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Identification and analysis of a novel serine β-lactamase-like plant protein by a bioinformatic approach

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We have searched through plant databases for nucleotide and protein sequences sharing a significant similarity to bacterial serine β-lactamases and metazoan LACTB. The search resulted in the identification of novel serine β-lactamase homologues in both Gymno- and Angiosperms. However, unlike bacterial β-lactamases and metazoan LACTB, plant homologues are dual domain proteins composed of an N-terminal ABC1 domain containing serine / threonine protein kinase motifs, followed by a C-terminal β-lactamase domain containing the active site motifs present in all serine β-lactamases. Multiple sequence alignments and secondary structure modelling of the β-lactamase domain revealed conserved regions that correspond to structural elements required for the serine protease active center formation. On the basis of sequence homology and secondary structure conservation, we propose that the chimeric plant protein is involved in apoptotic processes through its serine protease and serine / threonine protein kinase activity.

Key words: serine β-lactamases, LACTB, ABC1, computational modelling, apoptosis

INTRODUCTION

Serine β-lactamases, specific proteins located in the periplasmic space of gram-negative bacteria, provide resistance to β-lactam antibiotics and thus preserve peptidoglycan formation. The b-lactamase homologue LACTB is a mitochondrial protein that has been detected in mammals, fishes, amphibias and nematodes, but not in yeasts or insects [1]. Recently, mouse LACTB protein has been succesfully expressed in *Escherichia coli* [2]. Purified recombinant mouse LACTB underwent a rapid fragmentation suggesting that it can undergo autoproteolysis. Furthermore, LACTB appears to be associated with the mitochondrial ribosome [3]. However, the specific function of LACTB has yet to be defined.

In this study, we describe a novel putative plant protein containing a domain homologous to the bacterial serine β-lactamases and metazoan LACTB. Analysis of this β-lactamase homology domain sequence and its predicted secondary structure revealed conserved regions corresponding to structural elements essential for the serine protease active center formation. Furthermore, the plant β-lactamase homologue is fused to an ABC1 homology domain containing serine / threonine protein kinase motifs. We hypothesize that this novel plant serine β-lactamase-like protein has multiple functions and that it has adapted its serine protease and serine / threonine protein kinase activity to a novel function possibly associated with apoptosis.

MATERIALS AND METHODS

The sequence of full-length *E. coli* class C serine β-lactamase (UniProtKB accession number: P00811) was used to search for homologous plant proteins in genomic DNA databases: TIGR (www.tigr.org), PlantGDB (www.plantgdb.org) and NCBI (www.ncbi.nlm.nih.gov) using BLAST [4]. Multiple sequence alignments were built using ClustalX 1.83 [5] with the Gonnet series protein weight matrix. To improve the accuracy of the sequence alignments, known (i.e. bacterial) and predicted secondary structure data were used to set secondary structure-based gap penalties. Regions with a low sequence similarity and poor secondary structure alignment were

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edited manually. Retrieved sequences were analyzed for β-lactamase [6] and serine / threonine protein kinase [7] signature motifs. Targeting and transmembrane sequences of plant proteins were detected using TargetP 1.1 [8] and TMHMM 2.0 [9]. Secondary structure prediction for proteins was performed using JNet [10] from Jalview 2.07 [11].

RESULTS AND DISCUSSION

Identification of β-lactamase homologues in higher plants

Plant genomic and protein database BLAST searches using a bacterial class C serine β-lactamase as a bait led to the identification of two full-length proteins from thale cress (locus No. AT5g24810) and rice (Os06g48770.1) having a length of 1009 and 948 amino acids, respectively. The coding sequences were divided into 18 exons localized on chromosome 5 in cress and chromosome 6 in rice. These putative plant proteins shared 61% overall sequence identity and were composed of two domains (Fig. 1). The N-terminal part of the polypeptide chain contained a mitochondrial targeting sequence followed by a predicted transmembrane segment and an ABC1 homology domain. The C-terminal part of the polypeptide chain shared 26% amino acid identity with the bacterial serine β-lactamase. Further searches in expressed sequence tag (EST) databases of higher plants revealed 70 sequences from various green plant species. However, sequences only from barrelclover, common bean, Irish potato and loblolly pine coded at least one out of three β-lactamase signature motifs (Table). These ESTs were translated into 100–290 amino acid residue long fragments and assembled into longer sequences for each plant species. These putative proteins shared 38% to 63% amino acid identity with the serine β-lactamase homology domain of the cress and rice proteins. Collectively, these findings suggest that proteins containing a serine β-lactamase homology domain are widespread in the plant kingdom.

