Plant Physiol. (1992) 100, 1547–1553
0032-0889/92/100/1547/07/$01.00/0

Received for publication April 15, 1992
Accepted July 6, 1992

Light Dependence of Catalase Synthesis and Degradation in Leaves and the Influence of Interfering Stress Conditions[1]

Birgit Hertwig, Peter Streb, and Jürgen Feierabend*

Botanisches Institut, J. W. Goethe-Universität, P.O. Box 11 19 32, D-6000 Frankfurt am Main, Federal Republic of Germany

ABSTRACT

The enzyme catalase (EC 1.11.1.6) is light sensitive and subject to a rapid turnover in light, similar to the D1 reaction center protein of photosystem II. After 3 h of preadaptation to darkness or to different light intensities (90 and 520 μmol m^{-2} s^{-1} photosynthetic photon flux density), sections of rye leaves (*Secale cereale* L.) were labeled for 4 h with L-[^{35}S]methionine. From leaf extracts, catalase was immunoprecipitated with an antiserum prepared against the purified enzyme from rye leaves. Both incorporation into catalase and degradation of the enzyme polypeptide during a subsequent 16-h chase period increased with light intensity. At a photon flux density of 520 μmol m^{-2} s^{-1}, the apparent half-time of catalase in rye leaves was 3 to 4 h, whereas that of the D1 protein was much shorter, about 1.5 h. Exposure to stress conditions, such as 0.6 M NaCl or a heat-shock temperature of 40°C, greatly suppressed both total protein synthesis and incorporation of the label into catalase and into the D1 protein. Immunoblotting assays indicated that in light, but not in darkness, steady-state levels of catalase and of the D1 protein strongly declined during treatments with salt, heat shock, or translation inhibitors that block repair synthesis. Because of the common property of rapid photodegradation and the resulting dependence on continuous repair, declines in catalase as well as of the D1 protein represent specific and sensitive indicators for stress conditions that suppress the translational activities of leaves.

The enzyme catalase from both animal and plant sources is light sensitive (2, 4, 7, 23). Photoinactivation of catalase activity by visible light was demonstrated to occur also in intact cells or tissues, such as hepatocytes (5), cyanobacteria (26, 27), and leaves (6–8). The heme group of the enzyme (4) and, in green leaves, Chls as well (7) act as photoreceptors mediating catalase inactivation. From previous observations of catalase activity in leaves exposed to varying light intensities and to conditions suppressing protein synthesis (7, 29), we deduced that the steady-state levels of catalase maintained in mature leaves reflect an equilibrium between continuous photoinactivation with subsequent degradation and a concomitant new synthesis. Thus, for catalase a rapid turnover in light was postulated, similar to that described for the reaction center protein D1 of PSII (21, 22). To substantiate these conclusions at the molecular level, in our present work we investigated the dynamics of the catalase polypeptide with labeling and chase experiments and immunochemical techniques; for comparison, we investigated the dynamics of the D1 protein in rye leaves exposed to different photon flux densities.

The maintenance of proteins depending on rapid turnover will generally be sensitive to all environmental conditions that either overload or hamper the capacity of repair synthesis, as previously described for low temperature (29). Also, other stress conditions known to affect protein synthesis, such as salt (13, 16) or heat shock (28), can be expected to induce specific declines in catalase and possibly in the D1 protein even in only moderate light, simply by preventing repair. Therefore, their influence on catalase and on D1 was investigated.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Experiments were performed with leaf sections of 6-d-old rye seedlings (*Secale cereale* L. cv Halo). Seeds were surface sterilized by a 10-min vacuum infiltration and about 30-min soaking in a freshly prepared, filtered solution of 3% (w/v) calcium hypochlorite chloride, thoroughly washed with demineralized H_2O, and grown at 22°C in glass-covered plastic boxes on filter paper (Schleicher & Schull; No. 598) moistened with H_2O. After 1 d of germination in darkness, continuous illumination with white light was provided by fluorescent tubes (Osram L 36 W/36 Natura and Philips TL 40 W/47 de Luxe in alternating sequence), giving an incident PPFD of approximately 90 μmol of photons m^{-2} s^{-1}. For catalase purification, rye seedlings were, alternatively, grown for 6 d in vermiculite moistened with a modified Knop's nutrient solution, as previously described (7).

Experimental Treatments

Segments, 5 cm long, were cut from the middle of the primary leaves of 6-d-old rye seedlings, and each was dissected into halves. For each treatment, segments obtained from 10 leaves were placed in Petri dishes, 5 cm diameter, with 7.5 mL each of either H_2O, 0.6 M NaCl, 35.5 μM cycloheximide, or 6.3 mM chloramphenicol, and kept for 24 h, or as indicated, at 22°C in either light of 520 μmol of photons m^{-2} s^{-1} PPFD or in darkness. For heat-shock treatments, leaf sections kept on H_2O were exposed for 4 h to

[1] Financial support by the Bundesministerium für Forschung und Technologie, Bonn, FRG, and by the Fonds der Chemischen Industrie, Frankfurt, FRG, is greatly appreciated.

40°C and 520 μmol m^{-2} s^{-1} and subsequently for 20 h to 520 μmol m^{-2} s^{-1} light at 22°C.

