FEBS Letters 350 (1994) 159–163

FEBS 14366

Minireview

Structural features of plant chitinases and chitin-binding proteins**

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Received 18 May 1994; revised version received 5 July 1994

Abstract

Structural features of plant chitinases and chitin-binding proteins are discussed. Many of these proteins consist of multiple domains, of which the chitin-binding hevein domain is a predominant one. X-ray and NMR structures of representatives of the major classes of these proteins are available now, and are used to describe the structures of the other ones. Conserved positions of Cys residues can be taken as evidence for identically located disulfide bridges or cysteine residues. The current classification of chitinases is unsatisfactory and needs to be replaced by an evolutionarily more correct one. As the currently known three-dimensional structures of chitinases are those from barley and the rubber tree, *Hevea brasiliensis*, it is proposed to adopt the designation b-type (classes I, II and IV) and h-type (classes III and V) chitinases, respectively.

Key words: Chitinase; Chitine-binding protein; Lectin; Agglutinin; Disulfide bridge; PR protein

1. Introduction

During the last ten years many chitinases and chitin-binding proteins from plants have been discovered and further characterized [1–4]. Especially the study of pathogenesis-related (PR) and similar proteins from species like tobacco [5] stimulated many developments in this field [3,6]. A classification of chitinases and related proteins, often occurring in the same plant species, has evolved in the course of time. Multi-domain proteins consisting of several chitinase and chitin-binding protein domains occur frequently. Three-dimensional structures of reprensentatives of the major classes of these proteins have been determined either by X-ray diffraction [7–10] or NMR spectroscopy [11,12]. Structural features of chitinases and chitin-binding proteins can be compared on the basis of homology with these proteins with known three-dimensional structures.

2. b-type (class I, II and IV) chitinases

Fig. 1 shows a schematic representation of the structures of this family of homologous chitinases. Typical class I chitinases consist of an N-terminal chitin-binding hevein domain followed by a chitinase domain [13–20] and a vacuolar targeting sequence [21] which is cleaved off during processing of the proprotein [22]. Three-dimensional structures have been determined for the hevein domain in hevein and WGA [7,8,11] and for barley chitinase CHI26, which consists of only one single domain [9]. Disulfide bridges are very well conserved structural features in extracellular and other proteins synthesized at the rough endoplasmic reticulum [23], and conserved positions of Cys residues in chitinases can be taken as evidence of identically located disulfide bridges or free cysteine residues. Barley chitinase has three disulfide bonds and one free cysteine residue (at position 273 in the alignment of Fig. 1). It is clear from Fig. 1 that both Cys residues forming a disulfide bridge are either conserved in other members of this chitinase family or have been replaced or deleted together. There are a number of not very well conserved Cys residues in the other chitinase sequences (Fig. 1). However, inspection of the published stereo figure of the barley chitinase structure [9] does not suggest the presence of other disulfide bridges. There may be only an additional disulfide bridge between Cys^{15} in the hevein domain and Cys^{44} in the hinge region between the hevein and chitinase domains in chitinases from rice [24,25] and maize [26]. Inspection of the stereo figure of the barley chitinase structure also shows that extensive deletions in the sequences of class II [19,27,28] and class IV [26,29–31] chitinases are located in external loops of the structure. However, this cannot be said with equal certainty of the rather short deletion near residue 215 in class IV chitinases [31]. All residues between the first two Cys residues in the hevein domain, except one glycine residue, are deleted in class IV chitinases. This deletion can be accommodated in the structure of the hevein domain [7,8,11]. C-terminal extensions in rice CHT2 [25] and UDA [32] (lower case crosses in Fig. 1) may indicate vacuolar targeting signals.

Originally class I and class II chitinases were distinguished by the presence of a hevein domain and a vacu-

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**Dedicated to Dr. B.L. Archer, who discovered hevein in 1960 (Biochem. J. 75, 236–240).

