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Regulation of Ascorbate Peroxidase Activity in Dark-Grown Radish Cotyledons by a Catalase Inhibitor, 3-Amino-1,2,4-Triazole

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The present study was performed to see the physiological role of cytosolic ascorbate peroxidase (APX) and its relationship to other enzymes involved in the H_2O_2 scavenging metabolism, and also to elucidate the regulation of APX expression in dark-grown radish (*Raphanus sativus* L. cv Taiwang) cotyledons. To do so, 3-amino-1,2,4-triazole (aminotriazole), a known specific inhibitor of catalase, was used to simulate a catalase-deficient phenomenon in cotyledons. Aminotriazole, in very low concetration (10^{-4} M), inhibited remarkably the development of catalase activity in cotyledons during dark germination. This inhibition of catalase by aminotriazole, however, did not result in any significant changes in the growth response and the H_2O_2 level of developing cotyledons. In addition, the development of guaiacol peroxidase (GPX) activity was also not significantly affected. Unlike GPX, cytosolic APX activity was induced rapidly and reached a 1.7-fold increase in aminotriazole treated cotyledons at day 7 after germination. However, *in vitro* incubation of cytosolic APX preparation from cotyledons with aminotriazole did not result in any significant change in activity. One cytosolic APX isozyme (APXa) band involved in this APX activation was predominantly intensified in a native polyacrylamide gel by activity staining assay. This means that this APXa isozyme seems to play a key role in the expression of cytosolic APX activity. On the other hand, 2-day-old control seedlings treated with exogenous 1 mM H_2O_2 for 1 h showed a significant increase of cytosolic APX acitivity even in the absence of aminotriazole. Also, 2 μM cycloheximide treatment substantially inhibited the increase of APX activity due to aminotriazole. Based on these results, we suggest that a radish cytosolic APX could probably be substituted for catalase in H_2O_2 removal and that the expression of APX seems to be regulated by a change of endogenous H_2O_2 level which couples to APX protein synthesis in a translation stage in cotyledons.

Keywords: aminotriazole, ascorbate peroxidase, catalase, H_2O_2 scavenging metabolism, radish

Hydroperoxide metabolism in plants in general and particularly during seed germination has received little attention, although information about hydroperoxide production and utilization could be highly relevant to understanding the physiological and biochemical events taking place during the early growth of seedlings. In terms of this viewpoint, the enzymatic control of cellular H_2O_2 level is a major field in the regulation of hydroperoxide metabolism in plants. Since catalase and peroxidases are reported to be the most predominant H_2O_2-utilizing enzymes in plants (Puntarulo *et al.*, 1988; Asada, 1992; Prasad *et al.*, 1994), failure of these enzymes to remove H_2O_2 could potentially result in an accumulation of H_2O_2, subsequent free radical production, and cell damage (Ferguson and Dunning, 1986; Prasad *et al.*, 1994). There are however some obscure aspects on the physiological role of catalase and its relation to other enzymes involved in H_2O_2 scavenging metabolism. For example, the question of compensatory enzyme activity in relation to H_2O_2 removal is not fully understood (Tsaftaris and Scandalios, 1981; Ferguson and Dunning, 1986; Prasad *et al.* 1994). Catalase-deficient mutants of maize showed no differences in the expression of activities of superoxide dismutase or general peroxidases (Tsaftaris and Scandalios, 1981). Ferguson and Dunning (1986) have

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found that there was little increase in hydrogen peroxide content with severe catalase deactivation in suspension-cultured pear fruit cells treated with a catalase specific inhibitor, aminotriazole, without any significant effects on cell growth or respiration. MacRae and Ferguson (1985) suggested that the absence of a clear inverse relationship between catalase activity and peroxide content indicates the continued activity of other reactions that remove H_2O_2 and that these may be important in the tolerance of plants to oxidative stress. However, Prasad *et al.* (1994) showed a conflicting result, indicating an accumulation of H_2O_2 following a treatment with aminotriazole in maize seedlings. Based on their results, they insisted that catalase-3 isozyme seems to be the most important H_2O_2 scavenging enzyme in maize. In the meantime, the cytosolic fraction of endosperm of *Ricinus communis* was found to contain an ascorbate peroxidase (APX) for the scavenging of H_2O_2 which is nearly as active as catalase in H_2O_2 degradation (Klapheck *et al.*, 1990). This result suggests that the ascorbate-dependent H_2O_2 scavenging metabolism, which has been well documented to be responsible for the removal of photosynthetically derived H_2O_2 in chloroplasts, could also operate even in the cytosol of *Ricinus* endosperm. Recently, glyoxysomal membrane-localized APX was also immunocytochemically found in cotton cotyledons (Bunkelmann and Trelease, 1996). The presence of this enzyme with a monodehydroascorbate reductase within the glyoxysome reflects an essential pathway for scavenging H_2O_2.

