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CELLULASE AND COPPER CHLORIDE TREATMENTS OF PLANT CELLS INDUCE CHANGES IN THE COMPOSITION OF THE PLASMA MEMBRANE LIPIDS

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SYNOPSIS

Treatment of tobacco cell suspensions with cellulase elicited the accumulation of phytoalexins and induced rapid changes in the levels of several lipid classes, especially the four steryl lipid classes. The levels of acylated sterol glycosides were increased in proportion to the concentration of cellulase added. In contrast, the levels of sterol esters and phytoalexins were only increased with the low concentrations of cellulase. Treatment with copper chloride did not elicit phytoalexins, but did cause a several-fold increase in the levels of acylated sterol glycosides.

INTRODUCTION

Tobacco cell suspensions produce phytoalexins when infected with certain pathogens or when treated with cellulase or fungal elicitor (Chappell et al., 1987, and Threlfall & Whitehead, 1988). Using a new HPLC technique (Moreau et al., 1990), we investigated the effect of treatment of tobacco cell suspensions with phytoalexin elicitor, cellulase, on the lipid classes in those cells. We recently reported that treatment of cells with cellulase (1 µg/10 ml cells) elicited capsidiol and other phytoalexins, caused a 2-3 fold increase in the levels of sterol esters (SE) and acylated sterol glycosides (ASG), with a concomitant decrease in the levels of free sterols (St) and sterol glycosides (SG) (Moreau & Preisig, 1991). These rapid changes in the composition of steryl lipids may have important physiological implications, since steryl lipids are thought to be localized in the plasma membrane. Kesselmeier et al. (1987)

reported a similar induction of ASG and SG during the preparation of protoplasts from oat leaves. Whitaker et al. (1990) also reported that ozone treatment of snapbean leaves caused an induction of ASG and SG. In this report we investigated the effect of various concentrations of cellulase on capsidiol elicitation and sterol "esterification" in tobacco suspension cells.

MATERIALS AND METHODS

Cell suspension cultures of *Nicotiana tabacum* Ky 14, were obtained from Dr. Joseph Chappell, University of Kentucky, and were maintained as previously described (Chappell et al. 1987). Cellulase (Onozuka RS from *Trichoderma viride*) was obtained from Karlan Chemical Co., Santa Rosa, CA.

After elicitor treatment, cells were separated from the culture filtrate by gentle vacuum filtration. The culture filtrate was extracted for phytoalexins 2 times; each with one volume of diethyl ether. The cells were homogenized with a Polytron homogenizer, and the lipids were extracted according to the method of Bligh and Dyer (1959). HPLC-FID analyses of lipid classes were performed as previously described (Moreau et al., 1990). Capsidiol was analyzed using a similar HPLC-FID method as recently reported (Moreau et al., 1992). Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

RESULTS AND DISCUSSION

Treatment of the cells with cellulase induced the accumulation of capsidiol in the medium (Fig. 1). Among the three cellulase concentrations tested, the intermediate treatment, 1 μ g cellulase/ 10 ml cells, elicited the highest levels of capsidiol at both 6 and 24 h.

Among the intracellular steryl lipids, cellulase treatment induced a pronounced concentration-dependent effect. Although the lowest concentration of cellulase, 0.1 μ g/flask, induced only low levels of capsidiol accumulation, it caused the highest increases in the levels of StE (a 20% increase at 6 h and an 80% increase at 24 h). The intermediate cellulase concentration (1 μ g cellulase/flask) also caused an increase in StE, but to a lesser extent than the lower concentration. In contrast, the highest cellulase concentration caused a significant (>40%) reduction in the levels of StE at both 6 and 24h.

The levels of ASG increased in proportion to the concentration of cellulase elicitor. The response was slight with the lowest cellulase concentration but it was very high (about 5-fold) with the highest cellulase treatment. The levels of St and SG were reduced in proportion to increasing levels of cellulase treatment.

