SHORT COMMUNICATION

Proteins induced by cadmium in soybean cells

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Summary

The cadmium (Cd)-induced changes in protein pattern and identification of metal-stimulated polypeptides were analyzed in soybean cell suspension culture. The cell cultures were treated with various concentrations of Cd\textsuperscript{2+} (3–10 \textmu M) for 24, 48 and 72 h. The synthesis of \textsuperscript{[35S]}-labeled proteins and their accumulation were analyzed by SDS-PAGE, whereas the identification of selected protein bands was performed by mass spectrometry. It is shown that Cd induced the appearance of the following proteins in soybean cells: superoxide dismutase, histone H2B, chalcone synthase and glutathione transferase.

Cadmium, classified as a human carcinogen (Waisberg et al., 2003), is known to affect various essential processes in plants (Sanita di Toppi and Gabbrielli, 1999). However, the molecular mechanism used by plant cells to counter cadmium toxicity is not well understood. Screening of cadmium-responsive genes in \textit{Arabidopsis thaliana} (Suzuki et al., 2001) has shown that plants activated a set of genes involved in metal detoxification, protein refolding and wound healing. So far, the proteome analysis of the cadmium response was performed in \textit{Saccharomyces cerevisiae} (Vido et al., 2001).

Our studies are focused on the molecular mechanisms of cadmium response in plant cells. We are using soybean cell suspension culture as a model system and concentrations of Cd corresponding to those observed in contaminated soil (1–10 \textmu M) (Sobkowiak and Deckert, 2003, 2004; Sobkowiak et al., 2004). Here we present the Cd-induced changes in protein pattern and the identification of proteins, which are induced in soybean cells under cadmium stress.

Suspension culture of soybean (\textit{Glycine max} L. cv. Naviko) cells was maintained in modified B5 Gamborg medium, as previously described (Sobkowiak and Deckert, 2003). For the cadmium treatment experiments, freshly diluted subcultures were grown for 4 days, followed by addition of CdCl\textsubscript{2}. The final concentrations of Cd\textsuperscript{2+} in different cultures were: 3, 5, 6 and 10 \textmu M. Two milliliters of...
cell culture were labeled with $^{35}\text{S}$-methionine (740 kBq) (NEN Du Pont) at 0, 24, 48 and 72 h of Cd$^{2+}$ treatment, on rotary shakers for 1 h at 24°C, 100 rpm. After washing three times with fresh medium, the cells were quick-frozen in liquid N$_2$. Labeled cells were homogenized in 100 mM Tris–HCl pH 7.4, 15 mM MgCl$_2$, 15 mM EDTA, 75 mM NaCl, 1 mM DTE, 0.5 mM PMSF and 1 mM NaF. After centrifugation for 20 min at 4°C and 13 800 g, the supernatant was collected and protein content was determined by Bradford method (1976). The radioactivity of $^{35}$S-methionine incorporated into protein was determined in Liquid Scintillation Analyzer (Tricarb 2100 TR, Packard), according to Gwoźdź and Deckert (1989). The extracted protein samples were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1). Protein accumulation was visualized after Coomasie Blue staining of the gels (Fig. 1A), whereas the synthesis of $^{35}$S-labeled protein was detected by autoradiography (Fig. 1B).

The most pronounced effect of cadmium on soybean cells was the preferential accumulation of polypeptides of molecular weight 16 kDa (Fig. 1A) and the synthesis of polypeptides of 26 and 41 kDa (Fig. 1B). The appearance of these three polypeptides was dose- and time-dependent, as they are mostly detectable in the presence of higher concentration of Cd$^{2+}$ ($\geq$5 µM) and during longer metal treatment (48 and 72 h). Furthermore, the Cd$^{2+}$ induced the synthesis and accumulation of polypeptides of 36–38 kDa and reduced the level of others polypeptides (19, 30, 46, 62, 69, 78, 82 and 90 kDa) (Figs. 1A and B).

Protein bands, the intensities of which were enhanced in the presence of Cd$^{2+}$ (16, 26 and

Fig. 1. SDS-polyacrylamide gel electrophoresis of proteins accumulated (A) and synthesized (B) in soybean cell suspension cultures treated with Cd ions. The Cd$^{2+}$ at concentrations of 3, 5, 6 and 10 µM was added to 4-day-old soybean cell cultures and cells were labeled with $^{35}$S-methionine for 1 h at 0, 24, 48 and 72 h of the culture. The samples containing either the same amount of proteins (30 µg) – (A), or similar radioactivity (3 x 10$^5$ c.p.m.) – (B) were subjected to 12.5% (v/v) polyacrylamide slab gels and then either stained with Coomasie Brilliant Blue R-250 (A) or exposed to X-ray film (B). The white arrows indicate the polypeptides, which are increased, whereas the black arrows, the proteins which decreases in Cd-treated cells. The protein bands indicated by asterisk were identified by mass spectrometry.
41 kDa), were excised from the gels and analyzed in Mass Spectroscopy Lab (Institute of Biochemistry and Biophysics Polish Academy of Science, Warsaw, Poland). The analysis includes: in-gel tryptic digestion, reverse-phase and liquid chromatography (RP-HPLC) fractionation of resulting peptides, molecular mass analysis performed by using the nano-Z-spray ion source of Q-TOF electrospray mass spectrometer (Micromass), working with the regime of data dependent MS to MS/MS switch, allowing for a 3-s sequencing scan for each detected peptide. The data were analyzed using the MassLynx (Micromass) program. The protein fragments were identified by searching protein database using the software Mascot (www.matrixscience.com) (Perkins et al., 1999).

