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Plant Gene Register

Cloning of a Class III Acidic Chitinase from Chickpea[1]

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From chickpea (*Cicer arietinum* L.), one β-1,3-glucanase and two chitinases were purified and characterized (Vogelsang and Barz, 1993). The β-1,3-glucanase and one chitinase each with a basic isoelectric point (class I) were shown to be localized in vacuoles, whereas the second class III acidic chitinase revealed an extracellular localization (Mackenbrock et al., 1992). Here we report on the cDNA cloning and nucleotide sequence of this chickpea class III acidic chitinase (Table I).

A λgt10 cDNA library was constructed from poly(A)$^+$ RNA extracted from chickpea cell cultures 7 h after elicitor treatment (Tiemann et al., 1991). An oligonucleotide was synthesized according to the N-terminal amino acid sequence of the purified class III chitinase (Vogelsang and Barz, 1993) and used to screen the cDNA library. Approximately 10^6 plaques were screened and about 40 plaques showed positive hybridization with the ^{32}P-labeled oligonucleotide. After plaque purification, the size of the inserts from 20 isolated phage plaques was determined by agarose gel electrophoresis. Inserts showed an average size of 1100 nucleotides. Insert DNA obtained from two phage plaques was prepared and sequenced (Sanger et al., 1977) after subcloning into a plasmid vector (pT3T7, Pharmacia, Freiburg, Germany).

The cDNA contained an open reading frame encoding a protein with a highly hydrophobic signal peptide of 23 amino acids (Kyte and Doolittle, 1982) and a mature protein of 270 amino acids. The N-terminal amino acid sequence previously determined for the chitinase (Vogelsang and Barz, 1993) lacked the signal peptide and has now been found to be in accordance with the amino acid sequence of the mature protein deduced from the cDNA. Furthermore, the calculated mol wt of the mature protein (28,668) was identical to the molecular mass (28 kD) determined for the purified acidic chitinase by SDS-PAGE (Vogelsang and Barz, 1993).

The deduced amino acid sequence of the chickpea chitinase was compared with class III chitinases from *Cucumis sativus* (Metraux et al., 1989), *Nicotiana tabacum* (Lawton et al., 1992), and *Arabidopsis thaliana* (Samac et al., 1990). When gaps that permit maximum homologies were introduced, amino acid homologies of 63, 67.7, and 61.5%, respectively, were observed.

Table I. *Characteristics of class III chitinase cDNA from chickpea*

Organism:
Cicer arietinum L. cv ILC 3279.
Function:
Acidic class III chitinase (EC 3.2.1.14), secreted into the extracellular space.
Clone Type:
cDNA, full length.
Source:
cDNA library in a λgt10 vector constructed from poly(A)$^+$ RNAs of chickpea cell cultures treated with elicitor for 7 h.
Techniques:
The cDNA library was screened with a radiolabeled oligonucleotide deduced from the N-terminal amino acid sequence of the purified acidic chitinase, subcloned into pT3T7 vector (Pharmacia, Freiburg, Germany).
Sequencing Strategy:
Denatured plasmid DNA, dideoxynucleotide chain termination method, primer walking.
Features of mRNA Structure:
cDNA contains open reading frame encoding a protein with 293 amino acids, polyadenylation signal, and poly(A) tail.
Method of Identification:
Sequence comparison with N-terminal amino acid sequence and published protein sequences of class III chitinases.
Structural Features of the Protein:
Signal sequence is 23 amino acids, hydrophobic; mature protein is 270 amino acids, calculated M_r of 28,668.
Antibodies:
Available on request.

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The GenBank accession number for the sequence reported in this article is X70660.

LITERATURE CITED

Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol **157:** 105–132

Lawton K, Ward E, Payne G, Moyer M, Ryals J (1992) Acidic and basic class III chitinase mRNA accumulation in response to TMV infection of tobacco. Plant Mol Biol **19:** 735–743

Mackenbrock U, Vogelsang R, Barz W (1992) Isoflavone and pterocarpan malonylglucosides and β-1,3-glucan- and chitin-hydrolases are vacuolar constituents in chickpea (*Cicer arietinum* L). Z Naturforsch **47c:** 815–822

Metraux JP, Burkhart W, Moyer M, Dincher S, Middlesteadt W, Williams S, Payne G, Carnes M, Ryals J (1989) Isolation of a complementary DNA encoding a chitinase with structural homol-

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ogy to bifunctional lysozyme/chitinase. Proc Natl Acad Sci USA **86:** 896–900

Samac DA, Hironaka CM, Yallaly PE, Shah DM (1990) Isolation and characterization of the genes encoding basic and acidic chitinase in *Arabidopsis thaliana*. Plant Physiol **93:** 907–914

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA **74:** 5463–5467

Tiemann K, Inze D, Van Montagu M, Barz W (1991) Pterocarpan phytoalexin biosynthesis in elicitor-challenged chickpea (*Cicer arietinum* L.) cell cultures: purification, characterization and cDNA cloning of NADPH:isoflavone oxidoreductase. Eur J Biochem **200:** 751–757

Vogelsang R, Barz W (1993) Purification, characterization and differential hormonal regulation of a β-1,3-glucanase and two chitinases from chickpea (*Cicer arietinum* L.). Planta **189:** 60–69