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Model of 3D structure of putative parasitism factor, expansin (EXPB2) from golden potato cyst nematode *Globodera rostochiensis*

Model 3D-strukture verjetnega parazitskega dejavnika ekspanzina (EXPB2) pri rumeni krompirjevi ogorčici *Globodera rostochiensis*

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Abstract: Expansins are a group of plant cell wall loosening proteins. In animals, functional expansin (EXPB1) has been discovered in the golden potato cyst nematode *Globodera rostochiensis*. In plant-parasitic nematodes expansins act as the parasitism factors or effectors. Molecular variability of another expansin (*exp*B2) gene was evaluated in diverse populations of the *G. rostochiensis*. 3D modelling of GR-EXPB2 protein sequences revealed variants with different tertiary protein structure. Superimposing PDB structures of the protein model of common type protein with two longer variants revealed difference in position of one loop in the two longer proteins. All longer GR-EXPB2 variants originated from South America.

Key words: Cell wall degradation, *Globodera rostochiensis,* effectors, expansin, parasitism factor, plant-parasitic nematode, potato cyst nematode, 3D structure.

Izvleček: Ekspanzini so skupina proteinov, ki zrahlja rastlinsko celično steno. Pri živalih so odkrili funkcionalni ekspanzin (EXP1) pri vrsti rumene krompirjeve ogorčice, *Globodera rostochiensis*. Pri rastlinsko parazitskih ogorčicah ekspanzini delujejo kot parazitski dejavniki oz. efektorji. Pri *G. rostochiensis* je bila ovrednotena molekulska raznolikost dodatnega ekspanzinskega gena (*exp*B2). Modeliranje 3D-strukture proteina GR-EXPB2 je razkrilo različke proteina z različno terciarno strukturo. Z nalaganjem strukture daljših različic proteina nad najpogostejšo krajšo različico proteina se je razkrila različna pozicija ene zanke pri obeh daljših proteinih. Vse daljše različice proteina GR-EXPB2 izvirajo iz Južne Amerike.

Ključne besede: razgradnja celične stene, *Globodera rostochiensis*, efektorji, ekspanzin, parazitski dejavnik, rastlinsko parazitske ogorčice, 3D-struktura.

Introduction

Expansins are a group of proteins that operate by loosening non-covalent interactions between components of the plant cell wall making the individual components vulnerable to attack by other cell wall degrading enzymes (Cosgrove 2000). These proteins were thought to be specific to plants; however an active expansin EXPB1 has unexpectedly been identified in the plant-parasitic nematode, golden potato cyst nematode *Globodera rostochiensis* (Woll.) Behrens (Qin et al. 2004). *G. rostochiensis* parasitize different Solanaceous plant species and affects potato production

worldwide. Therefore *G. rostochiensis* is subjected to strict quarantine regulations in many countries. In Slovenia *G. rostochiensis* has spread in the last decade (Širca et al. 2010).

When potato cyst nematodes (PCN) invade a plant, they produce a mixture of lytic enzymes and expansins in their oesophageal glands and secrete them through the stylet into the plant. These proteins assist in the migration of infective juveniles through the host plant's tissues, and in the feeding site formation. Additionally, the host plant's own expansin genes are up-regulated upon nematode infection (Fudali et al. 2008). Expansins B1 and B2 were determined in the EST analysis of *G. rostochiensis* (Popeijus et al. 2000, http://www.nematodes.org/nembase4/overview.shtml). Molecular variability of *exp*B2 gene was evaluated in the diverse populations of the *G. rostochiensis* (Gerič Stare et al. 2012). One-hundred thirty-eight determined sequences of the Gr-*exp*B2 gene (FJ705444, GQ152151 – GQ152166, GQ152168 – GQ152288) resulted in different protein sequences. A majority (126 out of 138; 91.3%) of the DNA sequences resulted in protein sequences of 154 amino acids (AA) in length. Two sequences resulted in slightly longer proteins (156 AA) due to an identical 6-bp insertion into the fourth exon. One sequence resulted in a 181 AA protein sequence due to a 1-bp insertion in the fourth exon and a subsequent frame shift which thwarted the normal stop codon. Nine sequences were determined as pseudogenes due to short deletions in exons, subsequent frameshift and premature stop codon.

The aim of this study was to determine tertiary structure in variants of GR-EXPB2 protein determined in our previous study (Gerič Stare et al. 2012).

Materials and methods

3D protein models of predicted proteins without signal peptide were constructed with the Swiss-Model Workspace (Arnold et al. 2006) while protein structures were compared by superimposing pdb files with TopMatch (Sippl and Wiederstein 2008, Sippl 2008).

