Amino-terminal sequence of ethylene-induced bean leaf chitinase reveals similarities to sugar-binding domains of wheat germ agglutinin

John Lucas*, Agnes Henschen, Fritz Lottspeich, Urs Voegeli+ and Thomas Boller+

Department of Protein Chemistry, Max Planck Institut für Biochemie, am Klopferspitz, 8033 Martinsried bei München, FRG and +*Botanisches Institut der Universität, Schönbeinstr. 6, CH-4056 Basel, Switzerland*

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Chitinase has been reported for many plant species. A role in plant defense has been suggested for plant chitinases, due to their ability to degrade fungal cell wall chitin. Increased levels of chitinase are induced in bean plants by the plant hormone ethylene [(1983) Planta 157, 22–31]. The amino acid sequence of positions 1–30 of ethylene-induced bean leaf chitinase was determined and found to possess considerable sequence homology to wheat germ agglutinin and hevein. The implication of a lectin-related structure for the enzymatic specificity of chitinase is discussed.

Chitinase (Bean) Ethylene Amino acid sequence Lectin Plant pathology

1. INTRODUCTION

Chitinase has been reported in such taxonomically diverse plant species as wheat and oak [1,2]. Several indications suggest that plant chitinase may play a defense role against pathogenic fungi. The enzyme has no apparent function in plant growth and development since its substrate, chitin, is uncommon in plants but is an important component of fungal cell walls [3,4]. Chitinase activity has been reported to increase in pea and tomato plants during fungal invasion [5–7]. The purified enzyme from bean plants is capable of releasing chitin fragments from isolated fungal cell walls [8]. In bean plants, chitinase activity is induced 30-fold by the plant hormone ethylene [8–10]. The properties of the chitinase purified from the leaves of ethylene-treated plants [8] resembles those so far reported for chitinases isolated from tomato stem and wheat germ [1,6,7]. They are basic proteins of M_r approx. 30000 and function as endochitinases by releasing oligosaccharide fragments 2 units or larger in length from insoluble chitin. To obtain information concerning the chitinase structure and to examine similarities to other plant proteins, the amino acid sequence of bean leaf chitinase was investigated by protein sequencing.

2. MATERIALS AND METHODS

Chitinase from the leaves of ethylene-treated bean plants (*Phaseolus vulgaris*) was isolated by affinity chromatography on a chitin column as in [8]. Cysteine residues of chitinase were converted to *S*-carboxymethylcysteine or *S*-methylcysteine by reduction with mercaptoethanol in denaturant buffer (8 M urea, 0.1 M Tris-acetate, pH 5) and alkylation with iodoacetic acid or methyl iodide (from Merck) [11,12]. Samples were desalted by dialysis (Spectrapor 2) against 1 M acetic acid and concentrated by evaporation of solvent with a stream of nitrogen. Amino acid compositions were determined from acid hydrolysates of native, *S*-

* Present address: Pharmaceutical Research Laboratories R-1056.3.30, Ciba-Geigy Ltd, CH-4002 Basel, Switzerland

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carboxymethyl and *S*-methylchitinase using a Biotronic amino acid analyser [13]. Protein sequencing by Edman degradation was carried out on a prototype sequenator [14]. Phenylthiohydantoin derivatives of amino acids were determined by high-pressure liquid chromatography using an isocratic system [15]. Searches for sequence homology were made by computer using the available program and sequence data base [16].

3. RESULTS AND DISCUSSION

Amino acid sequence analysis of the *S*-carboxymethylated chitinase revealed an unusually high frequency of cysteine residues. This sequence was verified by repeating the sequence analysis on *S*-methylchitinase which provided an additional derivative for the verification of cysteine residues [12,15]. The identity of the first 30 amino acid positions of chitinase could be identified and are illustrated in fig.1. Comparison of the sequence with presently known sequences compiled in the sequence data base revealed a close homology, at identical positions without insertions or deletions, to sequences of wheat germ agglutinin (WGA) and hevein (fig.1). WGA is a 20 kDa lectin with a binding specificity for aminoacetylated amino sugars in saccharides, glycoproteins and cellular surfaces [17,18]. Its structure consists of four 43-residue-long isostructural sugar-binding domains, each assuming a loop-like structure through

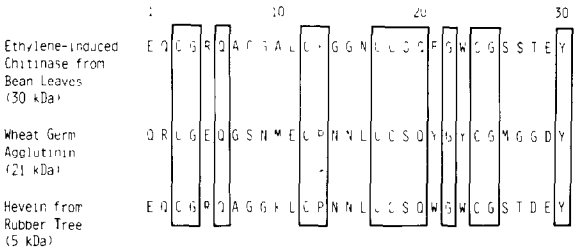

Fig.1. Sequence of the first 30 amino acid residues of ethylene-induced chitinase from bean leaves (single-letter code). Also shown is the alignment with the sequence positions 1–30 of wheat germ agglutinin [19] and hevein [20]. Identical amino acids in the sequences of chitinase, WGA and hevein are enclosed. The molecular masses of each protein are shown in brackets.

disulfide bonding [18,19]. A strong homology was also found between the sequences of chitinase and hevein, a 5 kDa protein of unknown function present in rubber tree latex [20].

Upon inspection of the sequences of chitinase, WGA and hevein, a considerable number of positions are preserved for the 3 proteins. These invariant positions are enclosed in boxes (fig.1) and involve primarily cysteine residues and residues adjacent to cysteine, thus suggesting a structural significance for these regions. In the case of the sugar-binding domains of WGA, X-ray crystallographic studies have shown that these disulfide bonds participate in sugar-binding modes to aminoacetylated aminosaccharides and are essential for the structural integrity of these domains [18,19]. In addition to the positions enclosed in boxes in fig.1, further homology is observed between the sequences of chitinase and the hevein. Whereas 43% of the chitinase sequence in fig.1 is identical to that of WGA, 79% identity is found between the chitinase and hevein sequences. This level of homology corresponds to the closer taxonomical relationship of the bean plant to rubber tree than to wheat.

Both WGA and hevein are cysteine-rich proteins having 18% cysteine, as deduced from their amino acid sequences [19,20]. However, the concentration of cysteine in bean leaf chitinase is only 4%, as deduced from the amino acid analysis of the *S*-carboxymethylated enzyme (not shown) which is comparable to the reported amino acid composition of wheat germ chitinase [1]. This discrepancy between the concentration of cysteine in chitinase and WGA suggests that the remaining uncharacterized parts of the chitinase sequence differ from WGA.

The finding that chitinase contains a structure resembling WGA provides an insight into the mode of chitinase specificity towards fungal cell walls. The microfibril network of many fungi consists primarily of glucans and chitin [4]. Chitin is composed of repetitive units of aminoacetylglucosamine which are recognized by lectins such as WGA. It can therefore be concluded that fungal chitin is recognized by the amino-terminal region of chitinase which, on the basis of sequence homology, bears considerable resemblance to the WGA domains which bind aminoacetylated aminosugars.

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