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Photoinactivation of Catalase Occurs under Both High- and Low-Temperature Stress Conditions and Accompanies Photoinhibition of Photosystem II[1]

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ABSTRACT

Severe photoinactivation of catalase (EC 1.11.1.6) and a decline of variable fluorescence (F_v), indicating photoinhibition of photosynthesis, were observed as rapid and specific symptoms in leaves exposed to a high heat-shock temperature of 40°C as well as in leaves exposed to low chilling temperatures in white light of only moderately high photosynthetic photon flux density of 520 μE m^{-2} s^{-1}. Other parameters, such as peroxidase (EC 1.11.1.7), glycolate oxidase (EC 1.1.3.1), glutathione reductase (EC 1.6.4.2), or the chlorophyll content, were hardly affected under these conditions. At a compatible temperature of 22°C, the applied light intensity did not induce severe photoinactivations. In darkness, exposures to high or low temperatures did not affect catalase levels. Also, decline of F_v in light was not related to temperature sensitivity in darkness. The effective low-temperature ranges inducing photoinactivation of catalase differed significantly for chilling-tolerant and chilling-sensitive plants. In leaves of rye (*Secale cereale* L.) and pea (*Pisum sativum* L.), photoinactivation occurred only below 15°C, whereas inactivation occurred at 15°C in cucumber (*Cucumis sativus* L.) and maize (*Zea mays* L.). The behavior of F_v was similar, but the difference between chilling-sensitive and chilling-tolerant plants was less striking. Whereas the catalase polypeptide, although photoinactivated, was not cleaved at 0 to 4°C, the D1 protein of photosystem II was greatly degraded during the low-temperature treatment of rye leaves in light. Rye leaves did not exhibit symptoms of any major general photodamage, even when they were totally depleted of catalase after photoinactivation at 0 to 4°C, and catalase recovered rapidly at normal temperature. In cucumber leaves, the decline of catalase after exposures to bright light at 0 to 4°C was accompanied by bleaching of chlorophyll, and the recovery observed at 25°C was slow and required several days. Similar to the D1 protein of photosystem II, catalase differs greatly from other proteins by its inactivation and high turnover in light. Inasmuch as catalase and D1 protein levels depend on continuous repair synthesis, preferential and rapid declines are generally to be expected in light whenever translation is suppressed by stress actions, such as heat or chilling, and recovery will reflect the repair capacity of the plants.

While green plants are dependent on light, they are threatened by its photooxidative actions. Although plant cells have developed a multiplicity of antioxidative mechanisms (3, 27), some components are obviously not fully protected from photodamage. Thus, the enzyme catalase is light sensitive in vitro and suffers from photoinactivation with subsequent degradation in intact leaves as well (7, 12, 31). To maintain a constant catalase level in light, its loss has to be continuously compensated for by an adequate concomitant resynthesis of the enzyme (12). When, however, light degradation exceeds the capacity for repair (25) or when resynthesis is impaired by inhibitors (7) or by stress conditions such as low temperature (31), an apparent loss of catalase is observed. The velocity of loss depends on light intensity.

Another rapid symptom of light stress is photoinhibition of photosynthesis, which similarly occurs either at excessively high irradiance or at only moderate light intensities when plants are simultaneously exposed to additional stress conditions. Photoinhibition was mostly related to a preferential inactivation of PSII (4, 16–18). Photoinhibition is accompanied by a decline of F_v[2], a parameter that is proportional to the quantum yield of PSII, because excitation energy is dissipated as heat by inhibited reaction centers (17). Whereas conceptions about the primary events in photoinhibition are still greatly diverging and the phenomenon may involve multiple mechanisms occurring under different experimental conditions (4, 16, 17), long-term photoinhibition of PSII has been related in various systems to a light-induced damage or modification and subsequent depletion of the plastoquinone Q_B-binding reaction center protein D1 of PSII under conditions in which photodegradation exceeds its repair (4, 11, 17, 18, 22, 26). Recovery from photoinhibition has been shown in various higher plants to require protein synthesis in the chloroplasts, which are the site of D1 protein synthesis (11, 14, 16, 18).

