The Role of D1* in Light-Induced D1 Protein Turnover in Leaves

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Light-induced degradation of the D1 protein of photosystem II (PS II) was determined by radioactive pulse-chase labelling experiments in intact leaves of *Schefflera polybotrya*. PS II photochemical efficiency was monitored by measuring chlorophyll fluorescence. A significant and consistent decline in the F_v/F_m ratio was taken to indicate photoinhibition. The formation and degradation of a modified form of the D1 protein, D1*, was different under photoinhibitory or non-photoinhibitory light conditions. At photoinhibitory irradiance greater amounts of D1* were formed relative to D1, and the degradation of D1* was slower when compared with non-photoinhibitory irradiance. The formation and degradation of D1* were therefore shown to be at least partly light intensity dependent. Higher light intensities appeared to slow D1* degradation, which suggests a modification in PS II turnover properties.

Introduction

The D1 protein is found in PS II, one of two pigment-protein complexes of the thylakoid membranes which convert harvested light energy into electrical energy. The D1 protein of PS II is of special interest because it is synthesized and degraded at very fast rates relative to other thylakoid membrane proteins [1, 2], and is also the binding site of some major plant herbicides such as atrazine and DCMU [3, 4]. D1 turnover is light regulated [5, 6], although physiological factors such as the growth light conditions of the plant appear to affect turnover [7–9]. The degradation of D1 is also temperature dependent, and is thought to be catalyzed by a serine protease found in the PS II reaction centre, possibly in the CP 43 peptide [10].

A modified form of D1, D1*, was first identified in 1990 [11]. A number of modifications of the D1 protein have been observed such as palmitoylation [12] and phosphorylation [13, 14]. The results of Callahan and co-workers [11] suggested that D1* is the degradable form of D1. Further work by Aro and co-workers [15–17] has shown that D1* is formed under photoinhibitory conditions, but that degradation of D1* under these conditions is retarded relative to degradation of D1. We present data in this paper from radioactive pulse-labelling experiments performed on whole leaves of the sub-tropical bush *Schefflera polybotrya* which show the formation of D1* under photoinhibitory and non-photoinhibitory light conditions. While the degradation rates of total D1 (*i.e.* D1 and D1*) are similar under both light conditions, much greater amounts of D1* are formed relative to D1 in excess light.

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Materials and Methods

Plant material

Schefflera polybotrya leaflets were harvested from a bush growing in the grounds of the University of Queensland, Brisbane, Australia. The leaflets were obtained from branches exposed to full sun conditions, with a maximum irradiance of 2200 μmol quanta m^{-2} s^{-1}.

Radioactive labelling experiments

In vivo pulse chase experiments using [^{35}S]methionine were conducted on detached leaflets at 90 and 1000 μmol quanta m^{-2} s^{-1}. The protocol for the pulse chase experiments was as follows:

In vivo labelling

Twelve leaflets were picked at 7 am and their fresh weight and area were measured. Their petioles were cut under water and each was transferred into a microfuge tube containing 200 μl of deionized water. To obtain sufficient transpiration rates the leaflets were incubated in a custom-built styrofoam cabinet where they were subjected to a light intensity of approximately 300 μmoles quan-

ta m^{-2} s^{-1}, and a temperature of 28–30 °C for approximately 2 h. This period was termed the "wake-up" period. When sufficient transpiration rates were obtained in most of the leaflets, six with similar transpiration rates were chosen and transferred to microfuge tubes containing [^{35}S]methionine (*trans*^{35}S-label, specific activity 1130–1140 μCi $mmol^{-1}$, ICN Biomedicals, Inc.) diluted 1:3 with deionized water. Uptake occurred over 45–75 min. To standardize between leaflets, as they varied significantly in size and thickness, the amount of radioactive label supplied to the leaflets was calculated individually for each leaflet from their area and fresh weight values such that each leaflet received approximately 6–7 μCi cm^{-2} of leaf tissue. The chase was performed with 2 mM cold methionine for 45 min. The temperature and light conditions for the pulse-chase were the same as those used for the 2 h wake-up period.

Time course

The leaflets were then placed under the experimental light regimes of 90 or 1000 (± 30) μmol quanta m^{-2} s^{-1} for 24 h, referred to as limiting and excess irradiances, respectively. The temperature during this time period was maintained between 20–26 °C.

Sectioning

A leaflet was hand sectioned into its palisade parenchyma and spongy mesophyll tissues after the initial labelling period, at the beginning of the time course, and then every 6 h over the 24 h. Sectioning took between 30 and 45 min. After sectioning, the tissues were frozen and stored in liquid nitrogen.