The identified proteins are arranged in Table 1 according to decreasing Masterscot scores in Cd²⁺-treated samples. The proteins derived from similar genes of various organisms have been omitted and only those with the highest score are included in Table 1.

The 16 kDa polypeptide bands from Cd²⁺-treated soybean cells contained proteins homologous to stress-induced protein SAM22, Cu,Zn-superoxide dismutase and histone H2B. SAM22 protein (Crowell et al., 1992), classified as pathogenesis-related PR-10 protein, was also present in control, Cd²⁺-untreated samples. The synthesis of PR10 proteins is enhanced by various biotic (Graham et al., 2003) and abiotic stress factors (Przymusiński et al., 2004), however their function in metal detoxification is still not known. On the other hand, antioxidant enzymes, such as Zn,Cu,Zn-SOD, are known constituents of plant defense reaction against metal toxicity (Rucińska et al., 1999; Schützendübel and Polle, 2002), also in soybean cells treated with Cd²⁺ (Sobkowiak et al., 2004). The appearance of Cd²⁺-induced histone H2B protein might be correlated with Cd²⁺-induced DNA damage and apoptosis (Fojtová and Kováří, 2000; Sobkowiak and Deckert, 2004; Wu et al., 2002). It was shown that the increased level of histone H2B is required in S. cerevisiae for repair of UV-induced DNA damage (Martini et al., 2002). The same role may play histone H2B protein in plant cells exposed to genotoxic stress factors, such as heavy metals. The 26 kDa polypeptide band contained various glutathione S-transferases (GST). Some of them are present both in control and Cd²⁺-treated cells (GST-8, GST-10, GST-12, GST-22), whereas the others (GST13, GST-14, GST-15) seems to be specifically induced by the metal. The function of GST enzymes in detoxification of wide range of xenobiotic compounds, including heavy metals, is well documented (Dixon et al., 1998; Frova, 2003). The third polypeptide band shows homology to chalcone synthase (CHS). The enzymes of phenylpropanoid pathway, including CHS, are activated in plants under various stress conditions.

### Table 1. Identification of proteins contained within three polypeptide bands (16, 26 and 41 kDa), which are induced in soybean cells by cadmium

<table>
<thead>
<tr>
<th>Protein name</th>
<th>NCBI accession no.</th>
<th>Mass</th>
<th>Presence (+) or absence (−) of protein within analyzed polypeptide band (Mascot score)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>16 kDa polypeptide band</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress-induced protein SAM22</td>
<td>134194</td>
<td>16 762</td>
<td>+(760)                                                               +(528)</td>
</tr>
<tr>
<td>Superoxide dismutase (Cu–Zn)</td>
<td>7433298</td>
<td>15 298</td>
<td>+(344)                                                               −</td>
</tr>
<tr>
<td>Histone H2B</td>
<td>21617908</td>
<td>16 364</td>
<td>+(232)                                                               −</td>
</tr>
<tr>
<td><strong>26 kDa polypeptide band</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione S-transferase GST-10</td>
<td>11385435</td>
<td>25 676</td>
<td>+(657)                                                               +(279)</td>
</tr>
<tr>
<td>Glutathione S-transferase GST-15</td>
<td>11385445</td>
<td>26 295</td>
<td>+(621)                                                               −</td>
</tr>
<tr>
<td>Glutathione S-transferase GST-8</td>
<td>11385431</td>
<td>25 893</td>
<td>+(543)                                                               +(218)</td>
</tr>
<tr>
<td>Glutathione S-transferase GST-22</td>
<td>11385459</td>
<td>24 315</td>
<td>+(289)                                                               +(243)</td>
</tr>
<tr>
<td>Glutathione S-transferase GST-12</td>
<td>11385439</td>
<td>26 700</td>
<td>+(230)                                                               +(256)</td>
</tr>
<tr>
<td>Glutathione S-transferase GST-14</td>
<td>11385443</td>
<td>25 340</td>
<td>+(218)                                                               −</td>
</tr>
<tr>
<td>Glutathione S-transferase GST-13</td>
<td>11385441</td>
<td>25 239</td>
<td>+(209)                                                               −</td>
</tr>
<tr>
<td><strong>41 kDa polypeptide band</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chalcone synthase 7</td>
<td>231799</td>
<td>43 189</td>
<td>+(1516)                                                              +(342)</td>
</tr>
<tr>
<td>Chalcone synthase 3</td>
<td>1345792</td>
<td>43 243</td>
<td>+(440)                                                               −</td>
</tr>
</tbody>
</table>
(Dixon and Paiva, 1995; Winkel-Shirley, 2002), although the Cd\textsuperscript{2+}-induced CHS protein synthesis has not been described so far.

Our results confirmed some of the existing data on plant cell response to heavy metals as well as suggest the participation of some other proteins in the complex pathway of plant cell reaction to abiotic environmental stress.

**Acknowledgements**

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**References**