Because of their common property of rapid turnover, both catalase and the D1 protein can be expected to be particularly sensitive to all conditions affecting the efficiency of protein synthesis in light. Loss of catalase activity due to photoinactivation at low temperature has been described (31). However, on the basis of the above considerations, not only low temperature but various other stress conditions should be equally effective in inducing a specific decline of catalase and photoinhibition of PSII whenever the capacity for repair

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[2] Abbreviations: F_v, variable fluorescence: $(F_m - F_o)/F_m$; F_o, initial fluorescence; F_m, maximum fluorescence.

synthesis is suppressed. Therefore, in the present investigation, we have also applied a high heat-shock temperature that is known to immediately block translation, except for the synthesis of specific heat-shock proteins (30). Furthermore, we have extended the analysis of the low-temperature action by comparing the effective temperature ranges in chilling-tolerant and chilling-sensitive plants. Under all conditions, the behavior of catalase was compared to that of F_v as an indicator of PSII performance and to that of other enzymes related to peroxide metabolism.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Experiments were performed with leaves or leaf segments of young plants of rye (*Secale cereale* L. cv Halo, F. v. Lochow Petkus GmbH, Bergen, FRG), pea (*Pisum sativum* L. cv Rheinperle, local commercial source), cucumber (*Cucumis sativus* L. cv Corona, TS Seeds, Ambacht, Holland), and maize (*Zea mays* L. cv Inra Corn, Raiffeisen Frankfurt eG, FRG). Seeds of rye were surface sterilized by a 10-min vacuum infiltration and soaking for about 30 min in a freshly prepared, filtered solution of 3% (w/v) calcium hypochlorite-chloride, thoroughly washed with demineralized H_2O and grown for 6 d at 22°C in glass-covered plastic boxes on filter paper (Schleicher & Schull, No. 598) moistened with H_2O. Peas, after a 2-h presoaking in H_2O, were grown for 14 d at 27°C; after 5 d in the presence of H_2O, a modified Knop's nutrient solution (9) was applied. Cucumber seeds, after a 1-h presoaking in H_2O, were grown for 14 d at 27°C; after 6 d, a modified Knop's nutrient solution was applied. Maize seeds, after a 3-h presoaking in H_2O, were grown for 10 d at 27°C; after 5 d, a modified Knop's nutrient solution was applied.

Rye seedlings were kept in continuous light provided by fluorescent tubes (Osram L/36N/36 Natura and Philips TL 47W/47 in alternating sequence) giving an incident PPFD of 90 μmol of photons m^{-2} s^{-1}. Cucumber, maize, and pea plants were grown in 16 h of light provided by an Osram Power-Star HQI-T 2000 W/D lamp giving an incident PPFD of 200 μmol of photons m^{-2} s^{-1}.

Experimental Treatments

Leaves or leaf segments were exposed for 24 h, or as specified in the text, to different temperatures and to a high light intensity of approximately 520 μmol m^{-2} s^{-1} PPFD provided by an Osram Power-Star HQI-T 1000W/D lamp in combination with a KG1 heat filter (Schott & Genossen). For the high light exposures, rye seedlings, lateral leaflets of the third, fully expanded leaf of pea seedlings, cucumber segments 35 mm in diameter from the primary leaves, or 5-cm segments of maize from 1 cm below the tip of the second leaf were laid flat on a filter paper moistened with H_2O.

Preparation of Cell-Free Extracts

The leaf tissues were homogenized under ice-cold conditions with mortar and pestle with acid-washed sea sand in 50 mM potassium phosphate buffer, pH 7.5. For each extraction, 10 primary leaves of rye or two leaf segments of cucumber or maize were used, and the homogenates were adjusted to a final volume of 5 mL. For pea, extracts were adjusted to a final volume of 10 mL and prepared from three lateral leaflets. The homogenates were centrifuged for 5 min at 7000*g* and 4°C. The resulting supernatants were used for the enzyme assays.

For immunoblotting, soluble supernatants and total membrane fractions of total leaf extracts were prepared as previously described (12).

PAGE

Electrophoresis under denaturing conditions in the presence of 0.4% (w/v) SDS was performed on polyacrylamide slab gels (10 × 13 × 0.15 cm^3) consisting of a 5% (w/v) stacking gel and a 10 to 15% (w/v) resolving gel in the buffer system of Laemmli (19).

Preparation of Antiserum and Immunoblotting

Antisera and immunoblotting procedures were described previously (12).

Radioactive Labeling

Labeling of leaf segments with L-[^{35}S]methionine, subsequent extraction, membrane preparation, and immunoprecipitation with anti-catalase serum was performed as described in the preceding publication (12).

Fluorescence Measurements

Fluorescence induction was measured at 25°C in leaf sections dark adapted for 10 min with a Hansatech Ltd. LD2 cuvette, equipped with a LS1 photodiode light source, fluorescence detector, and a TR1 transient recorder. Cucumber, maize, and pea leaf parts, as prepared for the experimental treatments (see above), were used. For rye, measurements were performed with 5-cm sections cut from the middle of three primary leaves.

Analytical Methods

Total Chl was determined from 80% (v/v) acetone extracts according to the method of Arnon (1).

