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Signal transduction events in aluminum-induced cell death in tomato suspension cells

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Summary

In this study, some of the signal transduction events involved in $AlCl_3$-induced cell death in tomato (*Lycopersicon esculentum* Mill.) suspension cells were elucidated. Cells treated with 100 μM $AlCl_3$ showed typical features of programmed cell death (PCD) such as nuclear and cytoplasmic condensation. Cell death was effectively inhibited by protease and human caspase inhibitors indicating a cell death execution mechanism with similarities to animal apoptosis. Cell death was suppressed by application of antoxidants and by inhibitors of phospholipase C (PLC), phospholipase D (PLD) and ethylene signalling pathways. The results suggest that low concentrations of heavy metal ions stimulate both PLC and PLD signalling pathways leading to the production of reactive oxygen species (ROS) and subsequent cell death executed by caspase-like proteases.

Abbreviations: AA, ascorbic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; Al, aluminum; AVG, aminoethoxy vinylglycine; CPT, camptothecin; DAG, diacylglycerol; FDA, fluorescein diacetate; imidazole, 1, 3-diaza-2, 4-cyclopentadiene; IMP, inositolmonophosphatase; L-gal, L-galactonic acid-γ-galactone; PA, phosphatidic acid; PCD, programmed cell death; IP_3, phosphatidylinositol-triphosphate; PLC, phospholipase C; PLD, phospholipase D; ROS, reactive oxygen species; TLCK, *N*α-ρ-tosyl-L-lysine chloromethyl-ketone; TPCK, *N*-tosyl-L-lysyl chloromethyl ketone; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling; U-73122, 1-[6-([17β)-3-metoxyestra-1, 3, 5(10)-trien-17-yl]amino)hexyl]-1H-pyrrole-2, 5, -dione; VPE, vacuolar processing enzyme; W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide; Z-Asp-CH_2-DCB, benzyoxycarbonyl-Asp-2, 6-dichlorobenzoyloxy-methylketone

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Introduction

Aluminum (Al) is among the most abundant toxic elements that contaminate the soil, waters, and food chain and represents a serious threat for human health and agricultural crops. Sources of Al are food additives, Al foil, cosmetics, and antacid medicines. In acid soils Al causes inhibition of cell division and elongation of the root tips thus negatively affecting the water supply and nutrient uptake (Samac and Tesfaye, 2003; Vitorello et al., 2005). In humans, Al is implicated in Alzheimer's and other neurological diseases and there are indications that Al toxicity is accompanied by features of programmed cell death (PCD) both in plant and animal systems (Savory et al., 2003; Meriga et al., 2004).

PCD is a functional term used to describe cell death aimed at eliminating redundant or harmful cells during the life cycle of multicellular organisms. PCD plays an essential role during development and morphogenesis by removing unwanted or misplaced cells in specific structures and organs. In addition, PCD is used in defensive mechanisms against infected or mutated cells. De-regulation of PCD is implicated in various human diseases, i.e. cancer, birth defects, ischemic vascular diseases, neurodegenerative and autoimmune diseases, AIDS and diabetes mellitus type I (Thompson, 1995). In animal cells, PCD is often associated with the occurrence of a specific set of cellular morphological features (Steller, 1995) such as condensation of the nucleus and the cytoplasm, nuclear and DNA fragmentation (DNA laddering) and the formation of cellular debris-containing vesicles called apoptotic bodies that are digested by neighbouring living cells. The cell death process abundantly showing such morphological features is called apoptosis. A small family of regulatory aspartate-specific cystein proteases named caspases is responsible for at least some of these specific features of apoptotic cells (Hengartner, 2000).

In plants, like in animals, PCD is an essential process during growth and development and it plays an important role in the response to a variety of pathogens (Lam, 2004) and to abiotic stresses such as ozone, UV radiation and a variety of toxic chemicals including heavy metals such as cadmium (Fojtová and Kovařík, 2000; Danon et al., 2004; Iakimova et al., 2005). Typical apoptotic features such as nuclear and DNA fragmentation and cytoplasmic shrinkage have been described in tobacco suspension cells grown in Al containing media. Al-induced cell death was efficiently abolished by the application of the serine protease inhibitor $N\alpha$-ρ-tosyl-L-lysine chloromethylketone (TLCK) (Yamaguchi et al., 1999). Al toxicity in plants provokes oxidative stress and a decrease in root and shoot elongation has been observed as a primary sign of Al injury. In Al-exposed seedlings the increase in hydrogen peroxide content is accompanied by a decrease in catalase activity (Yamamoto et al., 2001; Boscolo et al., 2003; Panda et al., 2003). An increase of electrolyte leakage and Al-enhanced peroxidation of lipids has been suggested to be a direct cause of cell death (Ikegawa et al., 2000). In addition, disturbance of mitochondrial functions has been found to occur (Yamamoto et al., 2002). In Al-treated soybean cell suspension culture, the administration of antioxidants reduced the number of dead cells (Rath and Barz, 2000). Using terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) Boscolo et al. (2003) showed that Al-induced death in root tip cells is accompanied by DNA breakdown. An Al-triggered increase of extracellular Ca^{2+} and a disturbance of phospholipid metabolism have recently been reported in suspension cell culture from *Coffee Arabica* L. (Martinez-Estévez et al., 2003; Sivaguru et al., 2005).

Despite the extensive ongoing research in this area, the molecular events involved in cell death in plants in response to Al stress are still poorly understood. This hampers the search for chemicals or technologies to counteract the damaging impact of Al on plant growth and development.

The aim of present work was to elucidate some of the signaling events involved in Al-induced PCD. A pharmacological approach was taken to study $AlCl_3$-induced cell death in suspension-cultured tomato cells and to identify the biochemical processes that contribute to the metabolic stress response involved in Al toxicity.

Materials and methods

The experiments were conducted with tomato (*Lycopersicon esculentum* Mill.) suspension cells, (line Msk8) grown in a liquid Murashige–Skoog medium, supplemented with 5 μM α-naphtalene acetic acid, 1 μM 6-benzyladenine, 3% (w/v) sucrose and vitamins (De Jong et al., 2002). The cells were subcultured every 7 days by 1:4 dilution with fresh medium and kept on a rotary shaker at 25 °C. For treatments, cells were used 5 days after subculture. Cell death inducers and inhibitors were added simultaneously to 5 mL of suspension culture in 30 mL flasks with a gas-tight screw cap. In general, inhibitors were tested in a range of concentrations (from nM to mM) with and without