Ca$^{2+}$ and Mg$^{2+}$ ions counteract the reduction by fosetyl-Al (aluminum tris[ethyl phosphonate]) of peroxidase activity from suspension-cultured grapevine cells

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Abstract

Grapevine (Vitis vinifera cv. Monastrell) cell suspension cultures were treated with 1.5 mM fosetyl-Al, a frequently used systemic fungicide for grapevine diseases caused by oomycetes. These cells showed a reduction in the level of peroxidase activity secreted into the culture media when compared to non-treated cells, the effect being mainly related to a decrease in the level of the basic B1 peroxidase isozyme. The effect of fosetyl-Al on peroxidase was analogous to that observed with the Ca$^{2+}$-channel blockers Cd$^{2+}$, Cd$^{2+}$ and La$^{3+}$, and was counteracted by Ca$^{2+}$ ions, but was not reversed when the Ca$^{2+}$-ionophore A23187 was added to the culture media. Moreover, the effect of fosetyl-Al on peroxidase activity and peroxidase isozymes was also partially reversed by Mg$^{2+}$ ions but not by Sr$^{2+}$, and was accentuated by Ba$^{2+}$ ions. These results suggested that Ca$^{2+}$ and Mg$^{2+}$ ions specifically overcome the inhibitory effect of fosetyl-Al on peroxidase. In this context, an apoplastic Ca$^{2+}$/Mg$^{2+}$-displacement hypothesis is proposed for the mechanism of action of fosetyl-Al on peroxidase from grapevine cells.

Abbreviations: IEF – isoelectric focusing

Introduction

Systemic fungicides possess properties that facilitate their movement in the xylem and, in the case of phosphonates, in the phloem as well (Cohen and Coffey, 1986). The alkyl phosphonate, fosetyl-Al (aluminum tris[ethyl phosphonate]), is the only phosphonate available commercially; it has proved effective against grapevine diseases caused by oomycetes (Raynal et al., 1980; Derks and Creasy, 1989).

In spite of the well-known effectiveness of fosetyl-Al in controlling some plant diseases, little is known about its molecular mechanism of action (Nemestothy and Guest, 1990; López-Serrano et al., 1994 and 1995). Elucidation of fosetyl-Al's mode of action is especially important since identification of the precise site(s) of metabolic disturbance caused by phosphonates may provide a new insight into the control at the host-parasite interface. Recently, we have reported that fosetyl-Al reduces the level of peroxidase (EC 1.11.1.7) secreted into the culture media by grapevine cells (López-Serrano et al., 1994). This effect of fosetyl-Al on peroxidase is mediated by Al ions (López-Serrano et al., 1994), partially reversed by ethylene (López-Serrano et al., 1996a), and seems to involve aluminum-mediated cell diffusible factors capable of inactivating the peroxidase secreted into the culture media (López-Serrano et al., 1996b). In this context, knowledge of the cellular target for Al in grapevine cells would contribute to understanding the mechanism of fosetyl-Al action.

Little is presently known of the molecular pathway that determines the plant cell's response to Al (Kinraide, 1991; Delhaize and Ryan, 1995). A crucial point emerges from the proposal that Al may compete with Ca$^{2+}$ for its binding to critical binding sites (Bennet and Breen, 1991). This interaction was implicated in Al toxicity (Reid et al., 1995). In this way, a possi-
ble antagonistic effect of fosetyl-Al and Ca\textsuperscript{2+} will be new and interesting towards understanding the mechanism of fosetyl-Al's action. For this reason, we decided to test the possible ameliorating effects of Ca\textsuperscript{2+}, and other alkaline cations, on the effect of fosetyl-Al on peroxidase. Based on the results obtained, the possible cellular targets for fosetyl-Al in grapevine cells are discussed.

Materials and methods

Chemicals

Fosetyl-Al (aluminum tris [ethyl phosphonate], 97\% purity) was generously supplied by Rhône-Poulenc Agrochimie S.A. (Madrid, Spain). Mg(NO\textsubscript{3})\textsubscript{2} was purchased from Merck (Darmstadt, Germany); Ba(NO\textsubscript{3})\textsubscript{2} and CaCl\textsubscript{2} from Probus (Barcelona, Spain); and Ca(NO\textsubscript{3})\textsubscript{2}, Sr(NO\textsubscript{3})\textsubscript{2}, Co(NO\textsubscript{3})\textsubscript{2}, Cd(NO\textsubscript{3})\textsubscript{2}, and La(NO\textsubscript{3})\textsubscript{3} from Fluka (Madrid, Spain). The calcium ionophore A23187 was obtained from Serva Feinbiochemica (Heidelberg, Germany).