Enzyme activities were assayed spectrophotometrically at 25°C. The assays for catalase (EC 1.11.1.6), GSH reductase (EC 1.6.4.2), glycolate oxidase (EC 1.1.3.1), and guaiacol-dependent peroxidase (EC 1.11.1.7) were previously described (31).

Between two and four independent experiments were performed, and SE values are indicated in the figures.

RESULTS

Photoinactivation at Heat-Shock Temperature

For exposures to a heat-shock temperature of 40°C, 6-d-old rye seedlings were used when the primary leaves were almost mature. Under a PPFD of 520 μmol m^{-2} s^{-1}, a 4-h heat-shock treatment induced a marked decline in catalase activity in rye leaves, which persisted after return to a normal

temperature (25°C), and an even more striking decline of F_v, which recovered slightly during the next 24 h after return to 25°C (Fig. 1). It was previously shown that the heat-shock exposure in light was accompanied by a substantial decline in the rate of synthesis, as well as in the amounts of both the catalase protein and the D1 protein of PSII (12). Incorporation into the D1 protein was suppressed less than incorporation into catalase (Fig. 2). At a temperature of 25°C, the PPFD applied did not induce major changes. In darkness, the heat treatment did not result in a loss of catalase, and the decline in F_v was much smaller than under high light intensity (Fig. 1). In addition, in darkness, not only a decrease in F_m but also a significant increase in F_o contributed to the decline in $(F_m - F_o)/F_m$, whereas at 40°C in light, the decline in this ratio resulted solely from a decrease of F_m. Similar to other heat-shock events, a preadaptation could be induced by a short 15-min treatment at 40°C in darkness, which markedly lowered the extent of the photoinactivation events observed during a later prolonged heat-shock and light treatment (Fig. 1). Labeling experiments with L-[^{35}S]methionine showed that after a short pretreatment at 40°C slightly higher rates of incorporation into both catalase and the D1 protein were maintained during a later, extended heat-shock period, as compared with an immediate exposure to 40°C (Fig. 2).

Photoinactivation of catalase and photoinhibition of photosynthesis were very specific effects of the 40°C treatments. During the same experimental time, the Chl contents (not shown) of the leaves remained unchanged for the plant material described in Figure 1, and another peroxisomal enzyme, glycolate oxidase, was not affected by any photoinactivation (Fig. 1). In later experiments with plants grown from another seed crop, gradual bleaching of Chl was ob-

