Cloning of a Class III Acidic Chitinase from Chickpea

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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 Xgt10 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 10⁶ plaques were screened and about 40 plaques showed positive hybridization with the ²⁵P-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.

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


