Effects of cellulase-containing enzymes on auxin heterotrophic and autotrophic tobacco tissue cultures

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ABSTRACT During the digestion of cell wall by cellulases, rapid cell death of auxin heterotrophic and autotrophic tobacco tissue cells has been observed. Under these conditions a considerable amount of H$_2$O$_2$ is secreted into the medium. It is suggested that the rapid cell death induced by cellulysin is partly a result of the hypersensitive defense reaction against cellulysin from the parasitic fungus Trichoderma viride. The absolute amount of secreted H$_2$O$_2$ is higher in the case of heterotrophic cells, but the quantity of H$_2$O$_2$ is greater in the medium of auxin autotrophic cultures as compared with control cells without cellulysin. These cells emit more cellulase-dependent ethylene than the auxin heterotrophic line. Cellulysin induces an increasing intracellular H$_2$O$_2$ level only in the cells of auxin heterotrophic tissues. There are differences between the two cell lines in the change in cytosolic Ca$^{2+}$ concentration in response to exogenous H$_2$O$_2$.

KEY WORDS cellulase, cell death, secreted H$_2$O$_2$, tobacco tissue cultures

The production of active oxygen species is a rapid response of plant cells to elicitors or pathogens. Specifically, H$_2$O$_2$ has been implicated in the direct killing of pathogen cells and host cells as part of a hypersensitive response.

Cellulysin treatment raises the endogenous jasmonic acid level after 30 min, this being followed by ethylene emission after 2-3 h (Piel et al. 1997). The glucan elicitor causes rapid insolubilization of two cell wall structural proteins, to the accompaniment of a reduction in the yield of protoplast (Brisson 1994). Pure pectic oligomer polygalacturonic acid can generate a localized H$_2$O$_2$ concentration of approximately 1.2 mM (Legendre et al. 1993).

These data suggest that plant cells can detect the cell wall-degrading enzymes used for protoplasting, or the wall compounds released by them at their surfaces, and initiate an oxidative burst reaction response, resulting in direct killing of the suspension cells.

We have compared the effects of cellulase on auxin heterotrophic and autotrophic tobacco cell lines. The auxin autotrophic line, developed by long-term cultivation from the heterotrophic cultures, has a higher stress tolerance (salt, osmotic and temperature) than the heterotrophic line (Csiszár 2004).

Materials and Methods

Plant material
The callus cultures of Nicotiana tabacum SR1 plants were grown on solid MS medium (Murashige and Skoog 1962; 2 μM kinetin, 17.5 μM IAA and 0.45 μM 2,4-D). The auxin autotrophic cultures were maintained on the same medium without auxin. The suspension cultures were prepared from the callus tissues by gentle shaking on the same medium. For the experiments, 2-week-old callus tissues or 3-day-old suspension cells were used.

Measurement of ethylene production
Callus tissues (0.5 g) were kept and treated with cellulysin (0.1%) in 10 ml closed test tubes. Samples were withdrawn from the gas phase above the plant material and were analyzed in a gas chromatograph equipped with a flame ionization detector.

Estimation of cell death
Cell death was estimated according to Koehl et al. (2003), using 0.05% (w/v) Evans Blue dye.

H$_2$O$_2$ measurement
The modification of method described by Gestelen et al. (2003) was used.

Intracellular reactive oxygen species detection
The oxidative stress-sensitive dye 2',7'-dichlorofluorescin diacetate was used for the estimation of H$_2$O$_2$ (Allan and Fluhr 1997; Myhre et al. 2003). The fluorescence was detected by means of an Axiovert 200 M fluorescent microscope equipped with a camera.

Estimation of cytosolic Ca$^{2+}$ content
The cells were loaded with the calcium-sensitive dye FLUO 3AM for 2 h in the presence of 0.05% pluronic acid (Irving et al. 1992). The fluorescence intensity was followed with a Zeiss Axiowert 200 M fluorescent microscope (excitation at 450-490 nm, emission at 520 nm).
Results and Discussion

In earlier experiments, we did not succeed in attaining viable protoplast from tobacco calli or cells of suspensions with several kinds of cellulase-containing enzyme combinations in a wide range of concentrations. The cytoplasm of the cells aggregated, the nucleus disrupted in the first few hours of the digestion and the cells finally collapsed. In the current experiments, we used cellulysin, a crude cellulase from the parasitic fungus Trichoderma viride. The tissue cultures are generally maintained in a medium with a sucrose concentration of about 0.1 M. Incubation for 3 h in the solution with higher sucrose content used for protoplast isolation decreased the viability of the cells. The auxin heterotrophic line displayed a dose-dependent increase in cell death after incubation for 3 h in cellulysin-containing solutions. The proportion of the cells of auxin autotrophic line that were killed was much lower at all cellulysin concentrations. Higher concentrations for a longer time are usually used for protoplast isolation. It has been suggested that the rapid cell death induced by cellulase is a result of a hypersensitive defense reaction. A number of studies have implicated the causal involvement of active oxygen species in hypersensitive reactions (Able et al. 2000; Koehl et al. 2003). 400 μM H₂O₂ induced a 2–2.5-fold higher extent of cell death in both cultures as compared with the control. Cellulysin elevated the concentration of H₂O₂ in the incubation medium of both types of tissues. Despite the larger amount of H₂O₂ secreted by the auxin heterotrophic cultures, the effect of cellulysin was higher on the auxin autotrophic cells relative to the control (in 0.4 M sucrose). Elimination of H₂O₂ from the protoplasting medium with the antioxidant ascorbic acid did not rise the protoplast yield. Other factors, e.g. cell wall components could also interfere in the cell death. Piel et al. (1997) reported that treatment with cellulysin raises the level of endogenous jasmonic acid after 30 min, this being followed by a transient emission of ethylene after 2–3 h. In our experiment, ethylene production was enhanced for 4 h in the case by auxin autotrophic cultures and continued at an elevated level for a further 4 h in the auxin heterotrophic tissues. Because of the high auxin concentration of the medium the heterotrophic cells always produced more ethylene than the autotrophic cells. H₂O₂ has been implicated in direct the killing of pathogen cells and host cells (as part of the hypersensitive response), in the oxidative cross-linking of the plant cell wall and as an intracellular signal regulating gene expression. An increased fluorescence intensity in 2′,7′-dichlorofluorescin diacetate loaded cells can be observed only in the auxin heterotrophic cells after cellulysin treatment. The accumulation of H₂O₂ in the auxin heterotrophic cells after cellulysin treatment, and the changes in cytosolic Ca²⁺ level induced by H₂O₂ in the auxin autotrophic cells, indicate the differences in signal transduction in the two types of culture under the stress conditions caused by the cellulase-containing enzymes.

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References