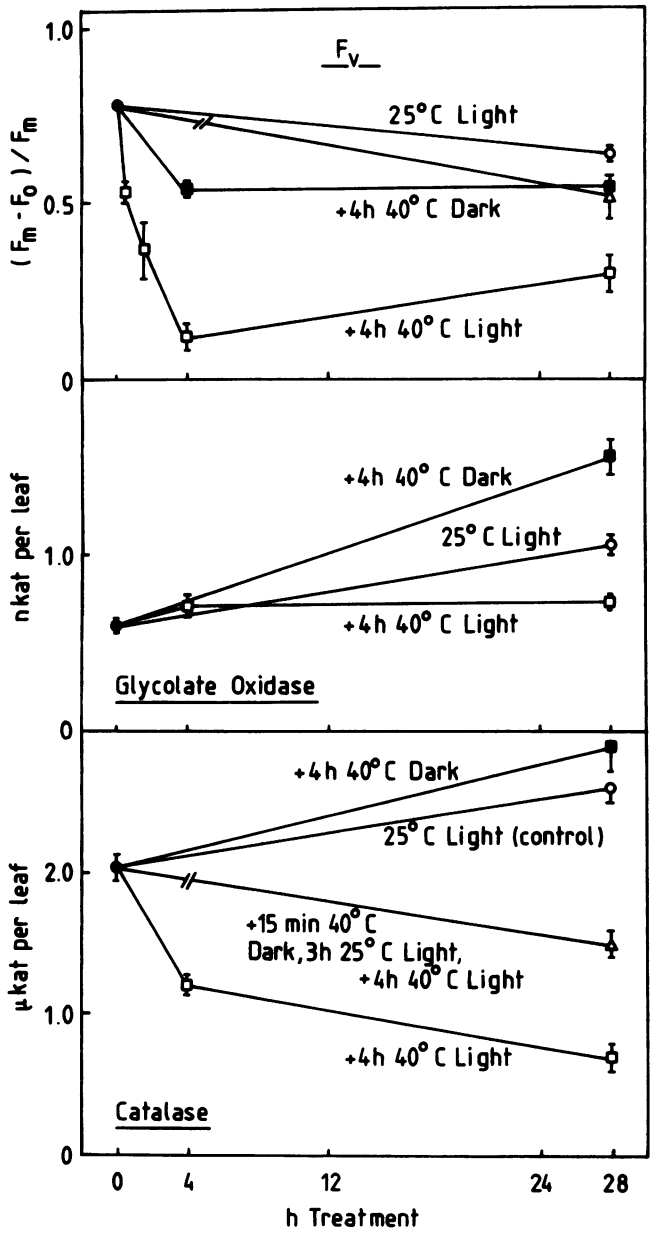

Figure 1. Changes in catalase, glycolate oxidase, and F_v after exposure of 6-d-old rye seedlings to a 4-h heat-shock treatment of 40°C. ●, Leaves before treatment; □, 4 h at 40°C and 520 μmol m^{-2} s^{-1} plus 24 h at 25°C and 520 μmol m^{-2} s^{-1}; ■, 4 h at 40°C in darkness plus 24 h at 25°C and 520 μmol m^{-2} s^{-1}; ○, controls without heat-shock, 28 h at and 520 μmol m^{-2} s^{-1}; △, 15 min at 40°C in darkness plus 3 h at 25°C and 520 μmol m^{-2} s^{-1} plus 4 h at 40°C and 520 μmol m^{-2} s^{-1} plus 21 h at 25°C and 520 μmol m^{-2} s^{-1}.

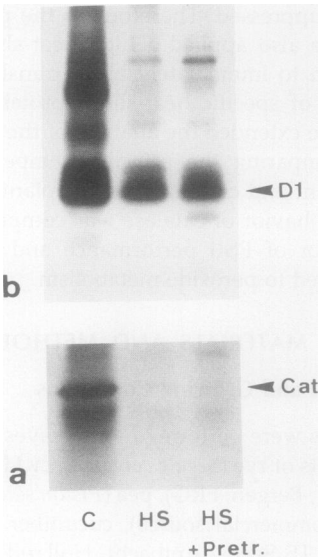

Figure 2. Comparison of the incorporation of L-[^{35}S]methionine into catalase (a, Cat) or into the D1 protein of PSII (b) in leaf sections of 6-d-old rye seedlings under heat-shock (HS) conditions in light of 520 μmol m^{-2} s^{-1} PPFD. C, Untreated control kept at 22°C; HS, during the 4 h labeling period leaf sections were exposed to 40°C; HS + pretreatment (Pretr.), during the 4-h labeling period, leaf sections were exposed to 40°C, but 3 h before the labeling period, plants had been exposed to a 15-min pretreatment at 40°C in darkness. Between pretreatment and the 4-h heat-shock labeling period, plants were kept at 25°C and a PPFD of 520 μmol m^{-2} s^{-1}. Shown are fluorograms after SDS-PAGE separations of (a) immunoprecipitates obtained with anti-catalase serum or (b) total cell membranes.

served during the high light exposure at 25°C following the heat-shock treatment.

Photoinactivation at Low Temperatures

To assay and compare photoinactivation events in the low-temperature range, seedlings or leaf sections of two chilling-tolerant plants, rye and pea, and of two chilling-sensitive plants, maize and cucumber, were exposed for 24 h to a PPFD of 520 μmol m^{-2} s^{-1} at different temperatures between 7 and 25°C. By this selection of species, each group included both a monocotyledonous and a dicotyledonous plant. In all plants, quite marked photoinactivation of catalase and of PSII, as monitored by the decline in F_v, was observed at the lowest temperature of 7°C. The effective temperature ranges were, however, clearly different for the tolerant and sensitive species, in particular, for catalase. In rye and pea, photoinactivation of catalase was seen only below 15°C, whereas the loss of catalase was already quite marked between 15 and 25°C in maize and cucumber (Fig. 3). In all plants, F_v gradually declined in light with decreasing temperature (Fig. 3). Also, for F_v, the decline at 15°C was greater in maize and cucumber than in rye and pea. However, the difference between chilling-tolerant and chilling-sensitive plants was not as pronounced as for the photoinactivation of catalase. In darkness, the 24-h low-temperature exposures had no effect on catalase. Only in cucumber leaf discs did F_v decline at 7°C in darkness. During the 24-h treatments at low temperature, photoinactivation of catalase and of photosynthesis were not accompanied by any bleaching of Chl (Fig. 3).

Of several other enzyme activities that have been compared, glycolate oxidase and GSH reductase were hardly affected by the low-temperature exposures, independently of the light conditions. In maize leaves, GSH reductase increased in light at temperatures above 10°C (Fig. 4). Peroxidase activity was not light sensitive. In pea leaflets and cucumber leaf discs, its activity increased during the treatments when the temperatures were higher than 10°C. These increases are presumably related to the detachment of the leaves (15). However, at 7°C, a loss of peroxidase occurred in the two chilling-sensitive species, maize and cucumber, both in light and in darkness (Fig. 4). These comparisons emphasize the high specificity of the chilling-induced photoinactivation of catalase and of PSII.

