Proceedings of the 7th Hungarian Congress on Plant Physiology, 2002
S1-P04

Volume 46(3-4):33-34, 2002
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Differences between the catalase isozymes of maize (*Zea mays* L.) in respect of inhibition by various phenolic compounds

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ABSTRACT Catalase isozymes of maize (*Zea mays*, L. hybrid Norma) were shown to differ in their sensitivity to phenolic compounds. Salicylic acid, benzoic acid, acetylsalicylic acid and o-coumaric acid caused significantly greater inhibition of CAT1 activity than of CAT2. The nature of inhibition was non-competitive for CAT1, while CAT2 was inhibited competitively. P-hydroxybenzoic acid (pHBA) was the only compound to inhibit both CAT1 and CAT2 activity in the same competitive manner. pHBA was also the only phenolic compound examined that failed to induce chilling tolerance in young maize plants. According to these results, CAT1 might be a candidate for mediating the effect of SA on the induction of chilling tolerance in maize.
Acta Biol Szeged 46(3-4):33-34 (2002)

KEY WORDS

catalase isozymes
maize
salicylic acid
chilling tolerance

Salicylic acid (SA) and some other phenolic compounds were shown to play a role in the induction of abiotic stress resistance. Young maize plants exhibited increased cold tolerance upon treatment with SA, aspirin (Asp) or benzoic acid (BA) (Janda et al. 1999, 2000). Exogenously added SA also increased the heat tolerance of mustard (Dat et al. 2000). Salicylic acid may also have a role in the defence against ozone stress, as demonstrated in *Arabidopsis thaliana* (Sharma et al. 1996). SA has a direct physiological effect through the alteration of antioxidant enzyme activities. Certain enzymes (guaiacol peroxidase and glutathione reductase) were activated by the salicylic acid treatment, while others, like catalase, were found to be inhibited. Catalase seems to be a key enzyme in salicylic acid-induced stress tolerance, since it was shown to bind SA *in vitro* (Chen et al. 1993) and to be inhibited by SA in several plant species (Sanchez-Casas and Klessig 1994; Conrath et al. 1995). However, there are doubts as to whether this binding is of biological importance, since it seems that it is not specific to catalase, but is a general feature of all iron-containing enzymes (Rüffer et al. 1995). Catalase is a tetrameric enzyme containing a haem prosthetic group in each of its subunits. It exists in three biochemically distinct isoforms in maize: CAT1, CAT2 and CAT3. The differential expression of these isozymes in different tissues and during plant development has been studied in detail (Scandalios et al. 1997). However, no distinction has been made between the isozymes in respect of their inhibition by phenolic compounds.

The aim of the present work was to compare the effect of in vitro SA and related compounds on the activity of the different catalase isozymes, and to determine the nature of the inhibition. The same phenolic compounds were also tested for their ability to induce chilling tolerance in young maize plants when added to the hydroponic solution.

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Material and methods

Plant material and growth conditions: To examine the protective effect of phenolic compounds against chilling stress, plants of the hybrid Norma were grown for 2 weeks in Hoagland solution at 22/20ºC with a 16/8-hour light-dark periodicity in a plant growth chamber at 200 μmol m^{-2} s^{-1} PPFD, and then treated with 0.5 mM phenolic compound added to the hydroponic solution [SA, BA, Asp, o-coumaric acid (CA) and p-hydroxybenzoic acid (pHBA)] for 1 day. The plants were then transferred to 5ºC and constant light.

For the scutellum preparations, seeds of the hybrid Norma were germinated at 22ºC on filterpaper for 9 days. For the 0 dpi (days postimbibition) preparations dry seeds were used. CAT1 and CAT2 isozymes were extracted from 0 dpi and 9 dpi scutella, respectively, according to Scandalios et al. (1997).

Enzyme extraction: 1 gram of the scutellum was ground in a mortar and pestle with 1 gram quartz sand and 3 ml 0.5 M TRIS buffer pH 7.5 and centrifuged for 30 minutes at 12,000 g. The protein concentration of the supernatant was determined according to the Bradford method, with bovine serum albumin as standard.