Therefore, the aims of this study are (a) to see an occurrence of a compensatory relationship between catalase, guaiacol peroxidase (GPX) and cytosolic APX in H_2O_2 removal, and (b) to elucidate the regulation of APX expression in dark-grown radish cotyledons which show a metabolic situation of extremely high turnover of H_2O_2.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Radish seeds (*Raphanus sativus* L. cv Taiwang) were surface-sterilized by soaking for 10 min in a solution of 1% (v/v) sodium-hypochlorite and then washed with sterilized water. These seeds were sown and allowed to germinate in glass-covered plastic containers (115× 115× 100 mm) which contained 3 layers of filter paper (Whatman No.2) moistened with 20 mL of distilled water or 20 mL of 0.1 mM aminotriazole solution. The radish seedlings were then placed in the growth chamber for 8 days at 25±1°C in darkness with an additional supply of 10 mL water on day 3 after sowing. Seedlings were sampled at daily intervals after sowing, and cotyledons were harvested for biochemical and enzyme assays, and growth measurements.

Preparation of Enzyme Extracts

Ten pairs of radish cotyledons were ground on ice by mortar and pestle using 4 mL of 100 mM potassium phosphate buffer (pH 7.0) containing 2 mM Na_2-EDTA, 5 mM ascorbate and 0.3 g of quartz sand. The supernatant, recovered at 4°C by centrifugation (27,000× g, 20 min), was used for enzyme source. Activities of catalase and ascorbate peroxidase were analyzed in particular after gel filtration of the enzyme extracts on Sephadex G-25 using a 100 mM phosphate buffer, pH 7.0, for elution. The protein content in the extracts was determined according to the method of Lowry *et al.* (1951) with BSA as a standard.

Enzyme Assay

Catalase activity was determined spectrophotometrically by following the decrease of absorbance at 240 nm (extinction coefficent 39.4 $M^{-1}cm^{-1}$) due to H_2O_2 consumption. The reaction mixture (3 mL) contained 50 mM potassium phosphate buffer (pH 6.5) and enzyme extract. The reaction was initiated by adding 10 mM H_2O_2 (Klapheck *et al.*, 1990). GPX activity was determined spectrophotometrically by monitoring the increase in absorbance at 430 nm due to the oxidation of guaiacol (Chance and Maehly, 1955). The reaction mixture (3 mL) contained 70 mM potassium phosphate buffer (pH 6.0), 0.6 mg guaiacol/mL, and enzyme extract. The reaction was initiated by adding 10 mM H_2O_2. Cytosolic APX activity was measured spectrophotometrically by monitoring the decrease in absorbance at 290 nm as ascorbate was oxidized (extinction coefficient 2.8 $mM^{-1}cm^{-1}$). The reaction mixture (1 mL) contained 50 mM potassium phosphate, pH 6.5, 0.5 mM ascorbate, 2.5 mM H_2O_2 and enzyme extracts. Correction was made for the low, nonenzymatic oxidation of ascorbate by H_2O_2 (Asada, 1984).

Native PAGE and Activity Staining of APX

Equal amounts of protein extract (80 μg) from cotyledons were subjected to discontinuous PAGE under nondenaturing, nonreducing conditions essentially as described by Laemmli (1970), except that SDS was omitted and the carrier buffer contained 2 mM

ascorbate (Mittler and Zilinskas, 1993). Electrophoretic separation was performed at 4°C for 2 h with a constant current of 10 to 15 mA per gel, using 10% polyacrylamide gels. The gels were prerun for 30 min to allow the ascorbate, present in the carrier buffer, to enter the gel prior to the application of the samples. Following electrophoretic separation, gels were equilibrated with 50 mM potassium phosphate buffer (pH 7.0) containing 2 mM ascorbate for 30 min. The gels were then incubated in 50 mM potassium phosphate buffer (pH 7.0) containing 4 mM ascorbate and 2 mM H_2O_2 for 20 min. The gels were subsequently washed with 50 mM potassium phosphate buffer (pH 7.0) for 1 min and submerged in a solution of 50 mM potassium phosphate buffer (pH 7.8) containing 28 mM TEMED and 2.45 mM nitroblue tetrazolium with gentle agitation. The reaction was continued for 10 min and stopped by a brief wash in water.