In rye leaves, the fate of the catalase and of the D1 protein was monitored by immunoblotting (Fig. 5). After a 24-h exposure of rye leaves to low temperature (0–4°C) and a PPFD of 520 μmol m^{-2} s^{-1}, the amount of the catalase subunit polypeptide was not significantly changed (Fig. 5b), although the enzyme activity was very low (Fig. 3; also see figure 1 of ref. 31). After termination of the cold treatment, when the leaves were kept for 1 h at 22°C and a PPFD of 90 μmol m^{-2} s^{-1}, a minor decline in the catalase polypeptide was detected. Thereafter, both enzyme activity (31) and catalase protein content recovered to normal levels. As documented before in experiments with purified enzyme in vitro (7), the catalase polypeptide was also not cleaved during the course of photoinactivation in vivo. Proteolytic breakdown, which presumably removed the damaged enzyme under heat-shock conditions (12), was apparently suppressed by the low temperature. In contrast, the D1 protein was already markedly degraded during the 24-h light exposure at 0 to 4°C (Fig. 5a). Care was taken that at the end of the treatment the leaf tissue was immediately frozen in liquid N_2 without any warming

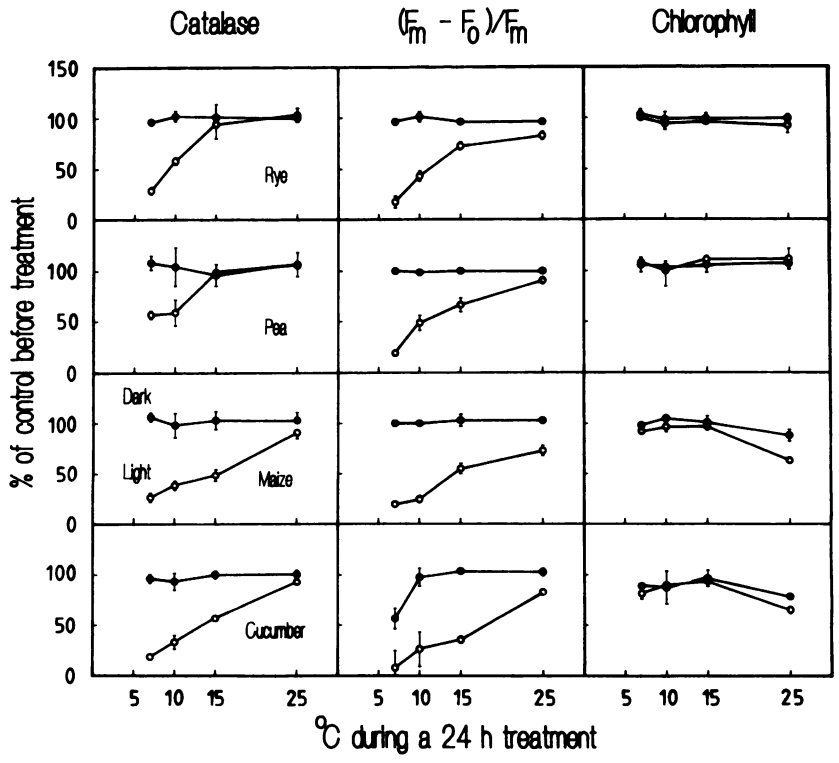

Figure 3. Changes of catalase activity, F_v, and the Chl contents in leaves or leaf sections from rye, pea, maize, or cucumber after a 24-h exposure to different temperatures either at a PPFD of 520 μmol m^{-2} s^{-1} (O) or in darkness (●).