Fluorescence Measurements

Fluorescence induction was measured at 25°C in 10-min dark-adapted leaf sections with a Hansatech Ltd. LD2 cuvette equipped with an LS1 photodiode light source, fluorescence detector, and TR1 transient recorder. Measurements were performed with three 5-cm sections cut from the middle of the primary leaves of 6-d-old rye seedlings.

Preparation of Cell-Free Extracts

The leaf tissue was homogenized under ice-cold conditions with mortar and pestle in 50 mM potassium phosphate buffer (1 mL per 1 g of tissue), pH 7.5, and centrifuged for 20 min at 48,000*g* and 4°C. The supernatants were used for immunoblotting with anti-catalase serum. Sediments were washed and, after another centrifugation, resuspended in 3.5 mL of 50 mM potassium phosphate buffer and used for immunoblotting with anti-D1 protein serum. For electrophoresis, the equivalent of either 10 μL of soluble supernatant or 30 μL of sediment suspension were applied to each lane.

Radioactive Labeling and Chase Experiments

For each treatment, 2-cm middle sections from 10 leaves of 6-d-old rye seedlings grown at 90 μmol m^{-2} s^{-1} PPFD were floated in Petri dishes, 3.5 cm diameter, on 0.6 mL of 5 mM sodium phosphate, pH 7.4, containing 0.01% (v/v) Tween 20 and preincubated for 3 h at 22°C either in darkness or in light at 90 μmol m^{-2} s^{-1} or 520 μmol m^{-2} s^{-1}. For radioactive labeling, leaf segments were then incubated for 4 h 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}S]-methionine (30 TBq·mmol^{-1}), starting with 2 min of vacuum infiltration. At the end of the labeling period, the leaf segments were 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. The suspension was centrifuged for 5 min at 9000*g* and 4°C. The supernatant was used for immunoprecipitation with antiserum against rye leaf catalase as previously described (1). For analysis of the D1 protein, the sediment was washed several times and, finally, suspended in 0.5 mL of 50 mM Tris-HCl, pH 7.4, and 1 mM EDTA and used for electrophoretic separations of total cell membranes in the presence of SDS and for subsequent fluorography.

For chase experiments after 3 h of preincubation and 4 h of labeling in 520 μmol m^{-2} s^{-1} PPFD of light, leaf segments were washed with distilled water and subsequently with excess unlabeled L-methionine (20 mM sodium phosphate [pH 7.4], 0.01% [v/v] Tween 20, 1 mM L-methionine) and incubated in the latter medium either in darkness or at a PPFD of 90 or 520 μmol m^{-2} s^{-1}. During the first 3 h of chase, the methionine-containing medium was renewed every 30 min. After 1, 3, and 16 h of chase, leaf samples were extracted as described above. The immunoprecipitate applied per lane of the electrophoresis gels was derived from one 2-cm leaf segment.

PAGE

Electrophoretic separations were performed in the presence of 0.4% (w/v) SDS on polyacrylamide slab gels (11 × 13 × 0.15 cm^3) consisting of a 10 to 15% (w/v) polyacrylamide gradient resolving gel and a 5% (w/v) stacking gel in the buffer system of Laemmli (20).

Electrophoresis under nondenaturing conditions was performed at 4°C with a 7% (w/v) resolving gel and a 2.5 (w/v) stacking gel, as previously described (7).

Preparation of Antiserum and Immunoblotting

Methods for the preparation of a rabbit antiserum against purified catalase from rye leaves, as described below, and immunoblotting after SDS-PAGE were as previously described (1, 30). The antiserum against the D1 protein of PSII was described by Johanningmeier (15).

Determination of Radioactivity

For the estimation of total incorporation into protein, extracts from labeled leaf segments were precipitated with 10% (w/v, final concentration) TCA. Precipitates formed at 4°C overnight were centrifuged for 15 min at 6000*g* and washed with 10% TCA and 1 mM L-methionine and subsequently with 5% TCA and 1 mM methionine. Sediments were washed twice with ethyl ether:ethanol (1:1, v/v) and dissolved in 200 μL of tissue solubilizer:H_2O (8:1, v/v; Zinsser, Frankfurt, FRG) for 20 min at 50°C under slow rotation. After the samples were cooled, they were neutralized by the addition of 200 μL of acetic acid and counted with Aquasol-2 (New England Nuclear) scintillation fluid.

Polyacrylamide gels were prepared for fluorography according to the procedure of Bonner and Laskey (3) and exposed for 1 to 5 d to Kodak X-Omat S film at −80°C. Labeled bands were excised from dried gels after fluorography, extracted for 2 h with tissue solubilizer as described above, and, after cooling and neutralization, counted with Aquasol-2 scintillation fluid.