Enzyme activity assay: catalase activity measurements were carried out in citrate buffer solution (pH = 6.8) as described by Sanchez-Casas and Klessig (1994). The evolution of oxygen was followed using a Clark-type oxygen electrode (Hansatech Ltd, England).

Chlorophyll fluorescence induction parameters: The chlorophyll fluorescence induction parameters of the youngest fully expanded leaves were determined at room temperature using a pulse amplitude modulated fluorometer (PAM-2000; Walz, Effeltrich, Germany) as described in Janda et al. (1994). Before the measurements, plants were dark-adapted for 30 min at room temperature. Measurements were carried out before and after 1 or 3 days of cold treatment.

Statistical analysis: The results are the means of at least 15 repetitions for the chlorophyll fluorescence induction

Table 1. Activity of CAT1 and CAT2 isozymes extracted from 0 and 9 dpi scutella, respectively, in the absence (control) and in the presence of 5 different phenolic compounds at a concentration of 2 mM. Values in parentheses represent ±SD.
*,**,***; significant at the P≤0.05, 0.01 and 0.001 levels compared to control activity, respectively.

	CAT1 ($\mu M\ O_2$/min*mg prot)	Inhibition(%)	CAT2 ($\mu M\ O_2$/min*mg prot)	Inhibition(%)
control	12.8 (±1.13)		12.3 (±0.73)	
SA	4.6 (±0.74) ***	64	9.9 (±0.64) *	20
BA	7.9 (±0.46) **	38	9.6 (±0.56) *	22
Asp	6.6 (±0.56) ***	48	8.2 (±0.92) *	33
Coumaric acid	4.5 (±0.21) ***	65	10.1 (±1.03) *	18
pHBA	10.8 (±0.68) *	15	11.2 (±0.42) *	9

parameters and of 3-5 repetitions for the enzyme activity studies for each treatment. The data were statistically evaluated using the standard deviation and *t*-test methods.

Results and discussion

SA, Asp, BA and CA showed the same pattern of inhibition of catalase isozymes: strong inhibition of CAT1, and feeble inhibition of CAT2 (Table 1). The nature of the inhibition was determined for SA and Asp. The Lineweaver-Burk plot of SA inhibition of CAT1 and CAT2 shows that CAT1 is inhibited non-competitively, while CAT2 is inhibited in a competitive manner. Asp showed the same kinetics of inhibition as SA. The only compound differing significantly was 4-hydroxybenzoic acid (pHBA), which showed very weak inhibition of both isozymes (15 and 9%, respectively). The nature of this weak inhibition by pHBA was found to be competitive for both isozymes. The weak competitive inhibition of catalase activity might be explained by the mechanism suggested by Durner and Klessig (1996), that all phenolic compounds can serve as electron donors for the slow peroxidative reaction of the enzyme. On the other hand, the strong non-competitive inhibition observed in the case of CAT1 cannot be explained by this mechanism and implies stronger binding of SA to the enzyme. CAT1 resembles dicot catalases in that its stronger inhibition is intolerant of substitution at position 4 of the benzene ring (Rüffer et al. 1995), since pHBA caused only weak, competitive inhibition of CAT1. This resemblance is in good correlation with the fact that maize CAT1 is more closely related to dicot catalases than CAT2 (Scandalios et al.1997). The K_I value of the SA inhibition of CAT2 (K_I=1.6 mM) is almost tenfold higher than that of CAT1 (K_I=0.2 mM). Thus CAT1 is more sensitive to SA, and is more likely to be inhibited by its physiological concentrations. Considering these facts, sensitivity to SA seems to be an important functional difference between the two catalase isozymes. All the phenolic compounds examined, except for pHBA, were found to induce chilling tolerance in young maize plants, since the chlorophyll fluorescence induction parameters measured after 3 days of cold treatment were significantly higher in plants pretreated with SA, Asp, BA or CA. This observation correlates well with the strong *in vitro* inhibitory effect of these compounds on CAT1 activity.

Acknowledgments

This work was supported by grants from the Hungarian National Scientific Research Foundation (OTKA T32653, T37195).

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