Results

Three-dimensional (3D) protein structure modelling

The 3D protein structure models were built to pinpoint differences between the predicted GR-EXPB2 proteins of different clones (pseudogenes were excluded from the analysis) (Fig. 1). An automated fold recognition technique constructed two different models based on the mature protein sequences in clone 5a which represented the most common protein sequence since 88 (64%) sequences resulted in such identical proteins. For clone 5a, the first 3D model was based on the Barwin lectin, a protein from barely seed (PDB ID: 1bw3A, E value $1.40e^{-7}$ and 18% identity) and the second on pollen allergen PHL P1 from *Phleum pratense* (PDB ID: 1n10A, E value $2.0e^{-20}$ and 17.5% identity). These two models differed significantly in their arrangement of local patterns (Fig. 2). The second model was a more likely candidate for the GR-EXPB2 structure on account of its lower E value and was also previously suggested as the best template for domain 2 of the GR-EXPB1 by Kudla et al. (2005). While GR-EXPB1 is composed of a carbohydrate binding domain coupled to an expansin domain (Kudla et al. 2005), the GR-EXPB2 3D model predicted in this study was composed only of expansin domain suchlike the putative expansins from plant parasitic nematodes *Bursaphelenchus xylophilus* and *B. mucronatus* (Kikuchi et al. 2009).

Models of clones 21b and 33b were constructed to search if the longer predicted proteins have structural properties similar to a common type of protein. Both clones resulted in 3D protein models based on template 1n10A (Fig. 2). Superimposing PDB structures of common type protein with longer proteins revealed a difference in the position of one loop of the two longer proteins (Fig. 2).

Discussion

Expansins cause loosening and extension of cell walls by acid-induced disruption of non-covalent hydrogen bonds between cellulose and hemicellulose fibers, promoting slippage between the polymers and allowing the cell to absorb

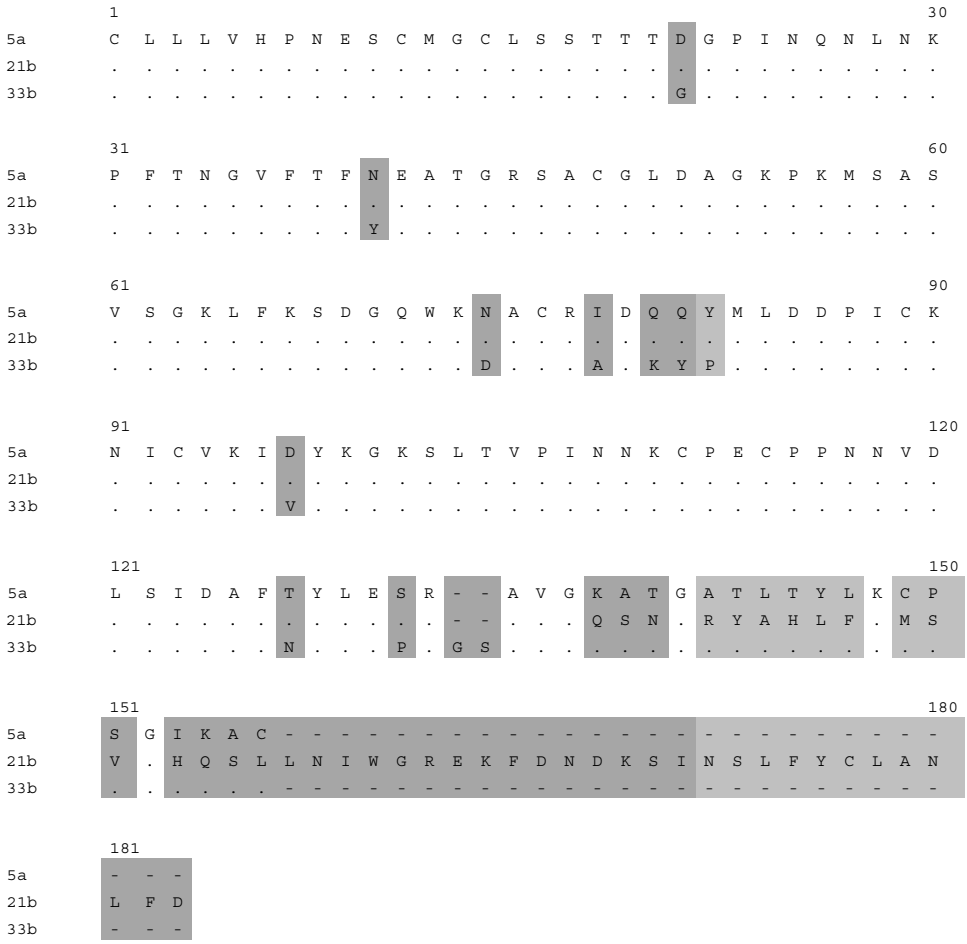
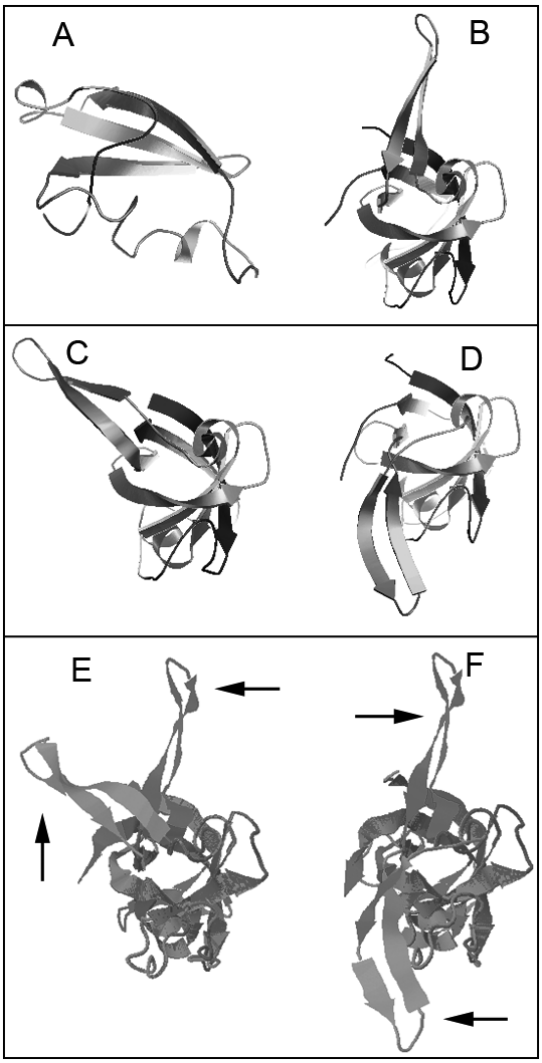