Growth Measurement

Fresh cotyedons after harvesting were rapidly weighed and also measured in length (long axis) for determinations of daily changes of weight and elongation growth.

Measurement of H_2O_2 Content

10 pairs of cotyledons were homogenized in 2 mL of 100 mM sodium phosphate buffer (pH 6.8) containing 0.3 g of quartz sand. The homogenate was centrifuged at 27,000×g for 20 min and the supernatant collected for assay. Determination of H_2O_2 content was performed basically according to Bernt and Bergmeyer (1974) using a peroxidase. A 0.5 mL aliquot of supernatant was mixed with 2.5 mL of peroxide reagent (0.12 M sodium phosphate, pH 7.0, 0.005% (w/v) o-dianicidine, 40 μg peroxidase/mL) and then incubated at 30°C for 10 min in a water bath. The reaction was stopped by adding a 0.5 mL of 1 N perchloric acid and centrifuged for 3 min at 3,000×g. The resultant clear supernatant was read at 436 nm and its absorbance was compared to the extinction of a H_2O_2 standard.

RESULTS

In vivo Inhibition of Catalase and Growth Response by Aminotriazole

The herbicide aminotriazole (3-amino-1,2,4-triazole) is capable of inactivating a catalase by direct binding to its protein moiety (Margoliash *et al.*, 1960). Aminotriazole, at a concentration of 10^{-4} M, completely prevented the normally observed increase in catalase activity in maize following seed imbibiton and ger-

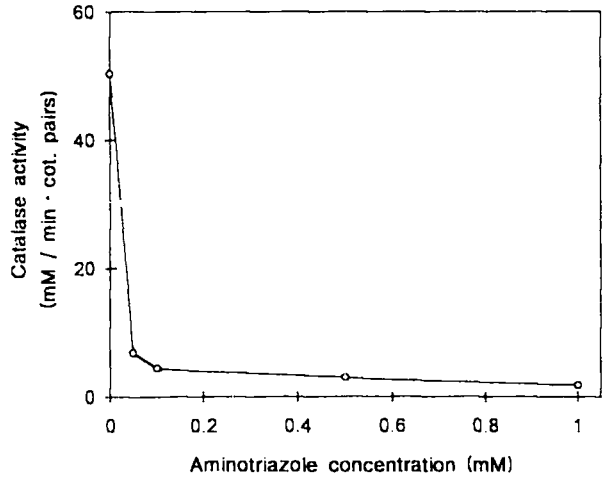

Fig. 1. *In vivo* inhibition of the catalase activity in 3-day-old radish cotyledons as a function of aminotriazole concentrations. The radish seedlings were grown at 25±1°C under dark. The data presented are average values of three replicate assays.

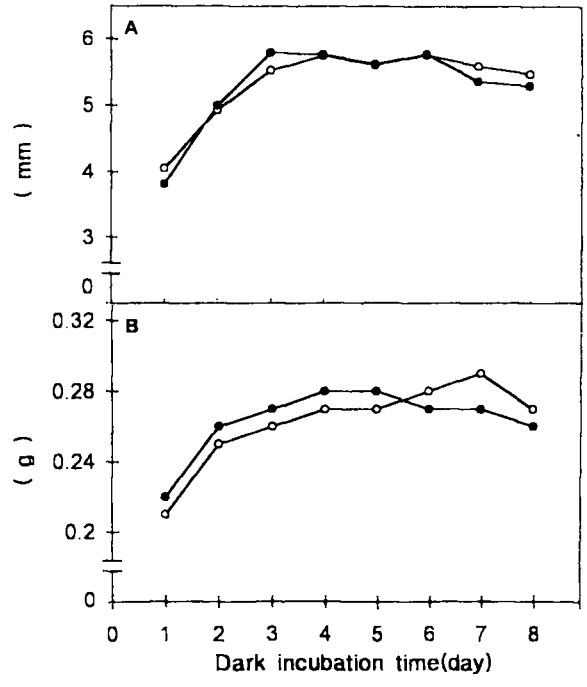

Fig. 2. Changes of the length (A) and fresh weight (B) in the cotyledons of radish seedlings during 8 days of dark germination in the presence of 0.1 mM aminotriazole (●) or not (○). The data presented are average values of three replicate assays.

