Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis, 2005

Volume 49(1-2):23-24, 2005
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

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

Margit Szabó*, Jolán Csiszár, Emese Illés, Ágnes Szepesi, Bernadett Bartha

Department of Plant Physiology, University of Szeged, Szeged, Hungary

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_2O_2 is secreted into the medium. It is suggested that the rapid cell death induced by cellullysin is partly a result of the hypersensitive defense reaction against cellulysin from the parasitic fungus *Trichoderma viride*. The absolute amount of secreted H_2O_2 is higher in the case of heterotrophic cells, but the quantity of H_2O_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_2O_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_2O_2.
Acta Biol Szeged 49(1-2):23-24 (2005)

KEY WORDS

cellulase,
cell death,
secreted H_2O_2
tobacco tissue cultures

The production of active oxygen species is a rapid response of plant cells to elicitors or pathogens. Specifically, H_2O_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_2O_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.

*Corresponding author. E-mail: szabo@bio.u-szeged.hu

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_2O_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_2O_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 Axiovert 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 H_2O_2 induced a 2-2.5-fold higher extent of cell death in both cultures as compared with the control. Cellulysin elevated the concentration of H_2O_2 in the incubation medium of both types of tissues. Despite the larger amount of H_2O_2 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 H_2O_2 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. H_2O_2 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 H_2O_2 in the auxin heterotrophic cells after cellulysin treatment, and the changes in cytosolic Ca^{2+} level induced by H_2O_2 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.

Acknowledgments

This work was supported by the Hungarian National Science Foundation, OTKA T 030 259. The authors are grateful to Ádámné Meszlényi Mária for valuable assistance.

References

Able AJ, Guest DI, Sutherland MW (2000) Hydrogen peroxide yields during the incompatible interaction of tobacco suspension cells inoculated with Phytophthora nicotinae. Plant Physiol 124:899-910.

Allan CA, Fluhr R (1997) Two distinct sources of elicited reactive oxygen species in tobacco epidermal cells. Plant Cell 9:159-1572.

Brisson LF, Tenhaken R, Lamb C (1994) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. Plant Cell 6:1703-1712.

Csiszár J, Szabó M, Erdei L, Márton L, Horváth F, Tari I (2004) Auxin autotrophic tobacco callus tissues resist oxidative stress: the importance of glutathione S-transferase activities in auxin heterotrophic and autotrophic calli. J Plant Physiol 161:691-699.

Gestelen P, Asard H, Horemans N, Caubergs RJ (1998) Superoxide-producing NAD(P)H oxidases in plasma membrane vesicles from elicitor responsive bean plants. Physiol Plant 104:653-660.

Irving HR, Gehring CA, Parish RW (1992) Changes in cytosolic pH and calcium of guard cells precede stomatal movements. Proc Natl Acad Sci USA 89:1790-1794.

Koehl J, Osswald W, Kohn H, Elstner EF, Heiser I (2003) Different responses of two tobacco cultivars and their cell suspension cultures to quercinin, a novel elicitin from Phytophthora quercina. Plant Physiol Biochem 41:261-269.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissues cultures. Physiol Plant 15:473-479.

Myhre O, Andersen JM, Aarnes H, Fonnum F (2003) Evaulation of probes 2',7'-dichlorofluorescin diacetate, luminal, and lucigenin as indicators of reactive species formation. Biochem Pharmacol 65:1575-1582.

Piel J, Atzorn R, Gabler R, Kühnemann F, Boland W (1997) Cellulysin from the plant parasitic fungus Trichoderma viride elicits volatile biosynthesis in higher plant via the octadecanoid signaling cascade. FEBS Letters 416:143-148.