Prolonged high light treatment of plant cells results in changes of the amount, the localization and the electrophoretic behavior of several thylakoid membrane proteins

Volkmar Schmid, Stefan Peter & Christian Schäfer
Lehrstuhl Pflanzenphysiologie, Universität Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany

Received 2 November 1994; accepted in revised form 27 April 1995

Key words: photoinhibition, D1 protein, PS I, PS II, protein modification, protein translocation

Abstract

The effect of a 30 h high light treatment on the amount and the localization of thylakoid proteins was analysed in low light grown photoautotrophic cells of Marchantia polymorpha and Chenopodium rubrum. High light treatment resulted in a net loss of D1 protein which was accompanied by comparable losses of other proteins of the PS II core (reaction center with inner antenna). LHC II proteins were not reduced correspondingly, indicating that these complexes are less affected by prolonged high light. High light influenced the distribution of PS II components between the grana and the stroma region of the thylakoid membrane, probably by translocation of the respective PS II proteins. Additionally, modifications of several thylakoid proteins were detected in high light treated cells of C. rubrum. These effects are discussed in relation to photoinhibitory damage and repair processes.

Abbreviations: BCA—bicinchoninic acid; chl—chlorophyll; CF1—coupling factor; CYC—cycloheximide; GT—grana thylakoids; HL—high light; LL—low light; PAGE—polyacrylamide gel electrophoresis; PFD—photon flux density; PS I—Photosystem I; PS II—Photosystem II; RC—reaction center; SDS—sodium dodecylsulfate; ST—stroma thylakoids; Thyl—unfractionated thylakoids

Introduction

Treatment of photosynthetic cells with photon flux densities (PFDs) which greatly exceed the light saturating value results in longterm reductions of maximum photosynthetic efficiency. This phenomenon has been termed 'photoinhibition' (Krause 1988). Concerning the molecular basis of photoinhibition studies focussed above all on the D1 protein, a component of the PS II reaction center (Aro et al. 1993). Reasons for this approach are the pronounced light-dependent turnover of the D1 protein and its central role in PS II photochemistry.

PS II is mainly restricted to the grana regions of the thylakoid membrane, whereas the insertion of newly synthesized D1 protein occurs in the stroma regions (Mattoo and Edelmann 1987). Hence D1 protein turnover should result in a massive exchange of D1 protein and possibly other PS II components between the stroma and the grana regions. Additionally, net loss of D1 protein can occur under light stress conditions (Schäfer et al. 1993) and this could result in a surplus of the remaining PS II components. It is conceivable that these effects result also in changes in the steady state distribution of PS II components. The occurrence of such changes is also suggested from experiments with isolated thylakoids (Barbato et al. 1992; Hundal et al. 1990) and unicellular algae (Adir et al. 1990).

Another aspect of high light (HL) effects on thylakoid proteins are modifications. Several PS II proteins including D1 can be modified by phosphorylation (Allen 1992). Again, the modification of the D1 protein (D1*) has been studied in considerable detail,
and it could have significance for D1 protein turnover (Anderson and Aro 1994).

The present study analyses the effect of prolonged high light treatment on thylakoid protein contents in whole thylakoids and in stroma and grana thylakoid fractions. The experiments were performed with photoautotrophic suspension cultured cells of Chenopodium rubrum and Marchantia polymorpha. Earlier investigations have shown that these cell lines are useful model systems in the study of light stress effects (Schäfer 1994; Schäfer and Schmid 1995). We chose PFDs which might occur under natural conditions and a treatment duration which results in clear reductions of the photochemical efficiency of PS II, zeaxanthin formation and a pronounced increase in xanthophyll cycle pigment pool size (Schäfer et al. 1994). The results provide evidence for the coordinated degradation of proteins from the PS II core (reaction center plus inner antenna) and for an increase in PS II components in the stroma thylakoids of photoinhibited cells. In one of the studied species these changes were accompanied by modifications of certain thylakoid proteins.

Materials and methods

Cell material and HL treatment

Cell culture was as described in Schäfer et al. (1994): CO2-enriched air (two-tiered flasks and carbonate/bicarbonate-buffer for C. rubrum, Fernbach-flasks and constant air stream for M. polymorpha), Murashige & Skoog medium (M 5519, Sigma, München, FRG), 25 °C, PFD about 80 𝜇mol m⁻² s⁻¹, 16 h light, 8 h darkness, continuous shaking (rotatory shakers). HL treatment was given for 30 h by halogen lamps (PFD about 900 𝜇mol m⁻² s⁻¹ for C. rubrum and 1200 𝜇mol m⁻² s⁻¹ for M. polymorpha). This treatment represents light stress because light saturation in C. rubrum (Schäfer and Schmidt 1991) and M. polymorpha (Peter and Schäfer, unpublished) is reached at a PFD of approximately 300 𝜇mol m⁻² s⁻¹.

Thylakoid membrane isolation, electrophoresis and immunoblotting

Thylakoid membranes were isolated and subfractionated as described in Schäfer et al. (1994), using digitonin and Triton × 100 for solubilization (Leto et al. 1985). Polypeptides corresponding to equal amounts of chl from Thyl, GT and ST of the differently treated cells were separated on the same gel by fully denaturing SDS-PAGE in the presence of 6 M urea (Schäfer et al. 1993). Following electrophoretic separation, the proteins were transferred electrophoretically (Trans Blot Cell; Bio Rad, München, FRG) to polyvinylidenefluoride membrane (Immobilon P; Millipore, Eschborn, FRG) or nitrocellulose membrane (BA 85, 0.45 μm, Schleicher & Schüll, Dassel, FRG) as described in Schmid and Schäfer (1994). Immunodetection was done with secondary antibodies conjugated to alkaline phosphatase (Schmid and Schäfer 1994). The immunosignal intensity was quantified by laser densitometry (Ultra Scan XL laser densitometer and Gel Scan XL evaluation software; Pharmacia LKB, Uppsala, Sweden). It was checked by dilution series (range: 20 to 1000 ng chl per lane) that the applied chl amounts were in the linear range of the immunosignal/chlorophyll relationship. The slope of the corresponding regression line was used as a relative estimate of the respective protein content (Schmid and Schäfer 1994). If immunoblots were used for consecutive detections they were dried after immunodetection, scanned and reequilibrated in blocking solution before exposure to new antibodies. With the chosen antibody combinations disturbances by cross-reactivity did not occur (Fig. 3).

Other procedures

Chlorophyll determination was done according to Porra et al. (1989), using dimethylformamide as solvent. Protein was determined with the BCA™ protein assay (Pierce, Rockford, Illinois, USA; Smith et al. 1985). Antibodies against the PSI reaction center and LHC Ib of C. rubrum were raised in rabbits according to standard procedures (Dunbar and Schwoebel 1990). The sources of other antibodies are given in the acknowledgements.

Results

Thylakoid membrane subfractionation

Table 1 summarizes the contribution of several proteins to the total protein in whole thylakoids (Thyl), grana thylakoids (GT) and stroma thylakoids (ST). Compared to Thyl, the contribution of PS II components was considerably increased in GT and reduced in ST. PS I components were increased in ST and the α+β-subunits of the coupling factor (CF1, α, β) were only found in this fraction (compare also Fig. 3).