mination (Tsaftaris *et al.*, 1981). In this experiment, germination of radish seedlings for 3 days with different and much lower concentrations of aminotriazole showed also the reduction in catalase activity in cotyledons by 95% (Fig. 1). Singer and McDaniel (1982) have suggested that aminotriazole diffuses rapidly into cells.

As shown in Fig. 1, there was a non-linear dose respose over a range of 0 to 1 mM concentrations.

With a 0.1 mM concentration of aminotriazole, an approx. 91% reduction in activity in radish cotyledons was consistently achieved. Although the development of the seedlings was slightly retarded by this treatment, 0.1 mM aminotriazole had no effect on the increase in length (Fig. 2A) or on the fresh weight (Fig. 2B) of the cotyledons during germination, indicating that the development of the cotyledon was not disturbed by this concentration of aminotriazole. So, aminotriazole concentration of this order (0.1 mM) was used in subsequent experiments.

Developmental Changes of Catalase and H_2O_2 Level

Since H_2O_2 is the primary substrate for catalase, one might expect that a consequence of reduced catalase activity would be the accumulation of H_2O_2 and other toxic products such as hydroxyl radicals. This might then account for cell damage from such active oxygen species. With respect of this viewpoint, we investigated the developmental changes of catalase activity and hydrogen peroxide contents in the cotyledons of dark germinating seedlings with aminotriazole application (Figs. 3A and B).

The time-course of catalase activity in the control cotyledons during 8 days of dark germination showed an increase of activity with a peak (52.38 mM/min·cot.pair) at day 3 then a gradual decline in activity. However, the development of catalase activity in aminotriazole-treated cotyledons was completely suppressed during the whole period, indicating that 0.1 mM aminotriazole acted effectively in catalase deactivation (Fig. 3A). In the developmental profiles of H_2O_2 content, there was no significant difference in either group of cotyledons although the catalase activity in aminotriazole-treated cotyledons was severely inhibited (Fig. 3B).

Developmental Change of GPX and Cytosolic APX Activities

Under severe catalase deactivation, one question arises as to whether this low level of catalase is sufficient for the destruction of hydrogen peroxide produced in dark-grown cotyledons. Various peroxidases such as GPX and APX are also reported to be the major H_2O_2-utilizing enzymes in plants (Puntarulo *et al.*, 1988; Asada, 1992; Prasad *et al.*, 1994). Ascorbate peroxidases are currently classified into two types; one of them is localized in chloroplasts (chloroplastic APX), and the other is found in the cytoplasm (cytosolic APX) (Chen and Asada, 1989).

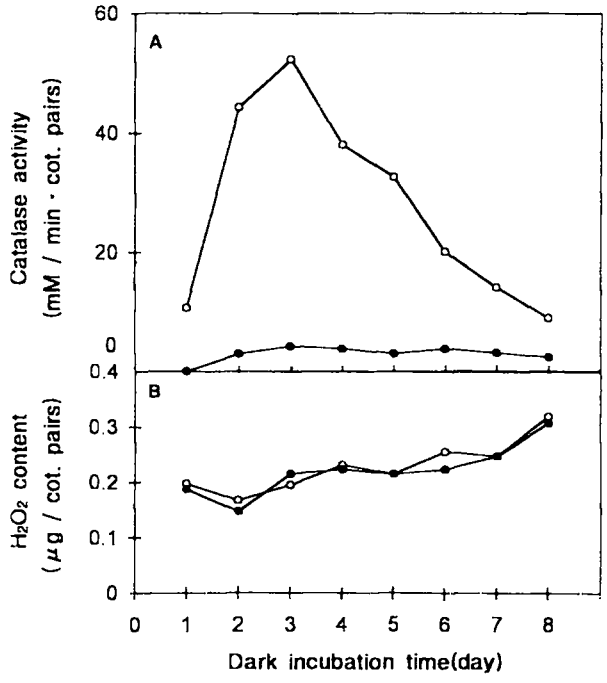

Fig. 3. The time-course of catalase activity (A) and H_2O_2 content (B) in the cotyledons of radish seedlings during 8 days of dark germination in the presence of 0.1 mM aminotriazole (●) or not (○). Each values are the means of three replicate experiments.