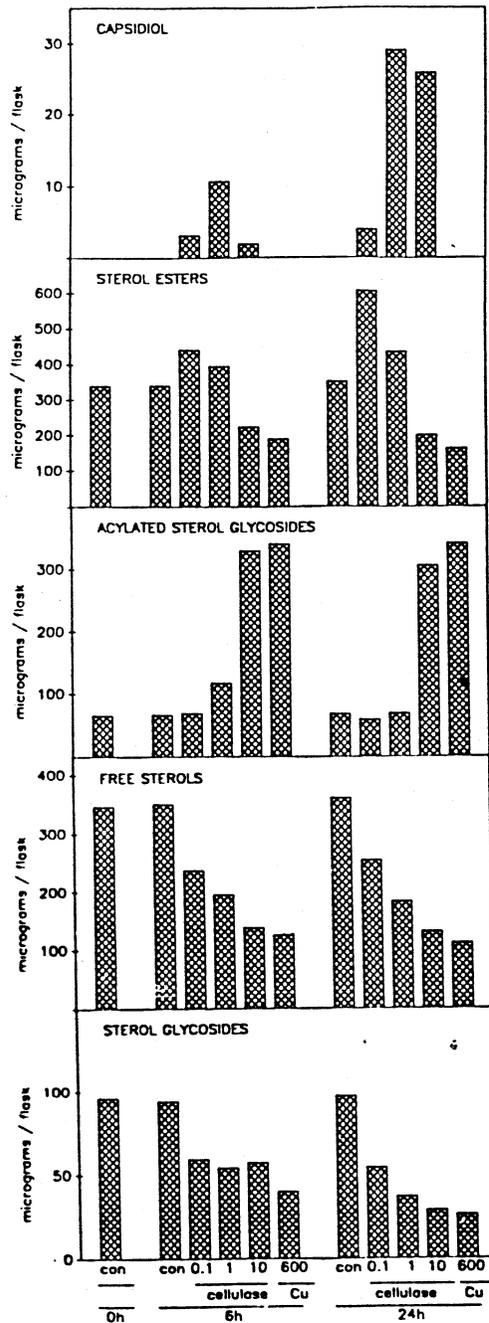


Figure 1. Changes in the levels of capsidiol (extracellular) and sterol lipids (intracellular) during the treatment of tobacco suspension cells by cellulase (0.1, 1.0, and 10 µg/10 ml cells) and copper chloride (600 µg/10 ml cells = 0.5 mM). Data presented are the mean values of duplicate samples from one representative experiment that was repeated three times.

Copper chloride has been reported to be an effective elicitor of isoflavonoid phytoalexins in peas and other legumes. Although treatment of tobacco cells with copper chloride did not elicit phytoalexins, it did cause significant changes in the levels of steryl lipids, with a very large increase in the levels of ASG (about 5-fold). For each of the four steryl lipids measured, the levels of each was similar at the two time points tested for both the 10 μ g cellulase/flask treatment or the CuCl₂ treatment.

In summary, these experiments indicate that two types of treatments can cause very different effects on the same plant cells. Treatment of the cells with cellulase elicited the production of capsidiol and caused a concentration-dependent induction of the esterification of steryl lipids. In contrast, copper chloride treatment caused a comparable induction in the esterification of steryl lipids, but did not elicit the production of phytoalexins.

In the previous report of induction of the levels of ASG and SG by cell wall degrading enzymes (Kesselmeier et al., 1987), much higher levels of cellulase were used (100,000 μ g cellulase/10 ml cells), in contrast to the 0.1 to 10 μ g / 10 ml cells used in this study. Since ozone treatment has also been reported to cause a similar esterification of steryl lipids (Whitaker et al., 1990), the possibility that other chemical and environmental treatments can induce the same types of changes needs to be considered. It has been reported that steryl lipids comprise about 55% of the total lipids in the plasma membrane (Lynch and Steponkus, 1987), however the actual physiological role of the various steryl lipid classes in this membrane is not known. Any chemical or environmental treatment that causes a net esterification of these lipids may have important physiological implications.

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