

# Slow turnover of the D1 reaction center protein of photosystem II in leaves of high mountain plants

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**Abstract** The D1 reaction center protein of photosystem II usually exhibits a rapid turnover in light. The D1 protein turnover was compared in three species of alpine plants, *Homogyne alpina*, *Ranunculus glacialis*, *Soldanella alpina*, and in the lowland plant *Taraxacum officinale* by radioactive labeling in light and subsequent chase experiments. The D1 protein of alpine plants could also be recognized by its more rapid labeling, relative to other membrane proteins. However, compared to *T. officinale* the turnover of the D1 protein was considerably slower in the alpine plants. The potential advantage of a slow D1 turnover for adaptation to the environmental conditions of high mountain plants is discussed.

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**Key words:** Antioxidative protection; D1 reaction center protein; Photoinhibition; Photosystem II; Stress tolerance; Alpine plant

## 1. Introduction

Excessive high light intensities are inhibitory to photosynthesis. Usually photoinhibition of photosystem II (PSII) is observed as an early symptom of photodamage [1–3]. However, the D1 reaction center protein of PSII is also inactivated and degraded in light [4] under non-photoinhibitory conditions in a dose-dependent manner, and must be continuously replaced by new synthesis. Therefore a rapid turnover of the D1 protein was generally observed in light in a wide variety of plant species [3,5–7]. It appeared that some damage of the D1 protein was inevitably coupled to electron transport through PSII and represented an intrinsic property of a photochemical reaction center operating in an oxygenic environment [2,8]. When the rate of inactivation exceeds the capacity for its repair, the content of functional D1 protein is depleted resulting in an apparent photoinhibition of PSII. This occurs under excessively high light conditions or when new protein synthesis is impaired by unfavorable environmental stress conditions, such as salt or high as well as low temperature [1,9,10].

Plants adapted to extreme environments must have developed strategies of resistance to, or avoidance of, photoinhibitory damage. Alpine high mountain plants may be exposed to high radiance and prevailing low temperatures for considerable time periods. Since only 2–3 months are available for their annual growth activity they can, however, not afford a prolonged or chronic photoinhibition of photosynthesis but depend on highly efficient carbon assimilation [11]. Previous

investigations have shown that three species of alpine high mountain plants studied were indeed very resistant to photoinhibition of PSII, even when the leaves were incubated on ice or when new synthesis of the D1 protein in the chloroplasts was prevented by application of the translation inhibitor chloramphenicol [12]. These observations strongly suggested that the light-dependent turnover of the D1 protein in leaves of these high altitude plants must have been very slow relative to lowland plants. In the present work we have now substantiated these conclusions by direct experimental evidence obtained from radioactive labeling of the D1 protein in leaves of alpine plants and subsequent chase experiments. Evidence is presented that leaves of these high mountain plants were indeed able to avoid photodamage and that the turnover of the D1 protein was greatly reduced, relative to non-alpine plants.

## 2. Materials and methods

### 2.1. Plant materials and experimental treatments

Experiments were performed with leaves of three species of alpine plants, *Homogyne alpina* (L.) CASS (alpine coltsfoot), *Ranunculus glacialis* L. (glacier crowfoot), and *Soldanella alpina* L. (alpine snowball), growing in the French Alps on a slope at about 2400 m altitude between the Lautaret Pass and the Galibier Pass. Conditions at the collection site were described previously [12]. Whole plants were dug up and transported in a moist and cool container to the laboratory in Frankfurt am Main. Labeling experiments were performed on the second and third day following the removal of plants from their field sites. Illumination with white light of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR during the labeling experiments was provided by an array of 75 W halogen lamps. Experiments were performed with plants collected during three subsequent years, 1995–1997. Only old leaves from the previous year were used for experiments with *H. alpina* and *S. alpina*. For comparison, labeling experiments were also performed with segments from fully expanded mature leaves of the lowland plant *Taraxacum officinale* WIGGERS, grown at sun-exposed sites of the Botanical Garden of the University of Frankfurt am Main.

