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Exogenous cadaverine induces oxidative burst and reduces cadaverine conjugate content in the common ice plant

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Summary

The effect of free cadaverine (Cad) on its conjugates formation was analyzed in roots of the common ice plants (*Mesembryanthemum crystallinum* L.). It was found for the first time that Cad could induce oxidative burst in the roots of adult plants, as was evident from the sharp decrease in the content of Cad soluble or insoluble conjugates. This unusual effect was associated with the increased oxidative degradation of exogenous Cad (1 mM, 1.5 h) and intense H_2O_2 production in the root cells of adult plants. Root treatment of both juvenile and adult plants with H_2O_2 (1 mM, 1.5 h) reduced the content of soluble Cad conjugates and increased the content of their components, free Cad and phenols. We also found that one of the possible reasons of the negative effect of exogenous diamine on the formation of conjugated forms in adult roots was alkalization of the root apoplast at Cad addition to nutrient medium and the unusual $O_2^{\bullet-}$ synthase function as a pH-dependent guaiacol peroxidase in the presence of a high content of H_2O_2. This was confirmed by the data on the accumulation of $O_2^{\bullet-}$ and enhanced superoxide dismutase activity in adult roots under treatment with Cad. It is possible that the accumulation of $O_2^{\bullet-}$ together with H_2O_2 was also responsible for oxidative burst, which induced a decrease in the content of Cad conjugates in adult roots of the common ice plants.

Introduction

Stress-induced production of reactive oxygen species (ROS) is an early response of plants to various abiotic factors (Hernandes et al., 2001).

Abbreviations: AG, aminoguanidine; Cad, cadaverine; DAO, diamine oxidase; Dpi, diphenylene iodonium; GuPX, guaiacol peroxidase; PA, polyamine; ROS, reactive oxygen species.

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Excessive intracellular generation of ROS ($O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet}$) may induce membrane damage and degradation of nucleic acids and proteins. ROS are also currently regarded as signal molecules capable of transport over short and long distances (Neill et al., 2002). However, the mechanisms underlying the formation and regulation of the antioxidant systems in plants are still not fully understood.

Polyamines (PAs) (putrescine, spermidine, spermine, and cadaverine (Cad)) are the components of cellular antioxidant systems that are usually regarded as ROS scavengers (Bors et al., 1989). It has been shown that the involvement of PAs in ROS quenching is based on their ability to bind proteins or to form acid-soluble conjugates with various phenol derivatives, rather than on autooxidation and oxygen-dependent enzymatic oxidation of their amino groups. In recent years, conjugated forms of PAs have attracted a considerable amount of attention as possible members of the plant defense systems against stresses (Martin-Tanguy, 1997; Dondini et al., 2003). It has been proposed that these conjugates might take part in PA translocation and may also be important for the regulation of free PA titer (Martin-Tanguy, 1997). The cellular PA content is determined by the rates of PA biosynthesis, degradation, conjugation, and intercellular transport. However, published data concerning the content of soluble PA conjugates in stressed plants are contradictory (Walters, 2000). Content could be determined by species specificity and by the amount of phenolic compounds and PAs as substrates for the production of acid-soluble PA conjugates (Martin-Tanguy, 1997). Several publications have focused on PA oxidative degradation under stress, but its consequences for conjugate formation remain to be elucidated.

One of the most appropriate plant models for studying stress-induced ROS production is the facultative halophyte *Mesembryanthemum crystallinum* L. (the common ice plant). Salinity treatment of this plant induces Crassulacean acid metabolism (CAM) accompanied by intense ROS generation (Miszalski et al., 1998). This plant species was found to be capable of synthesizing not only PAs of putrescine family but also Cad which occurs relatively rarely in the plant kingdom (Kuznetsov et al., 2006). A characteristic feature of this plant is a relatively low content of free Cad in juvenile plants and its relatively higher content in adult plants. It should be emphasized that plant cells contain a relatively low amount of Cad, but stress and ethylene treatment induce its rapid accumulation and interorgan translocation (Shevyakova et al., 2001). We have also shown (Aronova et al., 2005) that Cad exhibited its antioxidant effect by inducing the expression of the gene encoding Cu/Zn-superoxide dismutase (SOD, EC 1.15.1.1) at the level of its mRNA synthesis. This SOD isoform is one of the main antioxidant enzymes in the root apoplast (Hernandes et al., 2001). Cad, as a free radical scavenger, can protect against $OH^{\bullet}$-induced DNA degradation, which was shown *in vitro* in the hydroxyl-generating system (Kuznetsov et al., 2007). It is also not possible to rule out the possibility that the prooxidative effects of Cad, like all PAs, in the apoplast are mediated by H_2O_2 production during Cad oxidative degradation (Ogawa et al., 1997). In addition, the mechanisms of Cad antioxidative and prooxidative actions are poorly understood.

Recently, we have shown that the roots of adult ice plants (9–10-week old) grown under non-saline conditions or high salinity (400 mM NaCl, 1.5–48 h) contained perchloric acid (PCA)-soluble and PCA-insoluble forms of PA conjugates (Shevyakova et al., 2006). The content of all forms of conjugated Cad in adult roots sharply decreased when exogenous Cad was added to root medium (1 mM, 1.5 h). This negative effect was neutralized by Cad treatment in combination with aminoguanidine (AG), the inhibitor of diamine oxidase (DAO, EC 1.4.3.6). As reported previously (Shevyakova et al., 2006), activation of DAO and guaiacol peroxidase (GuPX, EC 1.11.1.7) by NaCl or exogenous Cad was observed in leaves and roots of the common ice plant. Thus, it may be expected that the unusual high content of free Cad in root cells due to exogenous Cad treatment of the ice plant can initiate a complex of oxidative and antioxidative responses.

The objective of this paper was to experimentally verify the hypothesis that the high content of H_2O_2, the end product of Cad degradation, prevents Cad conjugation. To this aim, a comparison between Cad and H_2O_2 treatments was carried out on the roots of juvenile and adult common ice plants. Changes in the activity of different forms of GuPX and total SOD, as well as the contribution of the H_2O_2 and $O_2^{\bullet -}$ generation, were studied using the inhibitory analysis. For comparison, the effect of salt shock on the content of Cad conjugates in the roots of juvenile and adult plants was also investigated.

Materials and methods

Plant material and growth conditions

Seeds of the common ice plant (*Mesembryantemum crystallinum* L.), available from the stock of our