SSDI 0014-5793(94)00753-5

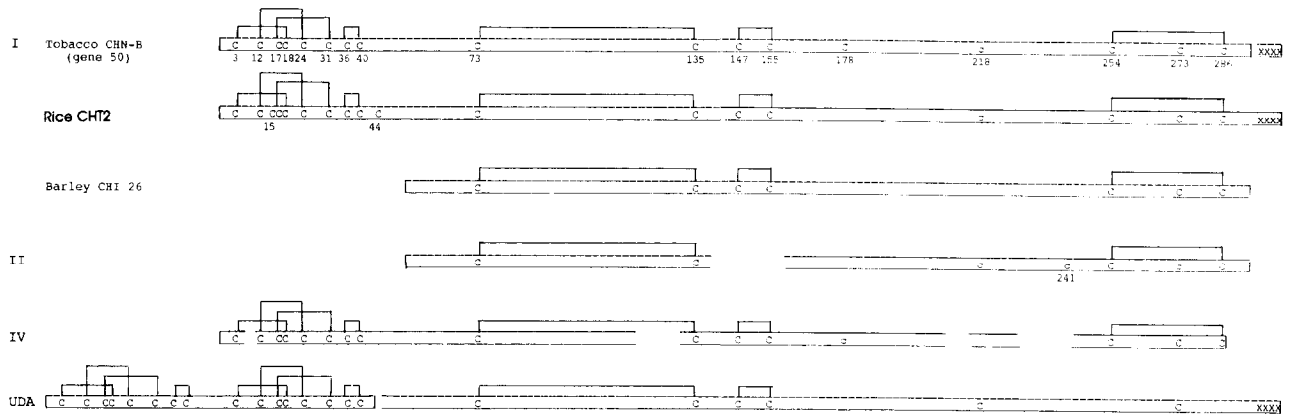

Fig. 1. Schematic representation of primary structures of several representatives of homologous b-type (class I, II and IV) chitinases from plants. Indicated are positions of (half-)cyst(e)ine residues numbered according to the sequence of tobacco CHN-B [14], and positions of disulfide bridges as derived from the X-ray structure of Barley CHI 26 chitinase [9,33], or from homology with this chitinase or with hevein domains in other proteins (legend Fig. 3). Lower-case c's indicate Cys residues which are not conserved, and interruptions in the bars are deletions. The crossed C-terminus in the tobacco sequence indicates the vacuolar targeting sequence, which has been removed from the mature enzyme [22]. Putative targeting signals in one of the rice sequences [25] and UDA [32] are in lower-case crosses.

olar C-terminal targeting signal in the basic class I enzymes and their absence in the acidic, extracellular class II enzymes. However, chitinases sequenced and characterized later did not fit into this classification, and other classes have been proposed [2,3]. Class IV chitinases are acidic and basic extracellular proteins with an N-terminal hevein domain [26,29–31]. They have the shortest chain lenghts, with four internal deletions which have already been discussed, and the C-terminus at the disulfide-linked Cys^{286} (Fig. 1). The primary structures of class IV chitinases are 35–50% identical with those of the class I and class II ones, and more than 60% among themselves.

The primary structures of class I and class II chitinases are 60–65% identical, while there is more than 70% identity within the classes. However, the classification of chitinases from grasses (rice [24,25], maize [26], and barley [33]) is less straightforward. They do not have the deletion near residue 150, and have more sequence similarities with the class I enzymes than with the class II ones. However, the presence or absence of an N-terminal hevein domain, or of a C-terminal vacuolar putative targeting signal is a variable feature, even among closely related enzymes from the same plant species [25,33].

A unique sequence is that of the precursor of stinging-nettle (*Urtica dioica*) agglutinin (UDA), which has two successive hevein domains in the mature lectin [34] followed by a chitinase domain which is cleaved off posttranslationally [32]. A bacterially expressed chitinase domain has chitinase activity. This domain has 40–46% sequence identity with the class I chitinases. Special structural features are the absence of the C-terminal disulfide bridge present in the other chitinases, and the presence of a putative vacuolar targeting signal at the C-terminus.

The hevein domain is not a targeting signal and does not play a role in the catalytic activity of chitinases; however, its presence is essential for chitin binding and for the substrate affinity and antifungal properties of these enzymes [35]. The presence of separate carbohydrate-binding and catalytic domains is a generally observed feature in enzymes hydrolysing solid carbohydrate substrates, and also occurs in cellulases and amylases [36]. Little is known about the active- site residues of these chitinases. Verburg et al. [37] have shown that a chitinase from maize becomes inactive after selective modification of a tyrosine residue. The authors suggest that this tyrosine is part of the catalytic site. However, it is replaced by phenylalanine or asparagine in other chitinases (class II chitinases). They reasoned that tyrosine may play a role as a general acid catalyst in the chitinase-catalyzed reaction, because it plays a similar role in the carboxypeptidase reaction [37]. However, the argument is not valid, since its role in the latter case is a textbook example of an incorrectly assigned active-site residue as demonstrated by site-directed mutagenesis [38].

Recently a striking similarity has been described between the three-dimensional structures of barley chitinase and lysozymes from animals and phages [39]. The very well conserved glutamic acid at position 117 may be the active-site residue, although this residue is replaced by alanine in the C-terminal domain of UDA , which has chitinase activity [32].

3. h-type (class III and V) chitinases

Class III and V chitinases do not show any homology with the chitinases discussed above. The X-ray structure

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                72                                                    127
Hevamine        IKVMLSLGGGIGSYTLASQADAKNVADYLWNNFL-GGKSSSRPLGDAVLDGIDFDIE
Serratia ChiB   LRIMFSIGGWYYSNDLGVSHANYVNAVKTPAARTKFAQSCVRIMKDYGFDGVDIDWE
Chitinase V     VKTFLSIAGGRADTTA------YGIMARQPNSRKSFIDSSIRLARQFGFHGLDLDWE
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Fig. 2. Distant sequence similarity of the putative active-site regions of a class III chitinase (hevamine [40]), a bacterial exochitinase (Serratia ChiB [48]) and class V chitinase from tobacco [49]. Identical residues in the three sequences (numbered according to the sequence of hevamine [40]) are indicated in bold face.

of hevamine, a class III chitinase/lysozyme from *Hevea brasiliensis* latex [40] has been determined [10]. Homologous chitinases or chitinase domains occur in plants [18,41–44] and fungi [45,46]. Although sequence identity can be as low as 35%, the positions of six Cys residues, which form three disulfide bridges in hevamine [10,40], are conserved. Probably glutamic acid at position 127 is the catalytically active residue. There is a distant sequence relationship with several prokaryotic exochitinases [47,48] in this region of the molecule (Fig. 2). Recently a new tobacco chitinase has been described [49], which was designated as a class V chitinase because no homology was found with other chitinases. However, a distant sequence relationship was reported with the same sequence fragments of prokaryotic exochitinases as those showing similarity with the class III chitinases (Fig. 2). This may imply that class III and V chitinases diverged from a common ancestor, perhaps before the split of eukaryotes and prokaryotes.