Because of the dark germination of radish cotyledons, the time-courses of activity development of GPX and cytosolic APX were examined in the cotyledons in order to see whether these might have a compensatory activity in H_2O_2 removal instead of catalase (Figs. 4A and B). Reduction in catalase activity in aminotriazole-treated cotyledons did not result in significantly the elevated GPX activity as compared with that of the control cotyledons (Fig. 4A). Unlike GPX, cytosolic APX activity in aminotriazole-treated cotyledons was induced more rapidly and then reached an 1.7-fold increase at day 7 compared to the control (Fig. 4B). This means that cytosolic APX might have a certain role in H_2O_2 removal under the situation of catalase deactivation due to aminotriazole.

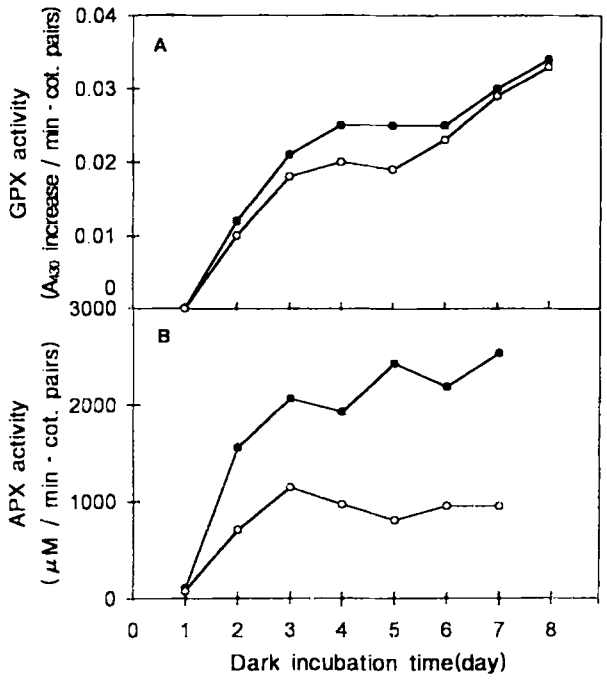

Fig. 4. The time-course of activities of guaiacol peroxidase (A) and cytosolic ascorbate peroxidase (B) in the cotyledons of radish seedlings during 7 to 8 days of dark germination in the presence of 0.1 mM aminotriazole (●) or not (○). Each values are the means of three replicate experiments.

Change in Cytosolic APX Isozymes

When cotyledon extracts were subjected to native PAGE and monitored for cytosolic APX activity, two different APX isozymes were observed (Fig. 5). Based on the elution profile, we referred to these two isozymes as APXa (Rf=0.39) and APXb (Rf=0.57), respectively.

As shown in Fig. 5, APXa occurred with major activity and APXb with minor. The expression of APXa activity was more enhanced in aminotriazole-treated cotyledons after 5 day germination, however it showed a downward tendency in activity at day 7 in the control cotyledons. The expression of APXb isozyme was also a little high at day 3 in aminotriazole-treated cotyledons although it showed minor activity. These results indicate that APXa seems to play a key role in the expression of total cytosolic APX activity in cotyledons.

Regulation of Cytosolic APX Activity

Cytosolic APX activity in radish cotyledons was increased following treatment with aminotrizole. However , little is known about the clear mechanism underlying its response to aminotriazole. In addition, there has been no *in vitro* studies with a cytosolic APX of plant orgin.