Fig. 1. Schematic picture of the thale cress and rice full-length protein derived from AT5g24810 and Os06g48770.1. The putative protein is comprised of a mitochondrial targeting sequence (Mt), a predicted transmembrane segment (Tr) and two domains: ABC1-like domain (ABC1) containing ATP-binding signature and serine / threonine protein kinase catalytic motifs, and serine β-lactamase (Ser-Lact) homology domain with β-lactamase active site motifs

Conservation of secondary structure and putative function of a novel β-lactamase-like protein

Multiple sequence alignment of the identified sequences revealed all proteins to contain the conserved catalytic site residues directly involved in the hydrolysis of β-lactams [6]. The conserved Ser and Lys residues constitute the first catalytic SXXK motif (Fig. 2). The second

Table. **Higher plant genomic or expressed sequence tag sequences used in the study**

Plant species	Genomic locus or EST acc. No.
Barrelclover (*Medicago truncatula Gaert.*)	CX532169, BF632878, CB894096
Common bean (*Phaseolus vulgaris* L.)	CB540324
Irish potato (*Solanum tuberosum* L.)	DN588921, BQ509449
Loblolly pine (*Pinus taeda* L.)	AW311690, CO166852, CX647798
Rice (*Oryza sativa* L. cultivar *Nipponbare*)	Os06g48770.1
Thale cress (*Arabidopsis thaliana* (L.) *Heynh.* ecotype *Columbia*)	At5g24810

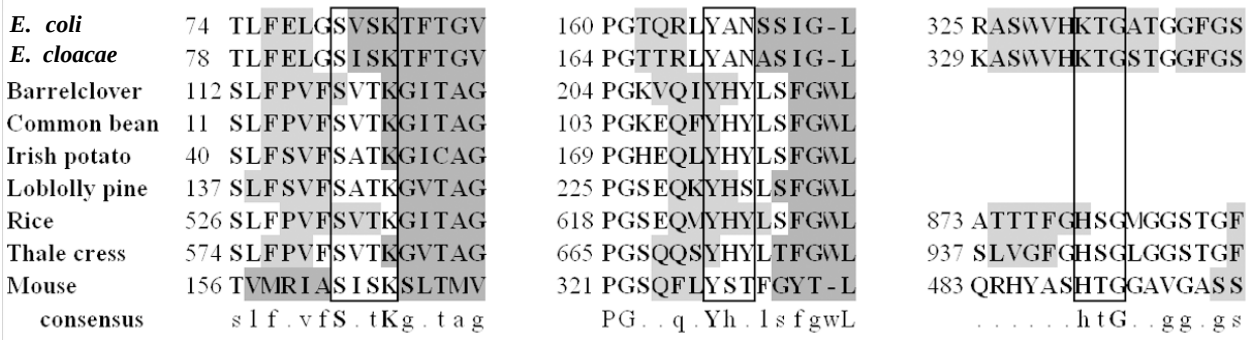

Fig. 2. Multiple alignments of serine β-lactamase active site motifs based on the secondary structure of bacterial class C β-lactamases. Dark gray colour indicates α-helices, light gray shows β-strands and white colour indicates loops. Sequences used for the alignment are from *E. coli* (P00811), *E. cloacae* (P05364), higher plants (see Table) and mouse (Q9EP89). Only two plant sequences, from thale cress and rice, represent full-length proteins, while others are translated from ESTs and lack the last serine β-lactamase catalytic motif. The catalytic motifs are marked with a text-box. Uppercase letters indicate conserved residues by the single-letter amino acid code, lowercase letters indicate nearly invariant residues. Numbers preceding the aligned regions refer to a position of the first amino acid in the peptide from the analyzed sequences