Figure 1: The alignment of the predicted variant GR-EXPB2 proteins from different clones. The sequence of clone 5a represents the most common protein, GR-EXPB2 type protein. The clones 21b and 33b represent the longer predicted proteins. Variable sites in the alignment are highlighted.

Slika 1: Poravnava predvidenih različkov proteina GR-EXPB2 iz različnih klonov. Zaporedje klona 5a predstavlja najpogostejši različek, tipični protein GR-EXPB2. Klona 21b in 33b predstavljata daljša različka proteina. Spremenljiva mesta v poravnavi so poudarjena.

water, ultimately leading to cell wall expansion (Cosgrove 2000, Whitney et al. 2000). No hydrolytic or other enzymatic activity has been found to account for expansin's unique effects on the cell wall. Functional binding target of expansin in the *Arabidopsis thaliana* cell walls was recently determined. Expansin binding to highly specific cellulose domains enriched in xyloglucan leads to loosening of the cell walls, whereas more abundant binding to pectins is unrelated to activity (Wang et al. 2013). It was proposed that the several highly-conserved tryptophans in the cellulose-binding module (CBM) may function in expansin binding to its target (Shcherban et al. 1995). The exact mechanism of action of the expansins is still not characterized in details. The shortage of functional information reflects the scarcity of informative structural data, given that the only



solved crystal structures represent one bacterial expansin and the expansin-like protein in maize (Dal Santo et al 2013). While GR-EXPB1 consists of an expansin domain and a carbohydrate-binding module, GR-EXPB2 consists solely of the expansin domain. GR-EXPB1 has considerable enzymatic activity only when both domains are present. Furthermore, CBM alone did not show expansin activity (Kudla et al. 2005). However it has been shown that also GR-EXPB2 consisting solely of the expansin domain exhibits the activity. The *in planta* expression and activity of GR-EXPB2 has been demonstrated in *Nicotiana benthamiana, Solanum lycopersicum* and *S. tuberosum* leaves (Dr. Shawkat Ali, personal communication). Further functional analysis of the GR-EXPB2 variants are planned to assess possible difference in activity between protein variants. It was suggested that the high conservation of this multigene family indicates that the mechanism by which expansins promote wall extension tolerates little variation in protein structure (Shcherban et al. 1995). Different position of the loop in the longer GR- EXPB2 variants (Fig. 2) could affect the interaction with polymers of the cell wall and the proteins function. Therefore it needs to be determined whether the longer protein varianst have the activity at all and if it is any different from the common type GR-EXPB2 protein.

