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Aminoaldehyde dehydrogenase activity during wound healing of mechanically injured pea seedlings

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Summary

Aminoaldehyde dehydrogenase (AMADH, EC 1.2.1.19) is an enzyme that, in association with amine oxidase, participates in polyamine catabolism. In plants, the enzyme is well characterized in pea seedlings. In this study, we used etiolated and light-grown pea seedlings as model plants to evaluate the possible AMADH role in response to stress caused by mechanical damage. In the beginning, the activity distribution of AMADH, amine oxidase and peroxidase in organs of 7-day-old intact pea seedlings was analyzed. To perform mechanical damage, stems of 10-day-old seedlings were each divided into four segments of equal length. The top (= fourth) segments were then longitudinally cut with a lancet. During healing, the injured segments and their control counterparts were harvested in 1-day intervals and analyzed for activity of the above enzymes, polyamine and 4-aminobutyrate (GABA) concentrations. The injury elicited increases in AMADH, amine oxidase and peroxidase activities in both etiolated and green seedlings, accompanied by parallel increases in putrescine, cadaverine, spermidine and GABA content. Histochemical experiments allowed visualization of increased AMADH activity in cross sections obtained from the injured stem segments. The activity was localized in cortical parenchyma and epidermal cells adjacent to the wound site in spatial correlation with an intensive lignification. In the control seedlings, AMADH activity or

Abbreviations: ABAL, 4-aminobutyraldehyde; AMADH, aminoaldehyde dehydrogenase; APAL, 3-aminopropionaldehyde; BADH, betaine aldehyde dehydrogenase, Cad, cadaverine, CAO, copper amine oxidase; Dap, 1, 3-diaminopropane; FW, fresh weight; GABA, 4-aminobutyric acid; NBT, nitroblue tetrazolium; PMS, phenazine methosulfate; POD, peroxidase; Put, putrescine; Spd, spermidine; Spm, spermine

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lignification in these tissues could not be visualized. Thus, we conclude that, in plants, AMADH may participate in processes of adaptation to stress events caused by mechanical injury, which involve polyamine catabolism, GABA production and lignification.

Introduction

Mechanical damage of plants is a stress event, which is accompanied by both increased loss of water and high probability of infection (Hammond-Kosack and Jones, 1996; Mehdy, 1994). In response reactions, hydrogen peroxide is utilized in peroxidase-mediated strengthening of structural cell wall components, which include the processes of lignification and wall stiffening (Angelini and Federico, 1989). H_2O_2 also plays an important role in the induction of expression of various defense-related genes and exerts toxicity to pathogens. On the other hand, taking into consideration the toxicity and limited lifetime of H_2O_2, there must be a precise mechanism that generates this substance *in situ* and regulates its level in tissues (Rea et al., 2002). H_2O_2 production in the apoplast is ensured by several enzymatic systems. In addition to the reactions of NAD(P)H oxidase (EC 1.6.3.1), superoxide dismutase (EC 1.15.1.1) or oxalate oxidase (1.2.3.4), the compound is directly generated by the oxidative deamination of polyamines (Cona et al., 2005).

Polyamine catabolism involves copper amine oxidases (CAOs, EC 1.4.3.6) and FAD-containing polyamine oxidases (PAOs, EC 1.5.3.11) (Boucher-eau et al., 1999). Plant CAOs oxidize diamine and polyamine substrates at primary amino groups, yielding the corresponding aminoaldehydes, hydrogen peroxide and ammonia. The oxidative cleavage of the polyamines spermidine (Spd) and spermine (Spm) by plant PAOs results in the formation of 4-aminobutyraldehyde (ABAL) and 4-(3-aminopropylamino)butyraldehyde, respectively, with the concomitant formation of 1,3-diaminopropane (Dap) and H_2O_2 (Šebela et al., 2000). Dap conversion produces 3-aminopropionaldehyde (APAL) (Duhazé et al., 2002). As has been recently shown, aminoaldehydes are further metabolized by NAD-dependent aminoaldehyde dehydrogenases (AMADHs, EC 1.2.1.19) (Šebela et al., 2000). Dap belongs to the precursors of β-alanine (via APAL), which can be further trimethylated to yield the osmoprotectant β-alanine betaine. It is also converted to uncommon polyamines associated with stress tolerance in plants (Duhazé et al., 2002; Koc et al., 1998). ABAL is oxidized to 4-aminobutyric acid (GABA), which participates in various physiological processes (Shelp et al., 1999).

AMADH was originally found in Fabaceae and Poaceae plants coexisting with high levels of amine oxidases (Flores and Filner, 1985). AMADHs from pea (*Pisum sativum*) and oat (*Avena sativa*) were purified and investigated in detail (Livingstone et al., 2002; Šebela et al., 2000). Their properties indicate a close relationship to betaine aldehyde dehydrogenases (BADHs, EC 1.2.1.8). Using activity staining, AMADH was localized in pea seedling tissues (Šebela et al., 2001). The enzyme was restricted to the central cylinder of the epicotyl and root. The intensity of staining was highest in vascular cambium cells, but the pericycle and endodermis were also stained. The primary structure of pea AMADH has been deduced from its cloned cDNA (Brauner et al., 2003).

In the present communication, we report on the distribution of AMADH activity in pea seedlings exposed to different light conditions, which is compared with the distribution of CAO and peroxidase (POD) activities. Whereas the functional synergy of CAO and POD in the cell wall has been subjected to detailed investigations (Angelini et al., 1990; Paschalidis and Roubelakis-Angelakis, 2005), the role of AMADH in physiological processes connected to polyamine degradation remains unclear. For this reason, we focused on modulations of AMADH activity levels during wound healing in mechanically injured pea plants. Both activity assays and histochemical staining using nitroblue tetrazolium revealed increased AMADH activity in the injured tissues. Time profiles of the increased AMADH activity were compared with those showing polyamine and GABA content. The results are discussed in the light of the previously described histochemical localization of the enzyme and with respect to the role of AMADH in polyamine catabolism.

Materials and methods

Chemicals

APAL diethylacetal (1-amino-3,3′-diethoxypropane) was purchased from Acros (Geel, Belgium). Benzoyl chloride, GABase (a mixture of GABA transaminase,