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Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells

Mohammad Muzahidul Islam, Md. Anamul Hoque, Eiji Okuma, Mst. Nasrin Akhter Banu, Yasuaki Shimoishi, Yoshimasa Nakamura, Yoshiyuki Murata*

Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

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Summary

Environmental stress, including heavy metal stress, can cause oxidative damage to plants. Up-regulation of the antioxidant defense system induced by proline and glycinebetaine (betaine) alleviates the damaging effects of oxidative stress in plants. Here, we investigated the protective effects of exogenously applied proline and betaine on growth, accumulation of proline and betaine, lipid peroxidation and activity of antioxidant enzymes in cultured tobacco Bright Yellow-2 (BY-2) cells exposed to cadmium (Cd) stress. Cadmium stress (at 100 μM Cd) caused a significant inhibition of the growth of BY-2 cells, and both proline and betaine significantly mitigated this inhibition. In addition, the mitigating effect of proline was more pronounced than that of betaine. Cadmium stress leads to an accumulation of Cd and endogenous proline in cultured cells, increased lipid peroxidation and peroxidase (POX) activity, and decreased activity of superoxide dismutase (SOD) and catalase (CAT). Exogenous application of proline resulted in a decrease in lipid peroxidation and an increase in SOD and CAT activities without reducing Cd contents under Cd stress, while application of betaine resulted in a decrease in lipid peroxidation and an increase in CAT activity with reducing Cd accumulation. Furthermore, exogenous proline and betaine intensified the accumulation of proline and betaine in Cd-stressed BY-2 cells, respectively. The present study suggests that proline and betaine confer tolerance to Cd stress in tobacco BY-2 cells by different mechanisms.

Abbreviations: Betaine, glycinebetaine; CAT, catalase; MDA, malondialdehyde; POX, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase.

*Corresponding author. Tel.: +81 86 251 8310; fax: +81 86 251 8388.
E-mail address: muta@cc.okayama-u.ac.jp (Y. Murata).

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Introduction

Cadmium (Cd), a non-essential heavy metal element, is a major environmental pollutant that is toxic to organisms including plants. In plants, Cd disturbs various biochemical and physiological processes, leading to cell death and inhibition of growth (Chaoui et al., 1997; Guo et al., 2009; Popova et al., 2009; Sandalio et al., 2001; Toppi and Gabbrielli, 1999; Xu et al., 2009). Similar to NaCl stress, Cd interferes with uptake and distribution of nutrients and water, and induces oxidative stress (Chaoui et al., 1997; Dixit et al., 2001; Metwally et al., 2005; Popova et al., 2009; Romero-Puertas et al., 2004; Sandalio et al., 2001; Toppi and Gabbrielli, 1999). Plants have evolved a variety of adaptive mechanisms to respond to heavy metal stress including Cd stress. One of the main adaptive mechanisms to Cd stress in plants is the accumulation of compatible solutes (Ashraf and Foolad, 2007; Mehta and Gaur, 1999; Shah and Dubey, 1998; Sharma and Dietz, 2006). Proline and glycinebetaine (from now on betaine) are the most common compatible solutes that occur in a wide variety of plants. Most plant species can accumulate proline but some cannot accumulate betaine because they are deficient in enzymes involved in betaine biosynthesis (Holmström et al., 2000; Rathinasabapathi et al., 1993). Increased levels of proline and betaine accumulated in plants correlate with enhanced stress tolerance (Ashraf and Foolad, 2007; Hasegawa et al., 2000; Shah and Dubey, 1998; Sharma and Dietz, 2006; Siripornadulsil et al., 2002). In addition to their roles as osmoprotectants, proline and betaine contribute to the protection of membranes, proteins and enzymes from damaging effects of various stresses (Ashraf and Foolad, 2007; Hasegawa et al., 2000; Okuma et al., 2000, 2002; Shah and Dubey, 1998). Furthermore, proline and betaine provide protections against stress by maintaining redox homeostasis (Chen et al., 2006; Hoque et al., 2008) and scavenging free radicals (Hasegawa et al., 2000; Hong et al., 2000; Okuma et al., 2000, 2004) and reactive oxygen species (ROS) (Chen and Dickman, 2005; Chen et al., 2006; Sharma and Dietz, 2006). Hong et al. (2000) suggest that the role of proline as a free radical scavenger is more important in overcoming stress than its role as a simple osmolyte.

Exogenous proline and betaine improve stress tolerance by up-regulating stress-protective proteins (Khedr et al., 2003), preventing photoinhibition (Ma et al., 2006) and reducing lipid peroxidation (Banu et al., 2009; Demiral and Türkan, 2004; Okuma et al., 2004) and protein oxidation (Hoque et al., 2008). We have demonstrated that both exogenous proline and betaine mitigate the NaCl-induced growth inhibition of tobacco BY-2 cells, and that proline is more effective than betaine in this system (Hoque et al., 2007a).

Environmental stress, including Cd stress, induce the production of ROS such as singlet oxygen ($^1O_2$), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) in plant cells (Apel and Hirt, 2004; Banu et al., 2009; Dixit et al., 2001; Guo et al., 2009; Hasegawa et al., 2000; Popova et al., 2009; Rodríguez-Serrano et al., 2006; Romero-Puertas et al., 2004; Toppi and Gabbrielli, 1999; Xu et al., 2009). These ROS are highly reactive and toxic to plants, and can lead to cell death by causing damage to proteins, lipids, DNA and carbohydrates (Apel and Hirt, 2004; Banu et al., 2009; Guo et al., 2009; Noctor and Foyer, 1998), although they act as signaling molecules that mediate many key physiological processes. Plants possess an array of enzymatic and non-enzymatic antioxidant defense systems to protect their cells against the damaging effects of ROS (Apel and Hirt, 2004; Noctor and Foyer, 1998). The major ROS scavenging antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase. Superoxide dismutase catalyzes the dismutation of O_2^- radicals to H_2O_2, which is subsequently detoxified by CAT and POXs. A decreased activity of antioxidant enzymes has been associated with Cd toxicity in different plant species (Chaoui et al., 1997; Dixit et al., 2001; Guo et al., 2009; Milone et al., 2003; Rodríguez-Serrano et al., 2006; Sandalio et al., 2001).

An efficient antioxidant defense system provided by proline and betaine might play an important role in tolerance of plants to Cd stress. Proline and betaine enhance antioxidant defense systems in plant responses to various oxidative stresses (Demiral and Türkan, 2004; Khedr et al., 2003; Ma et al., 2006; Molinari et al., 2007; Okuma et al., 2004). The antioxidant protection role of proline has also been described in fungal pathogenesis during various oxidative stresses (Chen and Dickman, 2005). We have shown that both proline and betaine improve salt tolerance in tobacco BY-2 cells by increasing the activity of enzymes involved in the antioxidant defense and methylglyoxal detoxification systems (Hoque et al., 2007a, b, 2008). Furthermore, our earlier study suggests that both proline and betaine induce the expression of ROS scavenging antioxidant defense genes, and suppress ROS accumulation and cell death in cultured tobacco cells exposed to NaCl stress (Banu et al., 2009). It has been suggested that proline