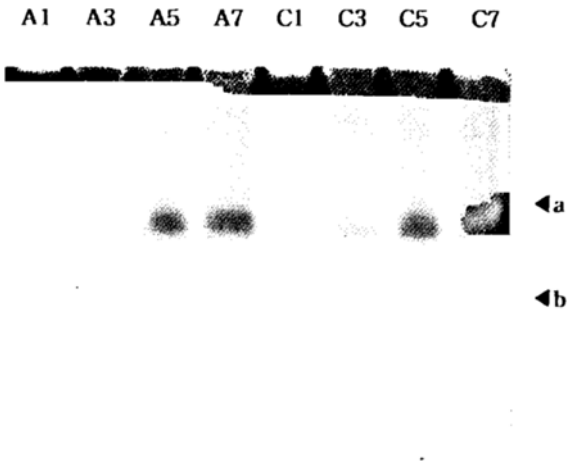

Fig. 5. Detection of cytosolic ascorbate peroxidase isozyme activities in soluble protein extracts from cotyledons of radish seedlings treated with 0.1 mM aminotriazole (A) or not (C). Numbers in each lane indicate the days of dark germination. Electrophoretic separation and analysis of enzymatic activities were performed as described under "Materials and Methods". All lanes contained 80 μg of soluble cotyledon protein extract.

Thus we investigated the *in vitro* effect of aminotriazole on the activity of partially purified APX preparation extracted from radish cotyledons. Table 1 shows that incubation of cytosolic APX preparation from radish cotyledons with aminotriazole did not result in any significant change in activity. In this case, the addition of H_2O instead of aminotriazole served as control. The fact that no increase in activity was seen by adding aminotriazole indicates that aminotriazole itself did not react directly with the APX enzyme. The activation of APX seems to be a result of metabolic changes in cotyledons by aminotriazole. And we examined the effects of ex-

Table 1. *In vitro* effect of the aminotriazole on the ascorbate peroxidase activity in the extracts from 3 day-old radish cotyledons. The radish seedings were grown at 25°C under the dark

Treatments	Distilled Water	Aminotriazole (0.1 mM)
Ascorbate peroxidase activity*	1.42	1.40

*mM/min · cot.paris

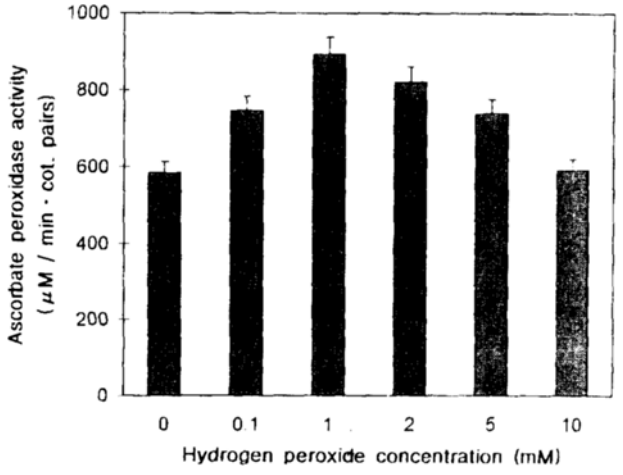

Fig. 6. Effects of exogenous hydrogen peroxide on ascorbate peroxidase activity. Two-day-old radish seedlings dark grown on water were treated for 1 hour with different concentrations of hydrogen peroxide. Seedlings grown continuously on water were used as a control.

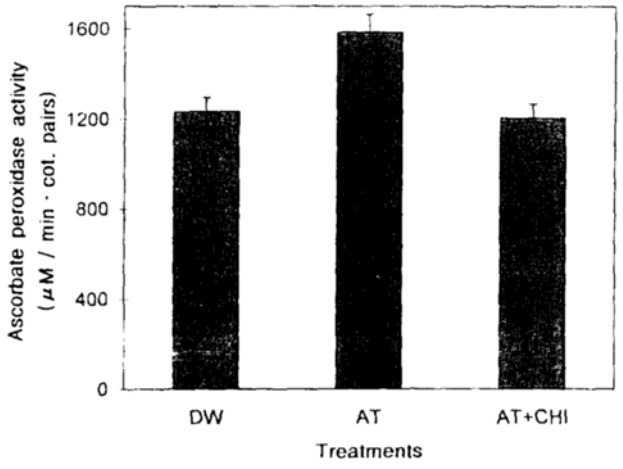

Fig. 7. Effects of cycloheximide on ascorbate peroxidase activity. The radish seedlings were grown on water for 2 days at 25±1°C under the dark, then transferred to each treatments for 1 day as follows: DW, water control; AT, seedlings treated with 0.1 mM aminotriazole; AT+CHI, seedlings treated with 0.1 mM aminotriazole+2 μM cycloheximide.

ogenous H_2O_2 on cytosolic APX expression in cotyledons to see whether it might play a role in the induction of the enzyme. To do so, 2-day-old seedlings grown on water were treated with different concentrations of hydrogen peroxide for 1 h in the absence of aminotriazole and then cytosolic APX activity was immediately measured.