Thylakoid membrane isolation

The thylakoid membranes were isolated from each sample at 4 °C. The tissue was ground in 2 ml of grinding buffer (0.33 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 25 mM Tricine/KOH pH 7.8) using an Ultra-turrax T25 grinder (Janke and Kunkel, IKA-Labortechnik) for approximately 8 sec. The homogenate was then filtered through two layers of Miracloth (Calbiochem, Sydney) and the filtrate was centrifuged at approximately 12000 × *g* in a Beckman Eppendorf Microfuge for 5 min. The pellet was washed (10 mM NaCl, 5 mM $MgCl_2$, 25 mM Tricine/KOH pH 7.8) and resuspended in grinding buffer. Samples were kept at −20 °C. Chlorophyll concentrations and Chl *a*/*b* ratios were determined in 80% acetone, according to Arnon [18].

Protein identification

The thylakoid membrane proteins for each sample were separated using LiDS polyacrylamide gradient gels as described by Ryrie [19], with modifications by Clarke and Critchley [20], using the Biorad Protean II electrophoresis system. Samples were loaded on equal cpm/μl. Proteins were visualized by staining with Coomassie Brilliant Blue R250 and identified by their migration relative to protein markers of known molecular weight (Dalton Mark VII-L, SIGMA). The gels were vacuum dried for 3 h (Biorad model 583) and the distribution of radioactivity visualized by exposing the dried gels to X-Omat RP-5 X-ray film (Eastman Kodak) at −70 °C for one to 14 days, depending on the amount of radioactivity loaded.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters F_v/F_m, F_o, F_m and the half-rise time taken to reach F_m ($t_{1/2}$) were measured at room temperature from the upper and lower surfaces of the remaining six leaflets (not radioactively labelled) with a Plant Stress Meter (PSM, Biomonitor, Umeå, Sweden). These leaflets were treated concurrently and identically with the labelled leaflets. Dark adaptation time was 30 min.

Results and Discussion

Significant degradation of radioactively labelled D1 protein was seen under both limiting and excess irradiances (Fig. 1 and 2). Fig. 2 shows that the rate of total labelled D1 degradation, *i.e.* D1 plus D1*, was very similar under both irradiances, and no significant difference was seen in the degradation rates between the two tissues. Approximately 40–50% of total labelled D1 remained after the 24 h light treatment. Table I shows the reduction in F_v/F_m due to photoinhibition of the leaves as a percentage of initial F_v/F_m (0.776–0.823). Limiting light had no effect on F_v/F_m, while excess light caused significant photoinhibition in

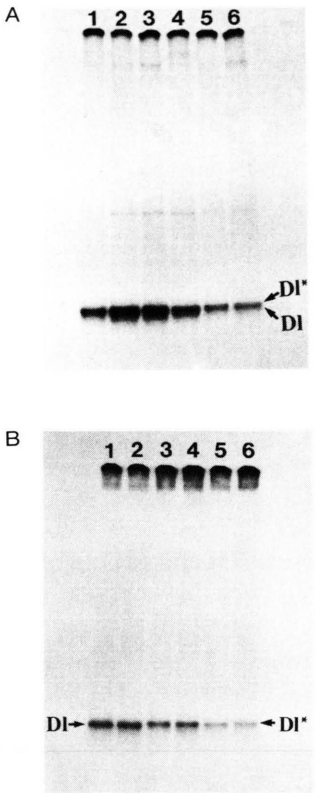

Fig. 1. Autoradiographs of typical experiments showing labelling of D1 and D1* over 24 h in *Schefflera polybotrya* sun leaves exposed to limiting (A) or excess (B) irradiances. Lanes 1: −45 min (end of labelling period); 2: 0 h (after 45 min chase, start of experimental irradiance treatment); 3: 6 h; 4: 12 h; 5: 18 h; 6: 24 h.

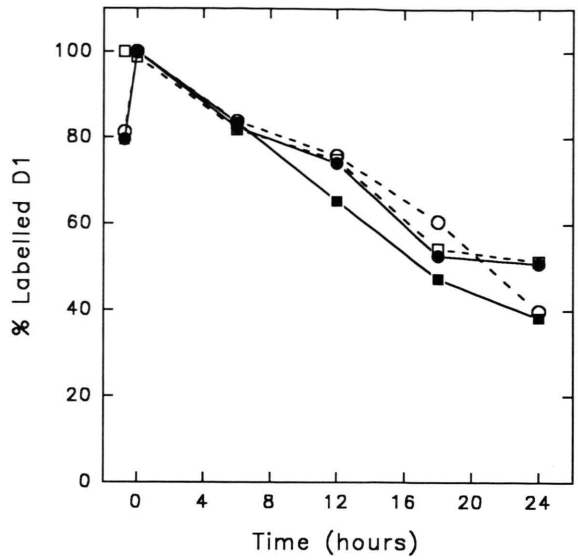

Fig. 2. The degradation of total D1 (D1 + D1*) in the palisade parenchyma (——) and spongy mesophyll (– – –) when illuminated over 24 h at limiting (● ○) or excess (■ □) irradiances.

Table I. Changes in the ratio of variable fluorescence (F_v)/maximum fluorescence (F_m) over 24 h measured from the upper or lower surfaces of *Schefflera polybotrya* sun leaves exposed to limiting or excess irradiances.

