Developmental and Comparative Immunology xxx (2017) 1–9

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Immune functions of insect βGRPs and their potential application

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ARTICLE INFO

Article history:
Received 4 October 2017
Received in revised form
6 December 2017
Accepted 8 December 2017
Available online xxx

Keywords:
Insect immunity
βGRP
Toll
Phenoloxidase

ABSTRACT

Insects rely completely on the innate immune system to sense the foreign bodies and to mount the immune responses. Germ-line encoded pattern recognition receptors play crucial roles in recognizing pathogen-associated molecular patterns. Among them, β-1,3-glucan recognition proteins (βGRPs) and gram-negative bacteria-binding proteins (GNBPs) belong to the same pattern recognition receptor family, which can recognize β-1,3-glucans. Typical insect βGRPs are comprised of a tandem carbohydrate-binding module in the N-terminal and a glucanase-like domain in the C-terminal. The former can recognize triple-helical β-1,3-glucans, whereas the latter, which normally lacks the enzymatic activity, can recruit adapter proteins to initiate the protease cascade. According to studies, insect βGRPs possess at least three types of functions. Firstly, some βGRPs cooperate with peptidoglycan recognition proteins to recognize the lysine-type peptidoglycans upstream of the Toll pathway. Secondly, some directly recognize fungal β-1,3-glucans to activate the Toll pathway and melanization. Thirdly, some form the 'attack complexes' with other immune effectors to promote the antifungal defenses. The current review will focus on the discovery of insect βGRPs, functions of some well-characterized members, structure-function studies and their potential application.

1. Introduction

Insects are frequently threatened by various pathogens in their living environment, among which, bacteria, viruses, fungi and parasites are fatal to insects with a compromised immune system. The vertebrate immune system is made up of the innate immune system and the adaptive immune system. In contrast, insects rely solely on the innate immune system against pathogens (Lemaitre and Hoffmann, 2007). Such innate immune system depends on germline-encoded pattern recognition receptors (PRRs) to detect the pathogen-associated molecular patterns (PAMPs) and to initiate the cellular as well as humoral responses. Cellular responses are comprised of phagocytosis, encapsulation and nodulation (Smith, 2010), whereas humoral responses include melanization and production of antimicrobial molecules (Zänker, 2010). Moreover, PRRs can be classified into several groups according to the particular PAMP type they recognize. Lectins can recognize carbohydrates (Barondes et al., 1994; Weis and Drickamer, 1996), peptidoglycan recognition proteins (PGRPs) can recognize peptidoglycans (PGs) (Kurata, 2014; Royet et al., 2011; Warr et al., 2008), while fungal β-1,3-glucans can be recognized by β-1,3-glucan recognition proteins (βGRPs) or gram-negative bacteria-binding proteins (GNBPs) (Brown and Gordon, 2005; Levitin and Whiteway, 2008). The Imd and Toll pathways in *Drosophila melanogaster* can regulate the expression of antimicrobial peptides (AMPs) (Ferrandon et al., 2007). The Imd pathway can mediate the recognition of gram-negative bacteria through the interaction between PGRP-LC and diaminopimelic acid (Dap)-type peptidoglycans (Choe et al., 2002; Gregorio et al., 2002; Lemaitre et al., 1995). The Toll pathway can be activated by lysine (Lys)-type peptidoglycans on the one hand, which is initiated by their interactions with circulating immune receptors GNBP1 and PGRP-SA. On the other hand, fungal β-1,3-glucans can be sensed by GNBP3 to initiate the protease cascade, thus leading to Spätzle processing by Spätzle-processing enzyme (SPE) and activation of the Toll pathway (Jang et al., 2006; Lemaitre et al., 1996; Valanne et al., 2011). Furthermore, some GNBPs are involved in activating the prophenoloxidase system, which is achieved by a similar mechanism through different proteases (Lu et al., 2014; Söderhäll and Cerenius, 1998; Wang and Jiang, 2017).