Purification of Catalase

For each preparation, 1 kg of rye leaves was cut into pieces and homogenized in a Waring Blendor (several intermittent treatments of 30 s at high speed) with 2 L of ice-cold 0.1 M K_2HPO_4 containing 0.5 mM PMSF. The homogenate was filtered through four layers of muslin and one layer of Miracloth. Protein precipitating between 0 and 20% and between 20 and 50% $(NH_4)_2SO_4$ saturation was collected, each by a 20-min centrifugation at 14,500*g* and 4°C. The sediment obtained between 20 and 50% saturation was dissolved in 100 mL of 50 mM K_2HPO_4, 0.25 mM PMSF and dialyzed for 2 h at 4°C against 50 mM K_2HPO_4. Undissolved protein was removed by 15 min of centrifugation at 48,000*g*. The supernatant was adjusted to 500 mL with 50 mM K_2HPO_4, kept on ice, and 400 mL of a 3:1 (v/v) mixture of ethanol and chloroform were slowly added with stirring. The

mixture was allowed to settle overnight. The resulting supernatant was decanted, and residual precipitate was removed by 10 min of centrifugation at 4000*g*. For each 1 L of supernatant, 1 g of Celite (type 545, 20- to 45-μm particle size; Serva, Heidelberg, FRG) was added. Then, two volumes of cold 95% (v/v) ethanol were slowly added with stirring. Stirring was continued for 1 h. The Celite was allowed to settle and, after the supernatant was decanted, washed two times with 60% ethanol in a Buchner funnel.

After the 60% ethanol was removed by filtration, protein was eluted from the Celite by suspension in about 10 mL of 0.1 M K_2HPO_4. After standing for 10 min at room temperature, the Celite was sedimented by 10 min of centrifugation at 480*g*. Extraction of the Celite was repeated, and the combined extracts were dialyzed against 5 mM Tris-HCl, pH 8.0. The solution was cleared by centrifugation (10 min at 27,000*g*) and applied to a DEAE-Sepharose CL-6B column (1.5 × 8 cm) equilibrated with 10 mM Tris-HCl, pH 8.0. After the solution was washed with 50 mL each of 10 mM Tris-HCl, pH 8.0, and 10 mM Tris-HCl, pH 8.0, containing 20 mM KCl, protein was eluted with 10 mM Tris-HCl, pH 8.0, and 0.25 M KCl. From the peak fractions with highest catalase activity, protein was precipitated by 60% $(NH_4)_2SO_4$ saturation. The precipitate was sedimented (10 min at 48,000*g*), dissolved in approximately 2 mL of 50 mM potassium phosphate, pH 7.5, applied to a Sephacryl S300 HR (Pharmacia) column (2.5 × 90 cm) equilibrated with 50 mM potassium phosphate buffer, pH 7.5, and 50 mM KCl, and eluted with 50 mM potassium phosphate, pH 7.5, containing 0.15 M KCl. Fractions with catalase activity were combined, adjusted to pH 7.4 and 1 M $(NH_4)_2SO_4$, and applied to a Phenyl-Sepharose CL-4B (Pharmacia) column (1.5 × 8 cm) equilibrated with 20 mM sodium phosphate, pH 6.8. After the column was washed with equilibration buffer and 50 mL of 25% (v/v) ethylene glycol in 10 mM sodium phosphate, pH 7.4, it was eluted with 100 mL of 45% (v/v) ethylene glycol in 10 mM sodium phosphate, pH 7.4. Protein from the combined fractions with the highest catalase activity was concentrated by $(NH_4)_2SO_4$ precipitation at 65% saturation or by centrifugation in Centriprep 30 (Amicon) tubes.

For a final purification, catalase preparations were separated by electrophoresis under nondenaturing conditions (see above). After electrophoresis was complete, strips (about 0.5 cm long) were cut from the gels, and catalase was localized by an activity stain (29). The zone containing the enzyme was excised from the unstained main gel, crushed into small pieces, loaded onto a 4-cm 4.5% polyacrylamide spacer gel in 8- × 150-mm glass tubes with a broad top part (20 mm), and eluted electrophoretically into a dialysis bag tightened at the bottom of the tube with a rubber ring (6–8 h, 5 mA per tube).

Between two and four independent experiments were performed. In photographs, results of representative experiments are shown; otherwise, SE values are indicated.

RESULTS

Light-Dependent Synthesis and Degradation

An antiserum was produced against the isolated catalase from green rye leaves (see "Materials and Methods"). Only a single native catalase form had been observed in light-grown rye leaves under nondenaturing conditions (29). Immunoblotting after separation of soluble leaf extracts in the presence of SDS detected the M_r 57,000 catalase subunit polypeptide (Fig. 1). Faint additional bands appeared at approximately M_r 180,000 and 240,000, which suggested that they represented residual undissociated trimers and tetramers of the tetrameric catalase holoprotein. Immunoblotting confirmed previous results obtained for catalase activity measurements (7). The enzyme level did not substantially change when rye leaf sections, grown at 90 μmol m^{-2} s^{-1} PPFD, were exposed for 24 h to either a higher PPFD of 520 μmol m^{-2} s^{-1} or darkness. In the presence of the translation inhibitor cycloheximide, however, the amount of catalase protein declined, but only in light (Fig. 1), suggesting that catalase suffered from photodegradation that was compensated for by new synthesis. This was more conclusively demonstrated by labeling experiments.