Figure 2: The 3D models of the predicted GR-EXPB2 protein from different clones. A – The first model of the common type protein (clone 5a) based on the barely seed protein (1bw3A). B – The second model of the common type protein (clone 5a) based on PHL P 1, a major Timothy grass pollen allergen (1n10A). C – Model of the first longer predicted protein (clone 21b) constructed on template 1n10A. D – Model of the second longer predicted protein (clone 33b) constructed on template 1n10A. E, F – Superimposing PDB structures of the second model of common type protein with longer proteins (clones 21b and 33b, respectively) revealed differences in position of one loop in the two longer proteins (shown by the arrows).

Slika 2: 3D zgradba modelov proteina GR-EXPB2 iz različnih klonov. A – Prvi model najpogostejšega zaporedja proteina (klon 5a) na osnovi proteina iz semena ječmena (1bw3A). B – Drugi model najpogostejšega zaporedja proteina (klon 5a) na osnovi proteina PHL P 1, alergena iz trave *Phleum pratense* (1n10A). C – Model prvega daljšega različka proteina (klon 21b) na osnovi šablone 1n10A. D – Model drugega daljšega različka proteina (klon 33b) na osnovi šablone 1n10A. E, F – Z nalaganjem strukture daljših različic proteina nad najpogostejšo krajšo različico proteina se razkrije različna pozicija zanke (kažejo puščice) pri obeh daljših proteinih (klona 21b in 33b).

All longer protein variants of GR-EXPB2 originate from the Bolivian populations. South America is the origin of potatoes as well as their nematode pests like PCN. PCN originate from the Andean Highlands of South America, where they co-evolved with their plant hosts (potatoes and other members of the family Solanaceae). The range of virulence of PCN present in that area is far greater than that present in European populations as their initial introduction into Europe represented bottle neck (EFSA 2012). Several studies have showed higher variability of South American PCN populations compared to European populations including our studies of variability of cell wall degrading proteins pectate lyase 2 and expansin B2 (Gerič Stare et al. 2011, 2012).

Conclusion

Comparison of GR-EXPB2 protein variants revealed differences in position of one loop in the two longer protein variants compared to the shorter common type protein.

Povzetek

Ekspanzini so proteini, ki z zrahljanjem nekovalentnih vezi pomagajo pri razgradnji rastlinske celične stene. Pri živalih so odkrili funkcionalni ekspanzin (EXP1) pri vrsti rumene krompirjeve ogorčice *Globodera rostochiensis* (Nematoda). Pri rastlinsko parazitskih ogorčicah ekspanzini delujejo kot parazitski dejavniki oz. efektorji. V predhodni študiji smo ovrednotili molekulsko raznolikost dodatnega ekspanzinskega gena (*exp*B2) pri različnih populacijah in patotipih *G. rostochiensis*. Pri večini (126 od 138; 91,3 %) zaporedij DNA smo s pomočjo »*in silico*« prepisa pridobili proteinska zaporedja dolžine 154 AK. Dve zaporedji sta dali malenkost daljši protein (156 AK) zaradi identične 6-bp dolge insercije v četrtem eksonu. Eno zaporedje je vodilo v daljši protein (181 AK) zaradi insercije 1-bp v četrti ekson in posledičnega premika bralnega okvirja, ki je pokvaril zaključni kodon. Devet zaporedij je predstavljalo pseudogene, saj so krajše delecije v eksonih vodile v premik bralnega okvirja in prezgodnji zaključni kodon. V tej študiji smo določili modelno 3D-strukturo proteina GR-EXPB2. Modeliranje je razkrilo različno terciarno strukturo različkov proteina različne dolžine. Z nalaganjem strukture daljših različic proteina nad najpogostejšo krajšo različico proteina se je razkrila različna pozicija ene zanke pri obeh daljših proteinih. Vse daljše različice proteina GR-EXPB2 smo določili pri populacijah *G. rostochiensis* iz Bolivije. Krompirjeve ogorčice izvirajo iz Južne Amerike, kjer so se razvile v ko-evoluciji s svojimi gostitelji. V Evropo je bilo vneseno manjše število cist iz majhnega območja Južnega Peruja, zato vse krompirjeve ogorčice v Evropi kažejo le manjši delež biotske raznovrstnosti trenutno prisotne v Južni Ameriki. Z našimi raziskavami smo dokazali večjo raznolikost Južno Ameriških populaciji nasproti evropskim glede na proteine za razgradnjo celične stene, ekspanzine in pektat liaze.

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