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https://doi.org/10.1016/j.dci.2017.12.007
0145-305X/

2. Pioneering studies on βGRPs

βGRPs were originally purified from the plasma of a lepidopteran model: the silkworm *Bombyx mori* (Ochiai and Ashida, 1988). Imler had briefly discussed the discovery of βGRPs in a review (Imler, 2014). In 1986, Ashida reported that the phenoloxidase activity in silkworm plasma depleted of β-1,3-glucan receptors through passing over a curdlan-type polysaccharide bead (plasma-CPB) column still could be triggered by peptidoglycans, rather than by β-1,3-glucans. This finding suggested that peptidoglycan receptors and β-1,3-glucan receptors existed as separate entities in the plasma, while the latter were necessary for β-1,3-glucan-triggered phenoloxidase activity (Yoshida et al., 1986). A β-1,3-glucan recognition protein (BmβGRP) with a molecular mass of 62 kDa and an isoelectric point of 4.3 was then purified from silkworm hemolymph biochemically (Ochiai and Ashida, 1988). BmβGRP was able to restore the phenoloxidase activity in β-1,3-glucan-triggered plasma-CPB. The immunocytochemical localization study suggested that BmβGRP was enriched in granules of granulocytes and spherules of spherulocytes (Ochiai et al., 1992). In 1996, Lee et al. reported the purification of a 50-kDa protein from silkworm plasma, which showed a strong affinity to the gram-negative bacterial cell wall (Lee et al., 1996). This protein was named gram-negative bacteria-binding protein (BmGNBP or BmβGRP2 in Table 1). The expression of *BmGNBP* was remarkably up-regulated by bacterial challenges. The deduced amino acid sequence displayed marked homology to the catalytic regions of bacterial β-1,3-glucanases. Following their preceding work, Ashida reported the cloning of *BmβGRP* cDNA (*BmβGRP1* in Table 1) and the binding properties of two α-chymotrypsin digested BmβGRP fragments (Ochiai and Ashida, 2000). Results of binding assays indicated that it was the N-terminal region (Tyr^{1} to Ala^{102}), instead of the glucanase-like region, that bound strongly to β-1,3-glucans. The comparison of amino acid sequences revealed significant similarities among BmβGRP1 (Thr^{264} to Pro^{386}), BmGNBP (Ser^{243} to Ala^{372}) and the catalytic regions of bacterial β-1,3-glucanases. BmβGRP1 and BmGNBP both lacked key glutamic acid residues for the glucanase activity. Homologs with similar functions were isolated during the same period from the crayfish *Pacifastacus leniusculus* (Duvic and Söderhäll, 1990), the cockroach *Blaberus craniifer* (Söderhäll et al., 1988), the tobacco hornworm *Manduca sexta* (Ma and Kanost, 2000), the pyralid moth *Plodia interpunctella* (Fabrick et al., 2003, 2004) and the fruit fly *Drosophila melanogaster* (Kim et al., 2000). These preliminary studies demonstrate that βGRPs and GNBPs belong to the same PRR family displaying specific affinity to β-1,3-glucans.