### 2.2. Radioactive labeling and chase experiments

For radioactive labeling sections of 5 or 7 mm diameter were cut from the leaves with the aid of a cork drill. For each experiment segments with a total fresh weight of 120 mg were collected and preincubated for 90 min at 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR on moist filter paper. Subsequently, the leaf segments were labeled for 2 h by incubation at 25°C with slow rotation on 0.6 ml of 5 mM sodium phosphate, pH 7.4, 0.01% (v/v) Tween 20 containing 3.7 MBq L- $^{35}\text{S}$ methionine (30 TBq  $\text{mmol}^{-1}$ ) in the presence of light of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, starting with 2 min of vacuum infiltration. When labeling was performed in the presence of 6.3 mM chloramphenicol, leaf sections were preincubated for 1 h in darkness in the presence of the inhibitor before the light and labeling periods. For chase experiments leaf sections were washed at the end of the labeling period with distilled water and subsequently with excess unlabeled 1 mM L-methionine (contained in 20 mM Na-phosphate, pH 7.4, and 0.01% Tween 20) and incubated in the latter medium in light of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR for up to 4.5 h. The methionine-containing medium was renewed every 30 min.

At the end of the labeling or chase periods the leaf segments were

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**Abbreviations:** PAR, photosynthetically active radiation; PSII, photosystem II; SDS, sodium dodecyl sulfate

thoroughly rinsed with distilled water. After excess water was removed, the tissue was frozen with liquid nitrogen and ground to a fine powder, which was suspended in 0.8 ml of 50 mM Tris-HCl, pH 7.4, and 1 mM EDTA. For *H. alpina* and *R. glacialis* the grinding medium contained, in addition, 10 mM Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>. The suspension was centrifuged for 5 min at 9000×g and 4°C. The sediment was washed two times, recentrifuged and, finally, suspended in 0.5 ml of grinding medium without Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>. The chlorophyll content was determined after extraction of samples of the membrane suspension in 80% acetone according to Arnon [13].

Samples of the membrane suspension were solubilized by addition of an equal volume of 6% (w/v) SDS, 10 mM dithioerythritol, 0.01% (w/v) bromophenol blue, and 5% (w/v) sucrose and 3 min heating at 95°C. After removal of insoluble material by 3 min centrifugation at 9000×g membrane samples were applied to polyacrylamide slab gels (11×13×0.15 cm<sup>3</sup>), consisting of a 10–15% (w/v) polyacrylamide gradient resolving gel and a 5% (w/v) stacking gel. Electrophoresis in the presence of 0.4% (w/v) SDS was performed in the buffer system of Laemmli [14]. Polyacrylamide gels were prepared for fluorography according to the procedure of Bonner and Laskey [15] and exposed for 30–90 min to Kodak X-Omat S film at –80°C.

**3. Results and discussion**

In order to compare synthesis and turnover of the D1 protein of PSII, segments from leaves of the alpine species *Homogyne alpina*, *Ranunculus glacialis* and *Soldanella alpina* and of the low-elevation plant *Taraxacum officinale* were labeled for 2 h in light with L-[<sup>35</sup>S]methionine. After fluorography of electrophoretic separations in the presence of SDS of crude membrane fractions obtained from labeled leaves, the by far highest incorporation of radioactivity was found in the lower of two closely adjacent bands in the range of 30 kDa in preparations from *T. officinale* (Fig. 1). By comparison with immunoblotting with an antiserum against the D1 protein, this band was identified as the site of the D1 protein, as observed previously [9]. After labeling in darkness incorporation into the D1 polypeptide was hardly detectable, thus illustrating the light-dependency of its synthesis (Fig. 2A). In membrane separations from the alpine species *H. alpina*, *R. glacialis* and *S. alpina* a comparable polypeptide of approximately 30 kDa represented the most strongly labeled band visualized under these conditions (Fig. 1). However, when membrane samples of equal chlorophyll contents were compared, incorporation into the D1 polypeptide was much high-

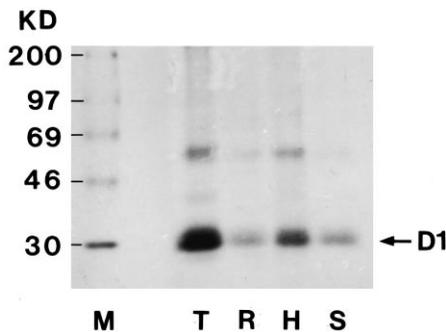


Fig. 1. Fluorogram after electrophoretic separation in the presence of SDS of fractions of total cell membranes from leaf sections of *Taraxacum officinale* (T), *Ranunculus glacialis* (R), *Homogyne alpina* (H), and *Soldanella alpina* (S) after 2 h labeling with L-[<sup>35</sup>S]methionine. Arrow indicates location of the D1 polypeptide of PSII. M, molecular mass markers. Membrane samples containing 4 µg chlorophyll each were applied per lane.