Following a 3-h preadaptation period, leaf segments under different light conditions were allowed to incorporate L-[^{35}S]methionine. If a lag phase is needed for a change of the rate of catalase synthesis, it should be completed within 3 h, according to previous observations of the recovery of catalase after photoinactivation (29). Incorporation into the catalase subunit protein was low in darkness but greatly increased with light intensity (Fig. 2). At a PPFD of 520 μmol m^{-2} s^{-1}, incorporation was more than 6 times higher than in darkness. Incorporation into total soluble protein precipitated by TCA was also about 3 times higher at the high light intensity, as compared with darkness (Fig. 3). When labeling at 520 μmol m^{-2} s^{-1} was followed by a chase in the absence of radioactive methionine, incorporation into catalase still increased during the first hour, presumably because the radioactive substrate

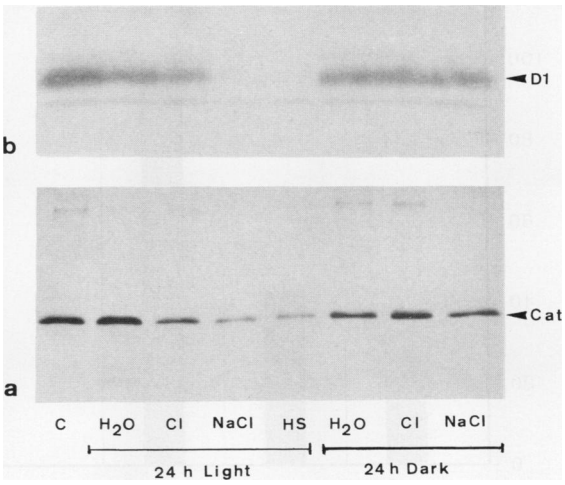

Figure 1. Immunoblotting analysis of catalase (a) and of the D1 protein of PSII (b) in rye leaves. C, Control: leaf sections before treatments. For experimental treatments, leaf sections were floated for 24 h on H_2O, 35.5 μM cycloheximide (Cl), or 0.6 M NaCl in either light of 520 μmol m^{-2} s^{-1} PPFD or darkness. For the heat-shock treatment (HS), leaf segments were kept at 40°C for 4 h and at 25°C for 20 h and 520 μmol m^{-2} s^{-1} PPFD. Arrows indicate locations of the catalase subunit (cat) or of the D1-protein of PSII.

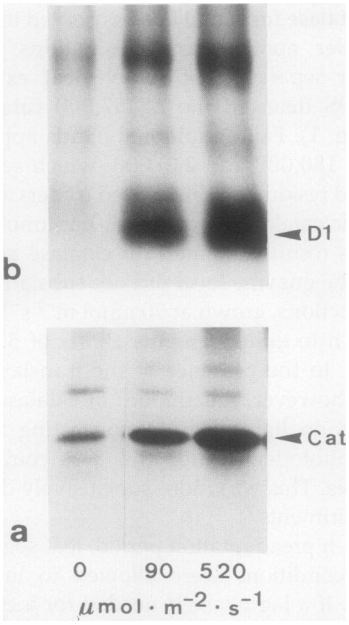

Figure 2. Fluorogram after SDS-PAGE of protein precipitated with anti-catalase serum (a) and of the total cell membrane factions (b) after labeling leaf sections of rye at different PPFDs with L-[^{35}S]-methionine. Arrows indicate locations of the catalase subunit (cat) or of the D1 protein of PSII.

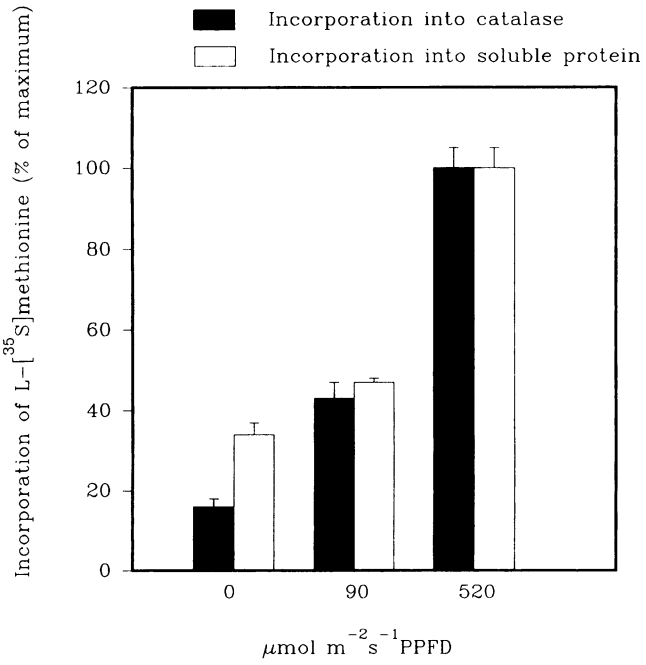

Figure 3. Comparison of the incorporation of L-[^{35}S]methionine into total soluble protein and into catalase polypeptides in middle sections from rye leaves during a 4-h labeling period at different light intensities. Catalase had been immunoprecipitated and separated by SDS-PAGE.

pool was not immediately depleted, but thereafter declined strikingly (Fig. 4). A half-time of the catalase protein between 3 and 4 h can be estimated for the light intensity of 520 μmol m^{-2} s^{-1}. When leaf sections labeled at the higher light intensity were subjected to a 16-h chase period at only 90 μmol m^{-2} s^{-1} or in darkness, turnover of the catalase was much slower (Fig. 5). In darkness, only minor degradation was observed.