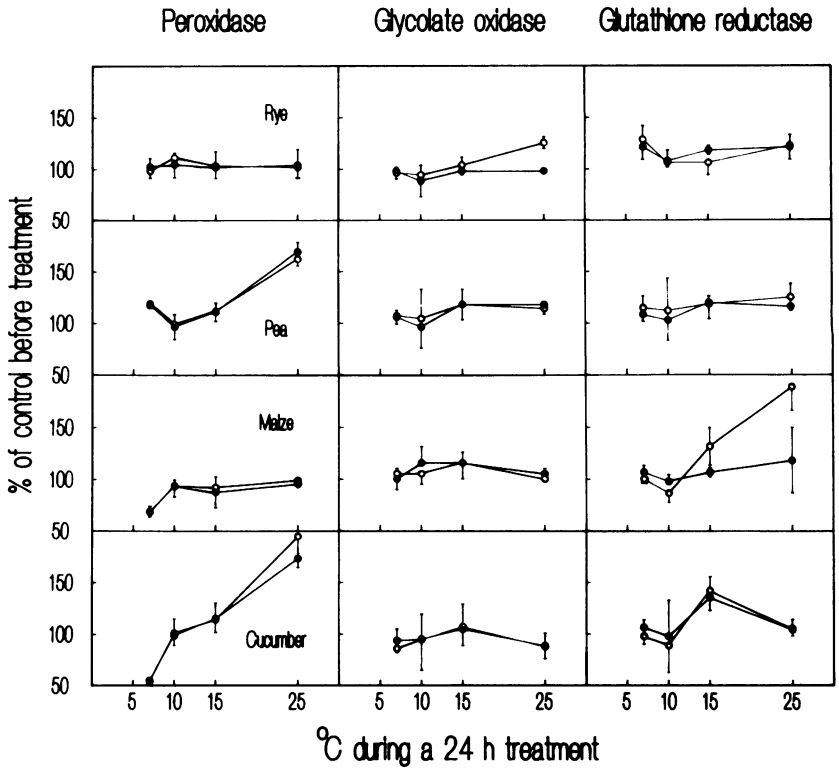

Figure 4. Changes of the activities of peroxidase, glycolate oxidase, and GSH reductase in leaves or leaf sections from rye, pea, maize, or cucumber after a 24-h exposure to different temperatures either at 520 μmol m^{-2} s^{-1} (O) or in darkness (●).

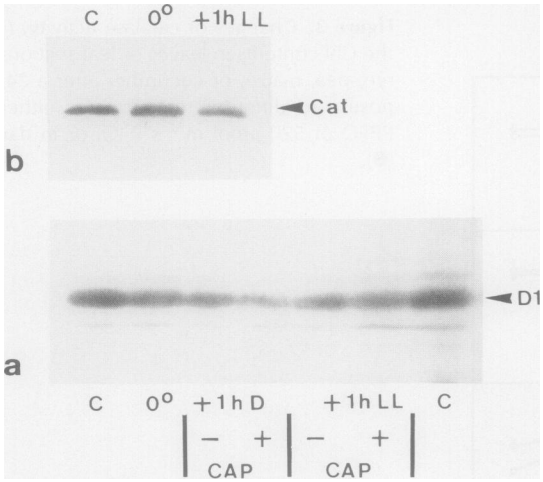

Figure 5. Immunoblotting of total cell membranes (a) and total soluble proteins (b) from rye leaves with anti-D1 serum (a) or anti-catalase serum (b). Leaves of 6-d-old rye seedlings grown at a PPFD of 90 μmol m^{-2} s^{-1} and 22°C (untreated control, C) were exposed to 0 to 4°C at a PPFD of 520 μmol m^{-2} s^{-1} (0°) for 24 h and subsequently transferred to a temperature of 22°C for 1 h either at low light of 90 μmol m^{-2} s^{-1} (+1 h LL) or in darkness (+1 h D) in the presence (+CAP) or absence (−CAP) of 6.3 mM chloramphenicol. Chloramphenicol was applied 1 h before the transfer from low to high temperature. Cat, Catalase.

before grinding and extraction. The results suggest that photocleavage was substantially contributing to the D1 protein breakdown under these conditions of long-term cold treatment. When the leaves were subsequently exposed to 22°C, some further degradation was seen at only low light intensity or in darkness, particularly in the presence of chloramphenicol (Fig. 5a). In the absence of a photoinhibitory low-temperature pretreatment, decline in the D1 protein did not occur within 1 h after chloramphenicol application at low light or in darkness (not shown). The observations suggest that part of the D1 protein still present at the end of the cold treatment was, nevertheless, photodamaged and could be degraded by a proteolytic or autoproteolytic process after return to a higher temperature, as described for isolated thylakoids (2, 4).

Recovery from Photoinactivation at Low Temperature

Because the striking loss of catalase in light under extreme temperature conditions must severely weaken the antioxidant defense mechanisms of leaves, it might be accompanied by an increase in general photooxidative damage and bleaching. However, previous studies (31) have shown that green leaves of chilling-tolerant rye do not exhibit major symptoms of enhanced general photooxidative injury, either during a period of catalase depletion at low temperature or during subsequent rapid recovery at normal temperature. Even in etiolated rye leaves, from which catalase activity was completely removed by a 24-h treatment at 0 to 4°C in high light, greening was not impaired in bright light after return to 22°C, and catalase recovered rapidly (Fig. 6). In contrast, in

