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Chitinase in bean leaves: induction by ethylene, purification, properties, and possible function

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Abstract. Ethylene induced an endochitinase in primary leaves of *Phaseolus vulgaris* L. The enzyme formed chitobiose and higher chitin oligosaccharides from insoluble, colloidal or regenerated chitin. Less than 5% of the total chitinolytic activity was detected in an exochitinase assay proposed by Abeles et al. (1970, Plant Physiol. **47**, 129–134) for ethylene-induced chitinase. In ethylene-treated plants, chitinase activity started to increase after a lag of 6 h and was induced 30 fold within 24 h. Exogenously supplied ethylene at 1 nl ml^{-1} was sufficient for half-maximal induction, and enhancement of the endogenous ethylene formation also enhanced chitinase activity. Cycloheximide prevented the induction. Among various hydrolases tested, only chitinase and, to a lesser extent, β-1,3-glucanase were induced by ethylene. Induction of chitinase by ethylene occurred in many different plant species. Ethylene-induced chitinase was purified by affinity chromatography on a column of regenerated chitin. Its apparent molecular weight obtained by sodium dodecyl sulfate-gel electrophoresis was 30,000; the molecular weight determined from filtration through Sephadex G-75 was 22,000. The purified enzyme attacked chitin in isolated cell walls of *Fusarium solani*. It also acted as a lysozyme when incubated with *Micrococcus lysodeikticus*. It is concluded that ethylene-induced chitinase functions as a defense enzyme against fungal and bacterial invaders.

Key words: Chitinase – Defense (against bacteria, fungi) – Enzyme induction – Ethylene – Lysozyme – *Phaseolus* (chitinase).

Abbreviations: ACC = 1-aminocyclopropane-1-carboxylic acid; AVG = aminoethoxyvinylglycine; GlcNAc = N-acetylglucosamine

Introduction

Much information is available on the action of ethylene in the growth and development of plants (Abeles 1973; Lieberman 1979). However, little is known about the significance of stress ethylene (Yang and Pratt 1978). One of its postulated functions is an induction of defense reactions against pathogens (Pegg 1976; Yang and Pratt 1978). In the hope of learning more about this possibility, we set out to examine more closely an etylene effect which appeared to be related to the plant's defense, i.e., the induction of chitinase in bean leaves (Abeles et al. 1970). Chitinase has no known function in growth and development. Since its substrate, chitin, is an important component of fungal cell walls, it has been postulated to have an antifungal function (Abeles et al. 1970; Pegg 1977).

Until now, the actual enzymatic properties of plant chitinase were not clear. The enzyme described and partially purified by Abeles et al. (1970) appeared to be an exochitinase which liberated monomeric N-acetylglucosamine from colloidal chitin. Abeles et al. (1970) devised an assay specific for exochitinase which was later employed by others to detect plant chitinase (Pegg and Vessey 1973; Wargo 1975; Nichols et al. 1980; Pegg and Young 1981). However, an earlier report described an endochitinase in bean seeds (Powning and Irzykiewicz 1965) and, more recently, an endochitinase was purified from wheat germ (Molano et al. 1979).

We initially determined which kind of chitinase was present in bean leaves and found that the exochitinase assay of Abeles et al. (1970) did not reflect the true chitinolytic potential of the tissue. The chitinase induced by ethylene was an endochi-