Synthesis and turnover of the D1 protein of PSII was, for comparison, studied in the same plant material that was used for the analysis of catalase. In separations of crude total membrane fractions, the highest radioactivity was found in the lower of the two adjacent bands in the range of M_r 30,000, which was identified as the site of the D1 protein by comparison with immunoblotting (Figs. 1b and 2b). This corresponds to previous observations for other plant species in which, even after labeling periods between 2 and 4 h, the M_r 32,000 D1 protein still represented the most strongly labeled polypeptide of whole cell membranes (14). The light dependence of D1 synthesis was much more pronounced than that of catalase, with virtually no incorporation in darkness (Fig. 2b). Correspondingly, the turnover during a chase experiment was also much faster than for catalase, and after a 3-h chase labeled protein was already totally degraded at 520 μmol m^{-2} s^{-1} (Fig. 4b). The resulting steady-state levels of the D1 protein were not significantly different whether leaf sections were kept in light of 520 μmol m^{-2} s^{-1} or in darkness (Fig. 1b).

Synthesis and degradation of a few other membrane proteins visualized on fluorographs of SDS gels (Figs. 2b and 4b) also appeared to be light modulated, although to a lesser extent, and the course of decline of radioactivity from total soluble leaf protein during the chase period was similar to that of catalase (Fig. 6). This contrasts with the behavior of

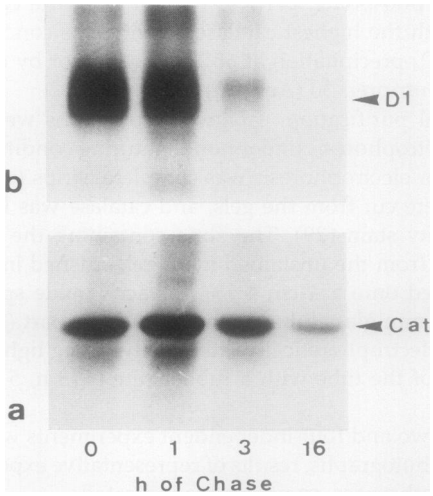

Figure 4. Fluorogram after SDS-PAGE showing the change of radioactivity incorporated by the catalase polypeptide (a) and the D1 protein of PSII (b) during a 16-h chase period in light (520 μmol m^{-2} s^{-1} PPFD) following 4 h of labeling of segments from rye leaves at 520 μmol m^{-2} s^{-1} with L-[^{35}S]methionine. Arrows indicate locations of the catalase subunit (cat) or of the D1 protein.

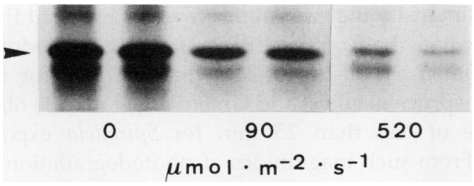

Figure 5. Comparison of radioactivity in the subunit polypeptide of catalase after 16 h of chase at different light intensities following 4 h of in vivo labeling of middle sections from rye leaves with L-[^{35}S]methionine at a PPFD of 520 μmol m^{-2} s^{-1}. Fluorogram after SDS-PAGE of the protein that had been precipitated by anti-catalase serum. Arrow indicates location of catalase subunit polypeptide.

major leaf proteins, such as Rubisco, which is known to be quite stable in light in rye leaves (30). Also, labeling of the major thylakoid protein, the light-harvesting Chl *a/b* protein, was still extremely weak or below levels of detection under our experimental conditions. Accordingly, the results suggest that several proteins with light-stimulated synthesis and turnover were still prevalent among the incorporation products obtained within 4 h in rye leaves.

The sampling times for the chase experiments (Fig. 4) were adjusted to the course of catalase turnover and did not allow a more accurate estimation of the half-life of the D1 protein. The results do, however, indicate that the half-life was markedly shorter than that of catalase and presumably less than 2 h at 520 μmol m^{-2} s^{-1}. The D1 protein is, in contrast to catalase, synthesized on 70S ribosomes within the chloroplast. When a translation inhibitor for 70S ribosomes, chloramphenicol, is applied to leaves, new synthesis of the D1 protein is prevented and the reaction center protein of PSII is depleted in light but not in darkness. Depletion of the D1 protein will result in a corresponding photoinhibition of PSII, which can be monitored by the decline of F_v[2] (17, 18). In rye leaves treated with chloramphenicol, F_v declined, as expected, only in light, and the rate of decrease depended on light intensity (Fig. 7a). At 520 μmol m^{-2} s^{-1}, the half-life for the decline of F_v was about 90 min, which is in good agreement with the estimate for the D1 protein half-life derived from the chase experiments. Immunoblotting confirmed that the decrease of F_v properly reflected the degradation of the D1 protein (Fig. 7b).

