reaction starts without further loss of activity. Obviously, there is triggering of the D_1 protein in the light, allowing it to be degraded once the protease becomes active at the higher temperature. By exploiting this experimental approach further, we have been able to

resolve the kinetics of the triggering event from both the inhibition of electron transport and the actual D_1 protein-degradation reaction [10]. The kinetics of the triggering closely follow those of the disappearance of the D_1 protein, suggesting that the proteolysis may not be the limiting step during the degradative phase of the repair cycle.

Still another observation is that D_1 protein trapped in the triggered state at low temperatures loses its manganese, giving experimental support for the concept of a conformational change [10]. The characterization of such a conformation change will require spectroscopic methods such as FTIR, CD and LD. In that respect, the ability to isolate triggered reaction centres from PSII-enriched thylakoid material photoinhibited at low temperatures should provide an interesting approach [11].

Proteolysis of the D_1 protein

It is now becoming generally accepted that D_1 protein degradation can occur in vitro. Moreover, the reaction is of proteolytic nature and can be prevented by inhibitors of serine-type proteinases [2,12]. Photoinhibition studies with isolated PSII complexes showed quite unexpectedly high levels of light-triggered D_1 protein degradation and concomitant appearance of proteolysis fragments (Fig. 2) [2,13,14]. These results provided the first evidence that the PSII complex carries at least one proteolytic subunit. We have been using a radiolabelled proteinase inhibitor (diisopropylfluorophosphate, DFP), which covalently binds to serines in the catalytic site of serine proteinases, in an attempt to identify proteolytic PSII subunits [15]. The results identify a radiolabelled 43-kDa polypeptide, most likely the apopolypeptide of CP43, a chlorophyll-*a*-binding protein of the inner PSII antenna.

Notably, the DFP labelling does not require light, which suggests that the proteinase does not require activation [15] and that D_1 protein degradation is initiated through substrate activation by the damaging and triggering events discussed above.

Even though our recent results suggest CP43 as a candidate proteolytic subunit, there may be more than one proteinase responsible for degrading the D_1 protein. This may be necessary since the D_1 protein is a transmembrane protein with loops exposed at both sides of the thylakoid bilayer and a single protease would only have access to one side of the membrane. Such a proteolytic heterogeneity would be in line with recent studies on isolated PSII reaction centres devoid of or completely lacking the CP43 polypeptide which possess a proteolytic activity degrading the D_1 protein [14].

The experiments on D_1 protein degradation in isolated PSII complexes allowed the identification of proteolytic fragments (Fig. 2). Thus, D_1 protein fragments of 23, 16, 14, 13 and 10 kDa could be identified [2,12,15,16]. Despite concentrated efforts, it has not been possible to obtain any information concerning the cut sites from N-terminal sequencing of these fragments. However, by the use of site-specific D_1 protein antibodies and by radiolabelling of N-terminal phosphothreonine it has been shown that the 23-kDa fragment is of N-terminal origin whilst the 16- and 10-kDa fragments are of C-terminal origin [15,16]. These results imply a cleavage site on the loop exposed at the outer thylakoid surface which connects transmembrane helices D and E of the D_1 protein, in accordance with previous suggestions [1].

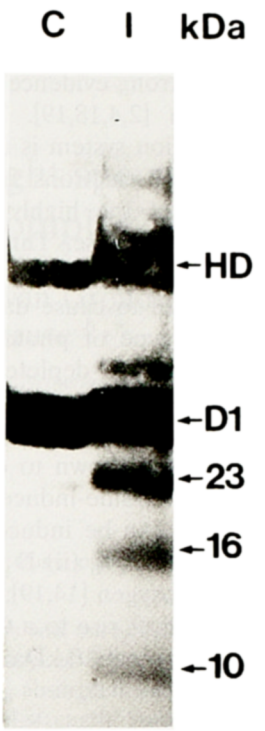

Fig. 2. Immunoblot showing light-induced degradation of the D_1 protein. Isolated PSII sub-core particles were illuminated under high light (5000 $\mu E/m^2$ per s) at 25°C at a chlorophyll concentration of 100 μg/ml. Lane C shows a dark-maintained control and lane I the illuminated material. The proteins were subsequently separated by SDS-PAGE, electroblotted onto a PVDF membrane and immunodecorated with anti-D_1-protein antisera and ^{125}I-protein A. HD: D_1-D_2 protein heterodimer. Fragment sizes are indicated.

It should also be stressed that the D_2 protein also suffers damage during high-light stress and undergoes degradation at a considerably slower rate than the D_1 protein [17]. The mechanism for D_2 protein degradation is currently not known and is an interesting topic for future research.

Donor-side-induced photoinhibition

The overreduction of the primary quinone acceptor Q_A (and the subsequent events described above) is not

the only mechanism that can lead to light-induced inhibition of electron transport and D_1 protein degradation. There is also strong evidence for donor-side-induced photoinhibition [2,4,18,19]. This can happen when the water-oxidation system is unable to keep up with the withdrawal of electrons from P680. If this occurs, the lifetimes of the highly oxidizing species $P680^+$ and Tyr_z^+ will increase. This creates a potentially dangerous situation, since both species have sufficient oxidizing potential to cause damage to the PSII reaction centre. This type of photoinhibition can be studied experimentally in Cl-depleted thylakoid material [20,21] or in isolated PSII reaction centres which lack the water-oxidizing system [14]. This donor-side photoinhibition has been shown to differ in some respects from the acceptor-side-induced photoinhibition described above: (i) it can be induced even at low or moderate light intensities [4]; (ii) D_1 protein degradation does not require oxygen [14,19]; (iii) the proteolysis of the D_1 protein gives rise to a C-terminal 24-kDa fragment and an N-terminal 10-kDa fragment [16].

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