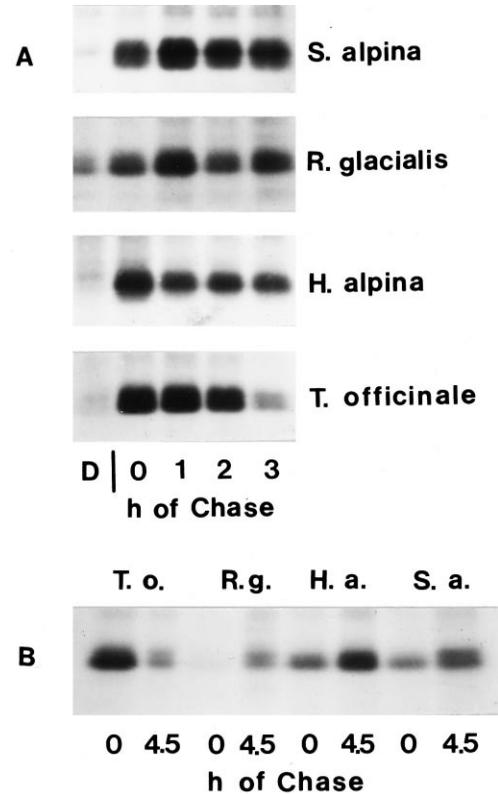


Fig. 2. Comparison of the radioactivity in the D1 polypeptide of PSII after 2 h labeling with L-[<sup>35</sup>S]methionine in darkness (D) or in light (all other samples) before the onset (0 h) or after a 1–3-h (A) or a 4.5-h (B) additional chase period in light (1000 µmol m<sup>-2</sup> s<sup>-1</sup> PAR). Fluorograms after electrophoretic separation in the presence of SDS of total cell membranes obtained from labeled leaf sections. In (A) longer film exposure times were applied for the fluorography of separations from the alpine plants (*H. alpina*, *R. glacialis*, *S. alpina*) than for those from *T. officinale*, in order to obtain similar maximum band intensities.

er in leaves of *T. officinale* than of the alpine species *H. alpina*, *R. glacialis* and *S. alpina* (Fig. 1). This observation already suggested that at an equal photon flux the turnover and the accompanying new synthesis of the D1 polypeptide appeared to be considerably slower in leaves of the alpine species than in leaves of *T. officinale*. Labeling of segments of *S. alpina* leaves was also performed in the presence of 6.3 mM chloramphenicol, an inhibitor of chloroplastic protein synthesis. Under these conditions incorporation into the D1 protein was totally blocked, as expected for a translation product of the chloroplasts.

Since the rates of L-[<sup>35</sup>S]methionine incorporation in different tissues may depend on differences of uptake or internal pool sizes, they may not reflect true rates of synthesis. Therefore, additional chase experiments were performed, subsequent to the labeling period, in order to document the turnover of the D1 protein. The time course of the decline of radioactivity from the D1 polypeptide band of *T. officinale* in light was equally fast, as previously observed for other low-altitude plants [4,7,9]. Within 3 h the radioactivity incorporated into the D1 polypeptide had almost disappeared, indicating its rapid degradation. After 3 h of chase the radioactivity in the D1 protein amounted to only 14% of that estimated at the end of the labeling period. This is in good agreement with the time course of the decline of activity of

PSII in *T. officinale* leaves, when its repair was blocked by the translation inhibitor chloramphenicol, as described by Streb et al. [12]. In the presence of the inhibitor the ratio of variable to maximum chlorophyll fluorescence ( $F_v/F_m$ ), which indicates PSII activity, decreased to about 10% within 3 h under day light conditions with a half time of less than 1 h [12].