Treatment with 1 mM H_2O_2 brought about a 56% increase in APX activity compared to water control (Fig. 6). This result together with the *in vitro* effect of aminotriazole (Table 1) means that aminotriazole could probably induce an expression of APX by control of endogenous hydrogen peroxide level in conjunction with catalase deactivation. In order to determine whether the increase of APX activity by aminotriazole is a result of *de novo* APX protein synthesis or is the outcome of enzymatic activation of preexisting APX, we also examined the effect of cycloheximide on cytosolic APX activity. So, 2-day-old seedlings grown on water were transferred to either water, 0.1 mM aminotriazole or 0.1 mM aminotriazole plus 2 μM cycloheximide, and incubated for an additional 1 day. As shown in Fig. 7, 2 μM cycloheximide significantly retarded the increase of APX activity resulting from a treatment of aminotriazole. Based on this result, cytosolic APX activity seems to be regulated at translational step in the APX protein synthesis process.

DISCUSSION

Aminotriazole, a known specific inhibitor of catalase, was used to simulate the catalase-deficient mutant by inhibiting catalase activity in maize (Tsaftaris and Scandalios, 1981). The usefulness of aminotriazole in catalase deactivation in plants has been amply demonstrated (Smith, 1985; Ferguson and Dunning, 1986; Havir, 1992: Prasad *et al.*, 1994). By using an aminotriazole, we herein intended to examine a metabolic role of cytosolic APX and its relation to other enzymes involved in the H_2O_2 scavenging pathway, and also to elucidate the regulation of cytosolic APX expression in dark-grown radish cotyledons.

Aminotriazole, in very low concentrations, severely inhibited the development of catalase activity in radish cotyledons (Figs. 1 and 3A). However, it did not result in any significant disturbance in the growth of cotyledons (Figs. 2A and B). There was little increase in H_2O_2 content concomitant with catalase deactivation in aminotriazole-treated cotyledons as well (Fig. 3B). A similar situation was observed in suspension-cultured pear fruit cells treated with aminotriazole (Ferguson and Dunning, 1986). In that case, almost no effects on cell growth or respiration were found even when catalase activity was severely decreased by 1 mM aminotriazole. From their results, they speculated that the existence of a range of peroxidases in a number of cellular locations provided some flexibility to the cell in responding to increased peroxide production. Taylorson and Hendricks (1977), in order to explain the role of cyanide in breaking seed dormancy, have proposed that the in-

hibition of catalase by cyanide could result in a higher steady state level of H_2O_2 which would lead, through an enhanced peroxidase actvity, to a stimulation of the pentose phosphate pathway. Therefore, our results together with the speculations of Ferguson and Dunning (1986) and Taylor and Hendricks (1977) led us to examine the roles of other peroxidases in H_2O_2 removal in radish cotyledons. Since GPX is well known as a general plant peroxidase, we first investigated the time-course of its activity development in the dark-germinating radish cotyledons (Fig. 4A). However, reduction in catalase activity by aminotriazole did not result in significantly the elevated GPX activity as compared with that of control cotyledons. It has been reported that APX is distinct from the typical plant peroxidases superfamily in terms of its structure and function, and it is also unique in having a preference toward ascorbate as a reductant (Chen and Asada, 1989; Mittler and Zilinskas, 1991). Thus we examined the development of cytosolic APX in cotyledons. While the function of chloroplastic APX as an H_2O_2-scavenger is relatively well documented in various studies (Foyer and Halliwell, 1976; Nakano and Asada, 1981; Jablonski and Anderson, 1982), there has been no detailed study on the physiological role of cytosolic APX nor to its relation to other enzymes involved in H_2O_2 scavenging metabolism. Unlike GPX development, there was a remarkable rise in cytosolic APX activity in aminotriazole-treated cotyledons (Fig. 4B). It is therefore conceivable that the role of catalase in H_2O_2 removal may be replaced by a cytosolic APX since even when the activity of catalase in aminotriazole-treated cotyledons is almost completely eliminated (Figs. 1 and 4A), the development of cotyledons appears to proceed normally during seedling growth (Fig. 2 and B). Our suggestion is strengthened by a recent report that the endosperm cytosol of *Ricinus communis* was found to contain an APX which is nearly as active as catalase in degradation of H_2O_2 at its physiological concentrations (Klapheck *et al.*, 1990). More recently, glyoxysomal membrane-localized APX was also immunocytochemically found in cotton cotyledons (Bunkelmann and Trelease, 1996).