Plant material and culture

Grapevine cell cultures were obtained from the pericarp tissue of immature grape berries (Vitis vinifera cv. Monastrell) as already described (Zapata et al., 1991; Calderón et al., 1994). Suspension cultured cells were grown in Gamborg’s B5 medium containing basal 1.0 mM CaCl\textsubscript{2}, 1.0 mM MgSO\textsubscript{4}, 0.1 \mu M CoCl\textsubscript{2}, 0.1 \mu M Co\textsubscript{4}, and supplemented with 20 g L\textsuperscript{-1} sucrose, 0.5 \mu M \alpha-naphthaleneacetic acid and 0.9 \mu M kinetin, pH 5.9. Cells (20 ml) were grown in 50 ml flasks with orbital shaking (110 rpm) at 25 °C in the dark.

Treatments

Grapevine cell cultures were treated for 48 h with 1.5 mM fosetyl-Al during the log phase of the growth cycle (López-Serrano et al., 1994 and 1995). Controls were treated identically except for the absence of fosetyl-Al. The log phase was reached for a packed cell volume of 45\% (v/v). Calcium-channel blockers [Co(NO\textsubscript{3})\textsubscript{2}], Cd(NO\textsubscript{3})\textsubscript{2} and La(NO\textsubscript{3})\textsubscript{3} and alkaline cations [Mg(NO\textsubscript{3})\textsubscript{2}, Ca(NO\textsubscript{3})\textsubscript{2}, Sr(NO\textsubscript{3})\textsubscript{2} and Ba(NO\textsubscript{3})\textsubscript{2}] were dissolved in Gamborg’s B5 medium as concentrate stocks and then filter-sterilized.

Table 1. Effect of 1.5 mM calcium-channel blockers (Co\textsuperscript{2+}, Cd\textsuperscript{2+} and La\textsuperscript{3+}) and of 1.5 mM fosetyl-Al on the level of peroxidase activity in the culture media of grapevine cells. Controls were performed in the absence of fosetyl-Al, but in the presence of basal concentrations of the calcium-channel blocker, Co\textsuperscript{2+} (0.2 \mu M). Means±SE.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Peroxidase activity (nkat ml\textsuperscript{-1})</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Control</td>
<td>8.6±0.8</td>
</tr>
<tr>
<td>Fosetyl-Al</td>
<td>3.1±0.2\textsuperscript{b}</td>
</tr>
<tr>
<td>Co(NO\textsubscript{3})\textsubscript{2}</td>
<td>10.6±2.7\textsuperscript{c}</td>
</tr>
<tr>
<td>Cd(NO\textsubscript{3})\textsubscript{2}</td>
<td>6.5±0.8\textsuperscript{d}</td>
</tr>
<tr>
<td>La(NO\textsubscript{3})\textsubscript{3}</td>
<td>3.6±0.8\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Measurement of peroxidase activity and separation of peroxidase isoenzymes by IEF

Peroxidase activity in the culture media of the cells was determined using 4-methoxy-\alpha-naphthol (Ferrer et al., 1990). Peroxidase activity was expressed in nkat (nkatal), this being defined as the amount of enzyme that oxidizes 1.0 nmol of 4-methoxy-\alpha-naphthol per s\textsuperscript{-1} (Ferrer et al., 1990). For this, cells were removed from the culture by centrifugation at 260 g for 5 min. Prior to IEF, protein samples were dialyzed overnight against 50 mM Tris-HCl (pH 7.5). IEF and staining of peroxidase isozymes were carried out as described earlier (López-Serrano et al., 1994).

Effect of alkaline cations on peroxidase activity

For this assay, alkaline cations [Mg(NO\textsubscript{3})\textsubscript{2}, Ca(NO\textsubscript{3})\textsubscript{2}, Sr(NO\textsubscript{3})\textsubscript{2} or Ba(NO\textsubscript{3})\textsubscript{2}] were individually added to the cell-free culture media at a final concentration of 10 mM each. After incubation at 25 °C for 48 h (pH 5.9), peroxidase activity (25 \mu l aliquots) was assayed with 4-methoxy-\alpha-naphthol in a final volume of 1.0 ml.

Statistical analysis

All the experiments were conducted at least three times (n = 3-25), three observations being made for each replicate. In the case of isozyme patterns (2 observations were made for each one of the two replicates), the results obtained were similar in each case. Statistical analyses were carried out using Student’s t test. In the tables, values followed by the same letter are not significantly different at P = 0.05.