Effect of Stress Conditions

As suggested by previous investigations (29), a decline of catalase is generally to be expected in leaves when they are subjected, even in only moderate light, to conditions interfering with the repair of the enzyme. Accordingly, the steady-state level of both activity (not shown) and protein of catalase strongly declined during exposure to a 40°C heat-shock temperature or to 0.6 M NaCl in light of 520 μmol m^{-2} s^{-1} (Fig. 1a). Total incorporation of L-[^{35}S]methionine into soluble protein was decreased by 95% in the presence of 0.6 M NaCl and by about 70% at 40°C, relative to untreated controls, and incorporation into catalase was strongly suppressed under these conditions (Fig. 8). Similarly, the D1 protein almost disappeared in the presence of salt or under heat shock in light (Fig. 1b), and its synthesis was virtually blocked in the presence of 0.6 M NaCl (Fig. 8). At 40°C, incorporation into the D1 protein was also suppressed but to a lesser extent. When the stress conditions were applied during the 3 h of preadaptation but not during the subsequent 4-h labeling period, the strong inhibition by salt persisted, but subsequent to a heat-shock exposure, protein synthesis was rapidly resumed (not shown). Following 3 h of heat shock, incorporation of radioactivity into catalase or into the D1 protein was only slightly lower than in untreated controls, and incorporation into total soluble protein was diminished by only about 15%. Therefore, it appears to be unlikely that the low level of D1 protein observed after 4 h at 40°C, and subsequently after 20 h at 22°C (Fig. 1b), resulted exclusively from an inhibition of new synthesis. Presumably, the rate of degradation must have been, in addition, increased by the heat treatment.

DISCUSSION

From the decline of catalase activity after application of translation inhibitors in light, we had concluded that the light-sensitive enzyme catalase is also not sufficiently protected from photoinactivation in intact leaves but is dependent on repair by new synthesis (7, 29). With the aid of an antiserum and labeling experiments, it has now been sub-

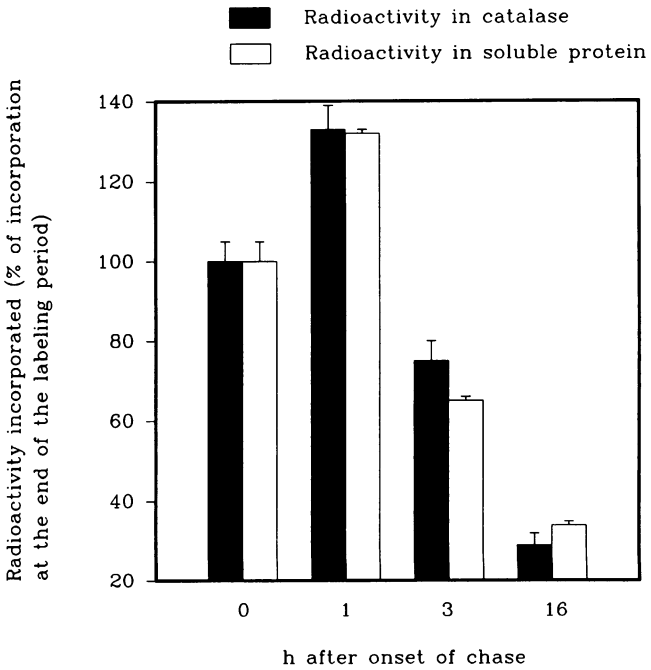

Figure 6. Change in the radioactivity that had been incorporated into total soluble protein and into catalase within 4 h of application of L-[^{35}S]methionine to middle sections of rye leaves at a PPFD of 520 μmol m^{-2} s^{-1} during subsequent 16 h of chase in the absence of radioactivity at the same light intensity. Catalase was immunoprecipitated and separated by SDS-PAGE.

[2] Abbreviation: F_v, variable fluorescence: (maximum fluorescence − initial fluorescence)/maximum fluorescence.

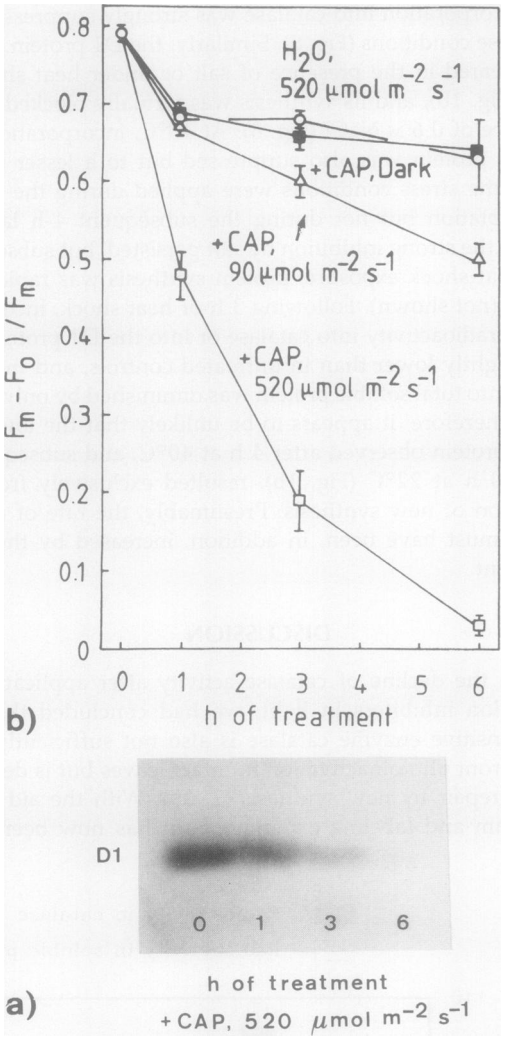

Figure 7. a, Disappearance of the D1 protein in middle sections of rye leaves during exposure to light of 520 μmol m^{-2} s^{-1} PPFD and 6.3 mM chloramphenicol (CAP). Immunoblotting with anti-D1 serum after SDS-PAGE. b, Changes of F_v in leaf segments of 6-d-old rye seedlings kept on: O, H_2O at 520 μmol m^{-2} s^{-1} PPFD; ■, 6.3 mM chloramphenicol (CAP) in darkness; △, 6.3 mM chloramphenicol at 90 μmol m^{-2} s^{-1} PPFD; □, 6.3 mM chloramphenicol at 520 μmol m^{-2} s^{-1} PPFD. The exposures were preceded by 1 h of incubation on H_2O or chloramphenicol solutions in darkness. F_o, Initial fluorescence; F_m, maximum fluorescence.