In low-elevation plants, such as *T. officinale* (Fig. 2A), highest radioactivity in the D1 polypeptide is usually observed immediately after the end of the labeling period and its decline starts at least within 1 h of chase [7,9], because the residual free radioactive methionine, that is contained in the tissue, is rapidly consumed by the fast turnover. In two alpine species, *R. glacialis* and *S. alpina* the radioactivity incorporated into the D1 polypeptide increased, however, more slowly to its maximum which was reached only after 1 h of chase (Fig. 2A). In all three alpine species that were investigated the degradation of the D1 polypeptide, as indicated by the loss of radioactivity, was much slower under identical experimental conditions than in *T. officinale* (Fig. 2A). Consequently, after 3 h of chase a high amount of radioactivity was still retained. In order to further confirm these results, labeling of the D1 polypeptide was compared at the end of the 2-h labeling time and after a more extended chase period of 4.5 h (Fig. 2B) in plants collected and analyzed in a different year than those used for the experiments of Fig. 2A. While only very little of the initial radioactivity was left in the D1 polypeptide of *T. officinale* after the chase, the labeling of the D1 polypeptide of all three alpine species was also after 4.5 h of chase considerably higher than before its onset in these experiments. Thus, altogether, the results of the present work provide clear evidence that under identical light conditions the turnover of the D1 protein, i.e. both its synthesis and degradation, was much slower in leaves of the three alpine species that were assayed than usually observed in low-elevation plants, such as *T. officinale*.

Since a dose-dependent inactivation and degradation of the D1 protein in light has been commonly observed among plants it was regarded as an unavoidable intrinsic property of the PSII reaction center [2]. Also in the leaves of the alpine species the D1 protein was recognized by its fast labeling in light, relative to other membrane proteins of these plants. However, in comparison to the majority of non-alpine plants its turnover was largely reduced at a given light intensity, indicating that photodamage in PSII can be prevented to a much greater extent than usually observed. This is in accordance with previous observations that the loss of PSII activity, indicated by the  $F_v/F_m$  ratio, was only very slow when its repair was blocked by the translation inhibitor chloramphenicol or by exposure to low temperature [12]. The ability of these high-altitude plants to largely avoid photodamage and turnover of their D1 protein provides an important mechanism of adaptation to their unfavorable environmental conditions with extended periods of high radiance in combination with low temperature. Such conditions would both enhance photoinhibitory damage and suppress repair activities, thus causing the danger of prolonged apparent photoinhibition of PSII [10,16–18]. Since PSII activity of isolated thylakoids from both *H. alpina* and *R. glacialis* (*S. alpina* did not allow reasonable chloroplast preparation) was as rapidly inactivated in vitro, as in thylakoids from lowland plants [12], the D1 protein of the alpine species appears to be equally sensitive to photodamage. However, in intact leaves more efficient

mechanisms than in lowland plants for the avoidance of, or protection from, photoinhibitory damage must be available. Also in cold-acclimated spinach leaves D1 protein degradation was found to be strongly diminished, relative to non-acclimated leaves [19]. Thus it appears that stabilization of the D1 protein may more generally accompany decreased sensitivity to photodamage in plants adapted to low temperature.

Acceptor and donor side mechanisms of the primary photo-damage in PSII have been discussed [2,3,20]. Both involve the production of either reactive oxygen species or highly oxidative radicals. A higher resistance to photoinhibitory damage may, therefore, be established by increased capacities of antioxidative scavenger systems [18,21] or by mechanisms that allow to maintain the primary electron acceptor  $Q_A$  in the oxidized state and thus to avoid acceptor side damage of PSII, for instance by thermal energy dissipation through non-photochemical quenching mechanisms. The higher stability of the D1 protein observed in young, as compared to old, sun leaves of the tropical forest tree *Anacardium excelsum* has been attributed to their higher capacity for thermal energy dissipation [22] which frequently seems to be related to high levels of xanthophyll cycle carotenoids [23]. A highly interesting feature of these alpine plants was that they have obviously developed quite divergent strategies of resistance to photoinhibitory damage. As shown previously [12], only *S. alpina* contained extraordinarily high levels of antioxidants and antioxidative enzymes as well as of xanthophyll cycle carotenoids which may indicate a high capacity for thermal dissipation of excitation energy. While these protective systems may be sufficient to provide strong light acclimation and PSII stability in *S. alpina*, their capacities were much lower in *H. alpina* and particularly in *R. glacialis*. While in *H. alpina* antioxidative protection may have been improved by the occurrence of a highly light-stable catalase, *R. glacialis* must predominantly depend on some other, yet unknown, mechanism of protection [12]. An alternative mechanism contributing to increased resistance of PSII to photoinhibitory damage, that was postulated for cold-hardened winter cereals, may result from the maintenance of strong sinks, also at low temperature, for the metabolic consumption of the photosynthetic electron flow that would be sufficient to prevent overreduction of the primary electron acceptor  $Q_A$  and thus help to avoid acceptor side damage of PSII [17].

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