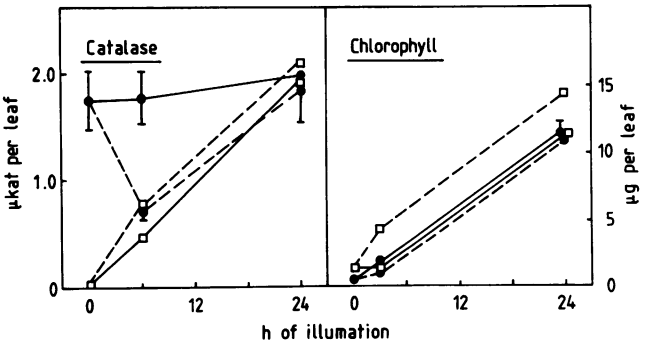

Figure 6. Changes in catalase activity and the total Chl contents in leaves of dark-grown 6-d-old rye seedling after exposure to 90 μmol m^{-2} s^{-1} (solid line) or 520 μmol m^{-2} s^{-1} PPFD (dashed line) with (□) or without (●) a 24-h pretreatment at 0 to 4°C and 520 μmol m^{-2} s^{-1} PPFD.

normal catalase-containing etiolated rye leaves continuously grown at 22°C, catalase suffered marked photoinactivation upon transfer to light (Fig. 6). At the lower light intensity, photoinactivation was indicated by an extended lag phase, as observed earlier (6), and at the higher PPFD, a strong transient decline in catalase activity occurred.

In cold-sensitive cucumber, prolonged cold exposures in bright light were followed by severe photooxidative bleaching and necrosis of the tissue after return to normal conditions. To avoid lethal effects and to keep the tissue in a condition allowing further analysis, we applied two cold treatments of 2 h each to cucumber plants in bright light on two consecutive days. These treatments resulted in limited injury. The loss of catalase activity was accompanied by Chl degradation to a similar extent (Fig. 7). The declines of catalase as well as that of Chl continued for about 1 d after return to a compatible temperature, before they started to recover. Recovery at 22°C was relatively slow. About 4 d were needed until catalase and Chl had again reached their original levels. The cold treatments of cucumber leaves were also accompanied by a small decline in F_v, which recovered immediately after the end of the cold treatments. Although other enzyme activities were not markedly affected, the cold treatments were typically followed by a major increase of peroxidase activity in cucumber leaves (Fig. 7).

DISCUSSION

Cold-induced decline in catalase has been observed (8, 21, 23, 29, 31), and in rye leaves this was demonstrated to be the result of photoinactivation (31). Results of our present comparative investigations support a more general understanding of this phenomenon. The occurrence of catalase photoinactivation is not specifically related to chilling temperatures. Because of the rapid turnover of this light-sensitive enzyme (12), loss of catalase through photoinactivation appears to occur, even when light is not excessive, whenever leaves are subjected to unfavorable stress conditions that suppress their ability to replace sufficiently the degraded catalase. Both heat-shock and chilling temperatures were equally effective in inducing declines in catalase at a moderate light intensity. The decrease of protein and catalase synthesis, which was documented in rye leaves at low (31) as well as at high temperature (12), appears to be sufficient to explain the marked and specific decrease in the catalase level. Other proteins or enzymes that are not light sensitive remain stable under such conditions.

Catalase photoinactivation generally paralleled the occurrence of photoinhibition of PSII, as indicated by the decline in F_v. Similar to the behavior of catalase, photoinhibition is known to occur not only under excessive but also under moderate light conditions when leaves are exposed to either