Light treatment	Tissue	Reduction in F_v/F_m*
Limiting (90 μE m^{-2} s^{-1})	palisade	0%
	spongy	0%
Excess (1000 μE m^{-2} s^{-1})	palisade	40%
	spongy	12%

* Reduction in F_v/F_m seen after 24 h exposure as a percentage of initial F_v/F_m (0.776–0.823).

the palisade layers, and mild photoinhibition in the spongy mesophyll.

The appearance and loss of D1* was quite different under these light intensities. D1* was formed rapidly, such that a small amount of D1* was seen immediately after the 45 min labelling period, and greater amounts after the 45 min chase (Fig. 1A and B, lanes 1 and 2). Under limiting light the ratio of D1*:D1 was never greater than 1, and the D1* degradation pattern was similar to D1 (Fig. 3A and B). Under excess light however, much greater amounts of D1* were formed relative to D1 such that D1*:D1 > 6 (Fig. 3C and D). The degradation of D1* relative to D1 was also slower, especially in the spongy mesophyll.

The role of D1* in PS II photochemistry has not been conclusively determined. It is probably the phosphorylated form of D1 [16, 17], and has been observed under both normal and photoinhibitory light conditions [21] which is consistent with the results presented here. Both D1 and D2 are phosphorylated at their amino termini by the same protein kinase, which is activated by reduction of the plastoquinone pool [13, 14, 22]. While this may suggest that electron transport is necessary for the formation of D1*, Callahan and co-workers [11]

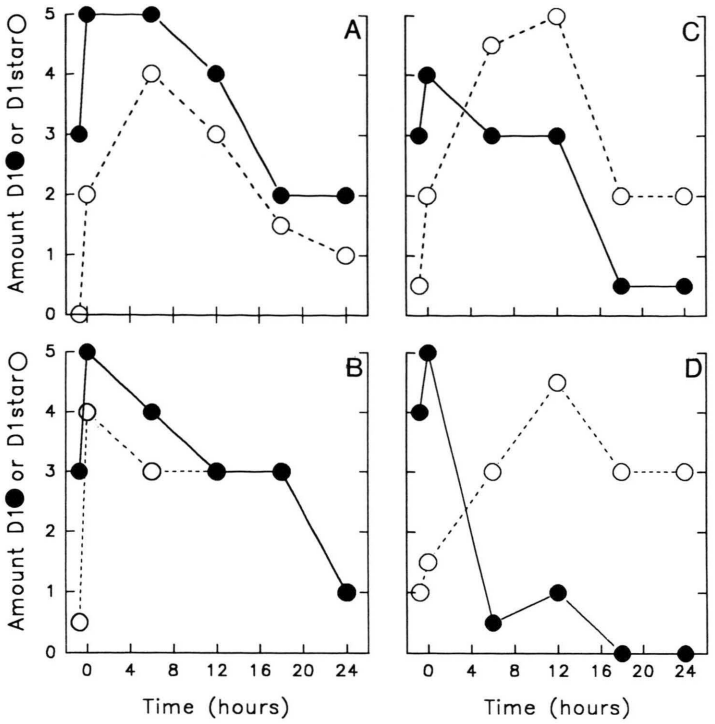

Fig. 3. D1 (●) and D1* (○) synthesis and degradation in sun-grown leaves under limiting (A, B) and excess (C, D) irradiance in the palisade parenchyma (A, C) and spongy mesophyll (B, D) tissues of *Schefflera polybotrya*.

have evidence which indicates that photosynthetic electron transport is not correlated with D1* formation, *i.e.* D1* is readily formed under UV light of 300 nm, a wavelength which does not support electron transport. They also present evidence for a role in D1 degradation, because D1* formation is inhibited in the presence of propylgallate, a free radical scavenger which inhibits D1 degradation but not linear electron transport. D1* formation is also inhibited in the presence of herbicides such as DCMU which prevent D1 degradation.

If D1* is the degradable form of D1, our results suggest that under limiting light, the rate of degradation of D1* is faster than, or approximately the same as, the rate of conversion of D1 to D1*, as $D1^*:D1 \leq 1$. At excess light the rate of degradation of D1* is much slower than the rate of conversion of D1* to D1. If D1* was degraded immediately upon being formed, the degradation rate would be considerably faster under excess light, which is not the case. Presumably, other factors must be required to cause its degradation, and these factors appear to retard its degradation under high light.

Aro *et al.* have shown that in photoinhibited thylakoids D1, and not D1*, is degraded [16]. It is possible however, that what appears to be degradation of D1 maybe conversion of D1 into D1*. Our results show that D1* is degraded under excess light conditions. However, although it is tempting to think of D1* as the degradable form of D1, our results taken together with those obtained by Aro and co-workers [15–17] suggest that while possibly playing a role in the regulation of D1 degradation, the formation of D1* in itself does not signal it for immediate degradation.

Acknowledgements

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