3. Features of the conserved domains and the phylogeny

The typical domain architecture of βGRPs was shown in Fig. 1A. The full-length βGRPs consist of a signal peptide, a carbohydrate-binding module (CBM) in the N-terminal and a glucanase-like domain (Glu) in the C-terminal. CBMs can be found in many carbohydrate-active enzymes, and they can enhance the catalytic efficiency of the carbohydrate-active enzymes against the soluble or insoluble substrates (Hashimoto, 2006). CBMs in insect βGRPs belong to family 32, which are further classified into 3 categories based on their respective substrate binding mechanisms: 'surface-binding' (type A), 'glycan chain-binding' (type B) and 'small sugar-binding' (type C). Type A CBMs can bind to insoluble ligands through a platform-like surface composed of aromatic residues (Boraston et al., 2004). Interestingly, 8 proteins with multiple CBM and Glu domains were identified from Lepidoptera and Coleoptera species, among which, 6 have 2 CBM and 2 Glu domains, while 2 have 2 CBM and 3 Glu domains (Fig. 1B). Protein sequences with the typical CBM-Glu domain architecture were aligned to construct an NJ tree (Fig. 2). The phylogenetic analysis revealed that βGRPs mainly exist in the phylum Arthropoda. They are also distributed in phylum Echinodermata, Hemichordata, Brachiopoda and Mollusca. The amino acid sequences are highly conserved within the same order of insects, and most orthologs derived from common ancestors before the divergence of insect orders. The non-Arthropoda

Table 1
A list of some insect βGRPs (GNBPs).

Gene	Organism	Accession#	Length (a.a.)	Reference
TmGNBP1	*Tenebrio molitor*	BAG14263.1	442	(Kim et al., 2008)
TmGNBP3	*Tenebrio molitor*	Q76DI2.1	481	(Lee et al., 2009; Yang et al., 2017; Zhang et al., 2003)
BmβGRP1	*Bombyx mori*	NP_001036840.1	495	(Ochiai and Ashida, 1988, 2000; Takahasi et al., 2009)
BmβGRP2 (BmGNBP)	*Bombyx mori*	NP_001037450.1	467	(Lee et al., 1996)
BmβGRP3	*Bombyx mori*	NP_001128672.1	489	(Tanaka et al., 2008)
DmGNBP1	*Drosophila melanogaster*	CG6895-PA	494	(Filipe et al., 2005; Gobert et al., 2003; Kim et al., 2000; Pili-Floury et al., 2004; Wang et al., 2006a)
DmGNBP2	*Drosophila melanogaster*	CG4144-PA	461	(Sackton et al., 2010)
DmGNBP3	*Drosophila melanogaster*	CG5008-PA	490	(Matskevich et al., 2010; Mishima et al., 2009a; Mishima et al., 2009b)
AgGNBPA1	*Anopheles gambiae*	AGAP006761-PA	490	(Warr et al., 2008)
AgGNBPA2	*Anopheles gambiae*	AGAP012409-PA, partial	450	(Warr et al., 2008)
AsβGRP	*Armigeres subalbatus*	AAT99011	500	(Wang et al., 2005; Wang et al., 2006b)
TcβGRP2	*Tribolium castaneum*	XP_969449	441	(Tribolium Genome Sequencing et al., 2008)
TcβGRP3	*Tribolium castaneum*	XP_972063	481	(Tribolium Genome Sequencing et al., 2008)
MsβGRP1	*Manduca sexta*	AAF44011	487	(Ma and Kanost, 2000; Rao et al., 2014)
MsβGRP2	*Manduca sexta*	AAN10151	482	(Jiang et al., 2004; Takahashi et al., 2014; Takahashi et al., 2015)
MsMBP	*Manduca sexta*	ADT82662, partial	482	(Wang and Jiang, 2017; Wang et al., 2011)
PiβGRP	*Plodia interpunctella*	AAM95970	488	(Dai et al., 2013)
SeβGRP	*Spodoptera exigua*	–	491	(Bang et al., 2013)
PxβGRP	*Plutella xylostella*	AFK24449	479	(Huang et al., 2015)
TpβGRP4a	*Thitarodes pui*	ADZ45541	496	(Sun et al., 2011)
AsβGRP	*Armigeres subalbatus*	AAT99011	500	(Wang et al., 2005; Wang et al., 2006b)
LmGNBP3	*Locusta migratoria*	AFD54027	496	(Zheng and Xia, 2012)

Please cite this article in press as: Rao, X.-J., et al., Immune functions of insect βGRPs and their potential application, Developmental and Comparative Immunology (2017), https://doi.org/10.1016/j.dci.2017.12.007