motif, taking part in the hydrolysis reaction, contains an YXX tripeptide. The last active motif is formed by a basic residue (Lys or His), followed by Thr or Ser and a Gly forming a [K/H][T/S]G motif. The same catalytic site pattern including several neighbouring residues also occurs in mouse LACTB and β-lactamase-like proteins from other metazoans [2]. Furthermore, secondary structure predictions suggest that all these motifs are located on distinct structural elements corresponding to those in the bacterial serine β-lactamase secondary structure [12]. As depicted in Fig. 2, the bacterial S[V/I]SK motif is located near the N-terminus of a long α-helix, and the YAN tripeptide is on a loop between the short β-strand and α–helix. Similar secondary structures were predicted for the regions containing these corresponding catalytic motifs in mouse and in several plant sequences. The motifs were located on the transition from a loop to the α-helix, except for the rice sequence where the SVTK and YHY peptides were found on predicted β-strands. The last catalytic [K/H][T/S]G motif lies on the first β-strand of three conserved C-terminal β-sheets or on a loop immediately after it. Noteworthy, the identified plant EST sequences did not contain this region and conclusions could be drawn only based on the genomic thale cress and rice sequences.

Thus, the sequence and secondary structure analysis shows that there are no apparent differences in all catalytic motifs among plant species representing Gymno- and Angiosperms. This also indicates that bacterial class C serine β-lactamases and plant as well as mouse serine β-lactamase-like proteins share the common catalytic site structure. Furthermore, our findings suggest that these plant and mouse proteins might retain catalytic activity characteristic of an ancestral protein. However, it certainly does not correspond to bacterial β-lactamase activity involved in bacterial cell wall maintenance, since eukaryotes do not synthesize peptidoglycan. According to a recent hypothesis [2], metazoan LACTB participates in peptide hydrolysis or transfer reactions of substrate molecules that resemble the β-lactam ring or the D-alanyl-D-alanine sequence. Moreover, LACTB may have other additional functions, since it has been related with the large 39S subunit of the mammalian mitochondrial ribosome [3]. This hypothesis is supported by the fact that two other mitochondrial ribosomal proteins, DAP3 and PDCD9/p52, are involved in the regulation of mitochondrial apoptotic events [13]. It should be noted that at an N-terminal part of the sequence of a putative plant protein identified from genomic sequences there is an ABC1 homology domain containing conserved serine / threonine protein kinase motifs (Fig. 1). It has been proposed that ABC1 participates in coenzyme Q biosynthesis by phosphorylation of monooxygenase or other necessary proteins in both prokaryotes and eukaryotes, and the protein is also supposed to be involved in p53-induced apoptosis in humans through the mitochondrial pathway [14, 15].

To our knowledge, the unique domain architecture of thale cress and rice protein does not occur in any bacterial, plant and metazoan protein identified so far. We hypothesize that either ABC1 protein kinase or LACTB-like serine protease domain may have an autoregulatory role for the whole protein function and may participate in proteolytic signalling cascades such as programmed cell death. However, future studies will be needed to elucidate the role of this novel plant serine β-lactamase-like protein in cellular processes.

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NAUJO, Į SERINO β-LAKTAMAZĘ PANAŠAUS AUGALŲ BALTYMO NUSTATYMAS IR ANALIZĖ BIOINFORMATIKOS METODAIS

Santrauka

Augalų genų ir baltymų duomenų bazėse ieškojome sekų, giminingų bakterijų serino β-laktamazių ir gyvūnų LACTB

baltymams, ir keletoje plikasėklių bei gaubtasėklių augalų rūšių identifikavome naują baltymą. Šio unikalaus daugiadomeninio baltymo polipeptidinės grandinės karboksi gale aptikome amino rūgščių liekanas, būdingas bakterijų serino β-laktamazėms ir gyvūnų LACTB, o amino gale – ABC1 homologišką domeną, turintį serino ir treonino proteinkinazės motyvus. Daugybinio panašių augalų, bakterijų ir pelės baltymų sekų palyginimo (*multiple alignment*) ir antrinės baltymų struktūros analizės dėka nustatėme baltymo sritis, būtinas serino proteazės aktyvaus centro formavimui. Remdamiesi gautais rezultatais mes iškėlėme hipotezę, kad šis naujas chimerinis augalų baltymas išlaikė proteazinį ir proteinkinazinį aktyvumą ir dalyvauja ląstelės apoptozės procesuose.