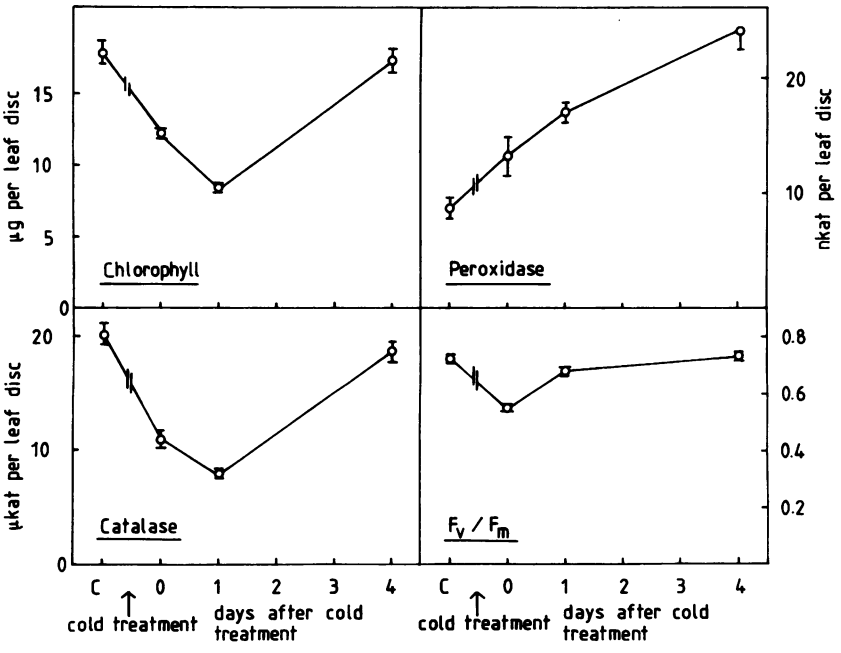

Figure 7. Changes in the Chl content, F_v, and catalase and peroxidase activities in leaves of cucumber at 22°C and 520 μmol m^{-2} s^{-1} PPFD after two exposures for 2 h each of whole 14-d-old cucumber plants to 0 to 4°C at 520 μmol m^{-2} s^{-1} PPFD on two consecutive days. C, Control values in the tissue before cold treatment. Space limitations of the growth cabinet did not allow plants to be assayed each day after the cold treatment in one series. Preliminary experiments have, however, provided evidence that the recoveries of Chl and catalase were not completed before day 4 after the cold treatment.

heat (20) or chilling temperatures (24). In accord with the behavior of photoinhibition (13, 28) photoinactivation of catalase was also not restricted to chilling-sensitive plants and not related to the susceptibility of the plants to chilling injury in darkness. Photoinactivation of both catalase and PSII appeared to represent quite general symptoms of light sensitivity and early photodamage in leaves.

We previously presented evidence that photoinactivation of catalase and of PSII are not causally interrelated but represent independent, although mostly concomitant, events (31). The parallel occurrence of both symptoms seems to be a consequence of the rapid turnover in light that is common to both catalase (12) and the reaction center protein D1 of PSII (18, 22). Depletion of the latter will result in photoinhibition (4, 11, 18). The decline in F_v at 40°C (Fig. 1) was as rapid as expected from our estimation of the half-life of the D1 protein at this PPFD in rye leaves (about 90 min). The disappearance of the D1 protein under these conditions has been documented (12). The increase in F_v (Fig. 1) and of the D1 protein level at normal temperature after heat-shock was, however, not as rapid as expected from the fast recovery of D1 protein synthesis (12), suggesting that the total response was more complex and involved an increased turnover of D1 after a heat-shock treatment. At low-temperature photoinhibition was also accompanied by a depletion of the content of functional D1. At approximately 0°C, the D1 protein was largely eliminated by photocleavage in our experiments with rye, whereas the inactivated catalase was not cleaved and its proteolytic breakdown retarded at low temperature. The possibility of a direct photocleavage of the D1 protein mediated by the production of triplet Chl, singlet oxygen, or oxygen radicals has been discussed (4) but was not observed with isolated thylakoids in vitro, even at 2°C (2). In isolated thylakoids, the photodamaged D1 protein was degraded by a subsequent endoproteolytic process that appears to be due to an autoproteolytic activity of the reaction center itself (4). Greer et al. (11) presented evidence that in barley leaves photoinhibition at low temperature was a consequence of the strong inhibition of repair processes. Photoinactivated reaction centers of PSII appear to be transformed in such a way that they mediate thermal energy dissipation and serve as a means of protection from excess excitation energy (11, 16, 17).

Further elucidation is needed for why chilling-tolerant plants in the range of 15°C are less light sensitive than susceptible species. This may involve more effective antioxidative protection or a greater capacity of repair as suggested by Greer (10). The rate of protein formation in leaves of rye, a cold-tolerant plant, is temperature dependent with a temperature coefficient of 5 to 6, but only in the range below about 17°C (5). The marked coincidence that photoinactivation of catalase also becomes apparent only below about 15°C is suggestive that its occurrence is restricted to the range in which protein synthesis and, consequently, repair processes are limited by temperature. Whether apparent catalase photoinactivation might reflect the range in which the rate of protein synthesis is temperature controlled needs to be further investigated in other plants. The induced thermotolerance after a heat-shock pretreatment appeared to some extent coincident with the maintenance of a slightly higher capacity for repair of both catalase and D1, although mechanisms of improved protection against photodegradation may also be involved. The limited and preliminary experiences with recovery after photoinactivation at low temperature would be in accord with the hypothesis (10) that repair processes are slower in a chilling-sensitive plant, such as cucumber, than in a chilling-tolerant plant, such as rye. Correspondingly, in rye leaves, periods of catalase deficiency were short, as compared to cucumber, after cold exposures in light. In addition, alternative antioxidants increased, H_2O_2 did not accumulate, and major photooxidative injury was not observed in rye (31). Further elucidation may indicate whether bleaching and photooxidative damage after cold exposures of cucumber leaves are related to the prolonged decline in catalase. Cucumber is the only plant for which an increase of the H_2O_2 content has been described after an experimental depletion of catalase (23).

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