stantiated that the catalase polypeptide in mature rye leaves was indeed increasingly degraded with increasing irradiance, although the steady-state level of the enzyme remained fairly constant. Degraded catalase was replaced by an attuned new synthesis that was also increasing with light intensity but was low in darkness. The behavior of catalase thus resembles that of the D1 reaction center protein of PSII, which has long been known for its light-dependent rapid turnover (21, 22). The direct comparison showed that the turnover of the D1 protein was more rapid than that of catalase in the same tissue. At a PPFD of 520 μmol m^{-2} s^{-1}, the half-life of catalase in rye leaves was 3 to 4 h, but that of D1 was only 90 min. At a light intensity that was about twice as high but that was still only about half of the PPFD of sunlight, Godde et al. (10) estimated a half-life of less than 30 min for the D1 protein in spruce needles, and Greenberg et al. (11) observed a half-life of less than 25 min for *Spirodela* exposed to sunlight. From such magnitudes of photodegradation, it can be expected that under field conditions the turnover of catalase must also be quite considerable during the daily sunlight periods. After application of cycloheximide to prevent new synthesis, marked photoinactivation of catalase has also been observed in field-grown plants (7).

In mature *Spirodela*, the light regulation of D1 protein synthesis appears to be mediated by posttranscriptional and presumably translational controls (9). Control of catalase repair synthesis in rye leaves has still to be elucidated. Skadsen and Scandalios (25) presented evidence that the light-induced synthesis of the major catalase isozyme of green maize leaves, cat 2, is regulated by a translational control. This is further supported by the observation that the level of the transcript for cat 2 does not vary markedly in maize leaves during diurnal light-dark changes (24). Translational controls would allow very rapid responses of both catalase and D1 protein synthesis to fluctuations of light intensity and protein turnover.

Levels of proteins, depending on an equilibrium of photodegradation and repair synthesis, will be particularly sensitive to conditions to which the plant is not adapted and which thus act as stress. Stress conditions stimulating photodegradation, such as excessive light and oxidative stress, may overload the capacity for repair synthesis, or, alternatively, stress conditions may inhibit repair synthesis. In both cases, apparent declines of the steady-state levels of catalase and of the D1 protein are to be expected in light, as already observed at low temperature (29). Various stress conditions may affect protein synthesis. As examples, heat-shock temperatures suppress translation of most normal proteins, ex-

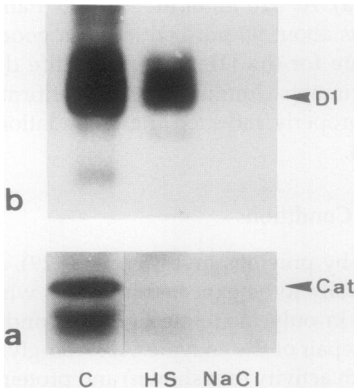

Figure 8. Comparison of the incorporation of L-[^{35}S]methionine into catalase (a, Cat) or into the D1 protein of PSII (b) in sections of rye leaves during 4 h of labeling in light of 520 μmol m^{-2} s^{-1} PPFD under stress conditions. C, Untreated control; HS, during the 4-h labeling period, leaf sections were exposed to 40°C; NaCl, during the 4-h labeling period, leaf sections were exposed to 0.6 M NaCl. Fluorogram after SDS-PAGE separations of (a) immunoprecipitates obtained with anti-catalase serum or (b) total cell membranes.

cept for a set of specific heat-shock proteins (28), and salt is also known to inhibit protein synthesis (13, 16). Consequently, the synthesis of catalase and of the D1 protein was inhibited at either 40°C or in the presence of NaCl, resulting in a striking decline of the steady-state levels of these proteins in light. The behavior of catalase and photoinhibition of PSII, because of their molecular properties (19), can thus serve as specific sensitive symptoms, indicating incipient photodamage under stress conditions to which leaves are not adapted. For photoinhibition of PSII, alternative mechanisms appear to exist that serve as transitory regulatory and protective mechanisms for the thermal deactivation of excess excitation energy and that may be independent of D1 protein degradation (17, 18). However, D1-depleted inactive PSII reaction centers also appear still to perform thermal energy dissipation and can serve as means of protection (12, 18). Whether any biological significance can be assigned to the light sensitivity of catalase remains elusive.

ACKNOWLEDGMENTS

We are grateful to Dr. Johanningmeier, Bochum, FRG, for providing the antiserum against the D1 protein. We thank Dr. M. Schmidt for the preparation of antiserum against catalase. Technical assistance of Beate Dahl is greatly appreciated.