Most studies in APX in response to oxidative stress have largely employed a solution assay for activity determination. Some recent reports, however, have demonstrated that the total activity of APX in leaf extracts which is usually measured by a crude enzyme solution assay would include several relatively stable isozymes (Mittler and Zilinskas, 1993; Rao *et al.*, 1996). In addition , Mittler and Zilinskas (1993) developed a very sensitive method for the detection of APX isozymes in native electrophoretic gels. Therefore, the APX gel assay appears to supply a valuable tool to distinguish between different isozymes and their responses to aminotriazole treatment. Using such a native-gel assay, we attempted to find an isozyme which seems to be more active in H_2O_2 removal. Our examination of a cytosolic APX isozyme profile revealed a significant enhancing effect of aminotriazole on the expression of the APXa isozyme (Fig. 5).

The result may be a clue in explaining incremental variations of total APX activity in aminotriazole-treated cotyledons. So, the APXa isozyme seems to play a key role in H_2O_2 scavenging metabolism. Rao *et al.* (1996) reported two isozymes of cytosolic APX in *Arabidopsis* leaves. They also found that exposures of rosette leaves to either UV-B or O_3 enhanced the expression of some isozyme activity. This result with our data may indicate a defensive role of cytosolic APX against H_2O_2 accumulation in radish cotyledons.

There is currently little information on the *in vivo* activation of APX activity by aminotriazole, although the uptake, metabolism and action of the compound in plants have been studied (Feierabend and Schubert, 1978; Singler and McDaniel, 1982; Ferguson and Dunning, 1986; Heim and Larrinua, 1989). According to our experiment on the *in vitro* effect of aminotriazole in partially purified APX preparation (Table 1), it may appear that aminotriazole itself would not act directly on APX as a simple enzyme activator.

It seems rather reasonable to assume that aminotriazole may cause an increase of APX activity through modulation of cotyledonary metabolic events. A possibility has been suggested by Klapheck *et al.* (1990) that the increased endogenous hydrogen peroxide levels in plant cells by various external stresses (i.e., drought, chilling or ozone exposure) may cause an induction of APX. Furthermore, hydrogen peroxide itself has recently been considered to be a second messenger and exogenous hydrogen peroxide can initiate plant defense responses (Prasad *et al.*, 1994; Price *et al.*, 1994). Therefore, the increase of APX activity resulting from treatment of radish seedlings with exogenous 1 mM H_2O_2 in the absence of aminotriazole (Fig. 6) could be explained by such a property of H_2O_2 as a signal in the induction response of APX.

In this case, one question arises as to how does hydrogen peroxides induce a cytosolic APX activation? So, we investigated the effect of a protein syn-

thesis inhibitor, cycloheximide, on the increase in APX activity in response to aminotriazole. Because it will allow us to determine whether the activation of APX activity by aminotriazole is a result of *de novo* APX synthesis or the outcome of enzymatic activation of preexisting APX proteins. Mittler and Zilinskas (1992) described the molecular cloning and characterization of a gene encoding pea cytosolic APX. In their experiment, they suggested a possibility that APX is regulated at least in part at the level of protein synthesis and/or enzyme activation. However, the fact that a decrease in activity was seen by the addition of cycloheximide (Fig. 7) indicates that the regulation of radish cytosolic APX activity could be mediated at least at the translational level in APX protein synthesis.

In conclusion, the increased activity of cytosolic APX accompanying a catalase deactivation without any significant changes in H_2O_2 level, growth response and GPX development in radish cotyledons by aminotriazole means that this cytosolic APX could substitute for catalase in H_2O_2 removal.

Furthermore, the expression of APX activity seems to be regulated by a change of endogenous H_2O_2 level which is coupled to APX protein synthesis in a translation stage in cotyleons.

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