4. Chitin-binding proteins

Schematic representations of chitin-binding proteins are presented in Fig. 3. The structure of wheat germ agglutinin (WGA) and similar proteins from barley and rice has been reviewed recently [1,50]. They consist of four hevein domains and a posttranslationally cleaved vacuolar targeting signal at the C-terminus, which is glycosylated.

Hevein is formed by processing of a larger precursor [51,52]. Wound-induced proteins from potato are homologous with the hevein precursor [53], but studies at the protein level have not yet been published. In tobacco and tomato, proteins homologous with the hevein precursor occur. Both vacuolar proteins with an N-terminal hevein domain, which is not cleaved off [54], and extracellular proteins without such a domain [55,56] have been identified. A protein homologous with the C-terminal domain of the hevein precursor is barwin, which has been isolated from barley seed [57]. Its three-dimensional structure has been determined [12]. It is not yet known whether barwin is formed from a precursor with an N-terminal hevein-like domain or not. Probably the same protein has been isolated and sequenced by Hejgaard et al. [58], although there is one difference in the published sequences. The sequences of the homologues of the C-terminal domain of the hevein precursor are 60–90% identical, with identical positions of six Cys residues, which form three disulfide bridges in barwin [12,57]. The DNA-derived sequences of the hevein precursor [51], of one of the wound-induced potato proteins [53] and of the vacuolar protein from tobacco [54] have C-terminal extensions compared to the other sequences. It has been demonstrated that, for the tobacco protein, this is a vacuolar targeting signal which has been removed from the mature protein [54].

Barwin has affinity for chitin oligomers [12] and chitin, and also has antifungal properties [58]. However, in the other members of this family these activities are dependent on the presence of the hevein domain [52,54–56].

The third group of chitin-binding proteins consists of the antimicrobial proteins Ac-AMP1 and Ac-AMP2 from the seeds of amaranth [59], which are formed from a larger precursor [60]. These proteins are homologous with hevein, but the loop between the first two Cys residues is shorter, and the C-terminal part with a separate disulfide bridge is deleted (Fig. 3).

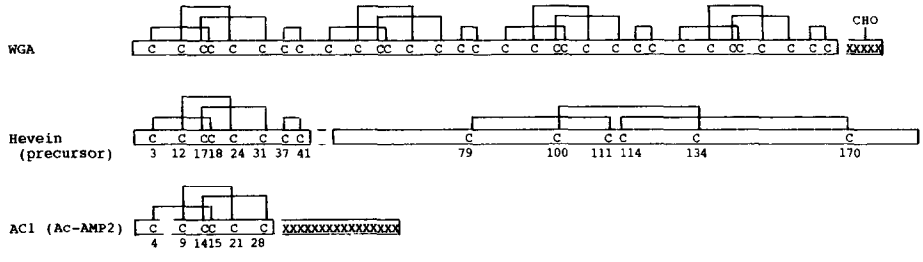

Fig. 3. Schematic representation of primary structures of lectins and chitin-binding plant proteins with hevein domains. WGA, wheat germ agglutinin [50]. The crossed C-terminus indicates the glycosylated (CH0) vacuolar targeting signal, which has been removed from the mature protein [50]. Positions of disulfide bridges in the C-terminal fragment of the hevein precursor are derived from homology with those in barwin [57]. AC1 (Ac-AMP2), antimicrobial chitin-binding protein from amaranth. The C-terminus has been removed from the mature protein. (The Asn-Pro-Thr sequence in this precursor probably is not glycosylated [61].)

5. Conclusions

It will be clear from the survey of chitinases given above that the classification which evolved in the course of time has become less satisfactory with time. Features like acidic or basic characteristics (a basic chitinase should not be called acidic for classification purposes [18]) or the presence of N-terminal hevein domains or vacuolar targeting signals are not very useful for classification purposes. With sufficient information, an evolutionary classification based on sequence similarity, with a high weight on shared deletions in the sequences, has to be preferred. As a first step in this direction it is proposed to use classes with roman numbers only for homologous chitinases (I, II and IV), and to designate the current class III and V chitinases in another way. As the currently known three-dimensional structures of chitinases are those of the enzymes from barley [9] and of hevamine [10], it may be useful to adopt the designation b-type (classes I, II and IV) and h-type (classes III and V) chiitinases, respectively.

Acknowledgements: Many thanks are due to Dr. R.N. Campagne, Groningen, for carefully reading the manuscript.

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