LITERATURE CITED

1. **Biekmann S, Feierabend J** (1985) Synthesis and degradation of unassembled polypeptides of the coupling factor of photophosphorylation CF_1 in 70S ribosome-deficient rye leaves. Eur J Biochem **152:** 529–535
2. **Björn LO** (1969) Photoinactivation of catalases from mammal liver, plant leaves and bacteria. Comparison of inactivation cross sections and quantum yields at 406 nm. Photochem Photobiol **10:** 125–129
3. **Bonner WM, Laskey RA** (1974) A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur J Biochem **46:** 83–88
4. **Cheng L, Kellog EW, Packer L** (1981) Photoinactivation of catalase. Photochem Photobiol **34:** 125–129
5. **Cheng LYL, Packer L** (1979) Photodamage to hepatocytes by visible light. FEBS Lett **97:** 124–128
6. **Eyster HC** (1950) Catalase activity in chloroplast pigment deficient types of corn. Plant Physiol **25:** 630–638
7. **Feierabend J, Engel S** (1986) Photoinactivation of catalase in vitro and in leaves. Arch Biochem Biophys **251:** 567–576
8. **Feierabend J, Kemmerich P** (1983) Mode of interference of chlorosis-inducing herbicides with peroxisomal enzyme activities. Physiol Plant **57:** 346–351
9. **Fromm H, Devic M, Fluhr R, Edelman M** (1985) Control of psbA gene expression: in mature *Spirodela* chloroplasts light regulation of 32-kd protein synthesis is independent of transcript level. EMBO J **4:** 291–295
10. **Godde D, Schmitz H, Weidner M** (1991) Turnover of the D-1 reaction center polypeptide from photosystem II in intact spruce needles and spinach leaves. Z Naturforsch **46c:** 245–251
11. **Greenberg BM, Gaba V, Canaani O, Malkin S, Mattoo AK, Edelman M** (1989) Separate photosensitizers mediate degradation of the 32-kDa photosystem II reaction center protein in the visible and UV spectral regions. Proc Natl Acad Sci USA **86:** 6617–6620
12. **Greer DH, Ottander C, Öquist G** (1991) Photoinhibition and recovery of photosynthesis in intact barley leaves at 5 and 20°C. Physiol Plant **81:** 203–210
13. **Hall JL, Flowers TJ** (1973) The effect of salt on protein synthesis in the halophyte *Suaeda maritima*. Planta **110:** 361–368
14. **Hoffman-Falk H, Mattoo AK, Marder JB, Edelman M, Ellis RJ** (1982) General occurrence and structural similarity of the rapidly synthesized, 32,000-dalton protein of the chloroplast membrane. J Biol Chem **257:** 4583–4587
15. **Johanningmeier U** (1987) Expression of the psbA gene in *E. coli*. Z Naturforsch **42c:** 755–757
16. **Kahane I, Poljakoff-Mayber A** (1968) Effect of substrate salinity on the ability for protein synthesis in pea roots. Plant Physiol **43:** 1115–1119
17. **Krause GH** (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol Plant **74:** 566–574
18. **Krause GH, Weis E** (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol **42:** 313–349
19. **Kyle DJ, Ohad I, Arntzen CJ** (1984) Membrane protein damage and repair: selective loss of a quinone-protein function in chloroplast membranes. Proc Natl Acad Sci USA **81:** 4070–4074
20. **Laemmli UK** (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
21. **Mattoo AK, Hoffman-Falk H, Marder JB, Edelman M** (1984) Regulation of protein metabolism: coupling of photosynthetic electron transport to in vivo degradation of the rapidly metabolized 32-kilodalton protein of the chloroplast membranes. Proc Natl Acad Sci USA **81:** 1380–1384
22. **Mattoo AK, Marder JB, Edelman M** (1989) Dynamics of the photosystem II reaction center. Cell **56:** 241–246
23. **Mitchell RL, Anderson IC** (1965) Catalase photoinactivation. Science **150:** 74
24. **Redinbaugh MG, Sabre M, Scandalios JG** (1990) Expression of the maize Cat3 catalase gene is under the influence of a circadian rhythm. Proc Natl Acad Sci USA **87:** 6853–6857
25. **Skadsen RW, Scandalios JG** (1987) Translational control of photo-induced expression of the Cat2 catalase gene during leaf development in maize. Proc Natl Acad Sci USA **84:** 2785–2789
26. **Tel-Or E, Huflejt ME, Packer L** (1986) Hydroperoxide metabolism in cyanobacteria. Arch Biochem Biophys **246:** 396–402
27. **Tytler EM, Wong T, Codd GA** (1984) Photoinactivation in vivo of superoxide dismutase and catalase in the cyanobacterium *Microcystis aeruginosa*. FEMS Microbiol Lett **23:** 239–242
28. **Vierling E** (1991) The roles of heat shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol **42:** 579–620
29. **Volk S, Feierabend J** (1989) Photoinactivation of catalase at low temperature and its relevance to photosynthetic and peroxide metabolism in leaves. Plant Cell Environ **12:** 701–712
30. **Winter U, Feierabend J** (1990) Multiple coordinate controls contribute to a balanced expression of ribulose-1,5-bisphosphate carboxylase/oxygenase subunits in rye leaves. Eur J Biochem **187:** 445–453