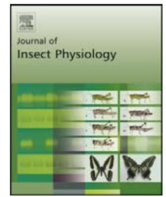
Journal of Insect Physiology 56 (2010) 1846–1856

Contents lists available at ScienceDirect

Journal of Insect Physiology

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



Integrin β1 subunit from *Ostrinia furnacalis* hemocytes: Molecular characterization, expression, and effects on the spreading of plasmatocytes

Jian Hu [a], Huafu Zhao [a], Xiaoqiang Yu [b], Jia Liu [a], Peng Wang [a], Jingya Chen [a], Qiuyun Xu [a], Wenqing Zhang [a,*]

[a] State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China
[b] Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110, USA

ARTICLE INFO

Article history:
Received 2 March 2010
Received in revised form 3 August 2010
Accepted 3 August 2010

Keywords:
Integrin β
Hemocytes
Plasmatocyte
Spreading
Ostrinia furnacalis

ABSTRACT

When lepidopteran larvae are infected by a large quantity of pathogens or parasitized by nonadaptive parasitoids, hemocytes in the hemocoel will encapsulate these foreign invaders. Cellular encapsulation requires hemocytes, particularly plasmatocytes, to change their states from nonadhesive, spherical cells into adhesive, spreading cells. However, it is unclear how the changes of plasmatocytes are regulated. Here we report that the integrin β1 subunit from hemocytes of *Ostrinia furnacalis* (*Of*int β1) plays an important role in regulating the spreading of plasmatocytes. The full length cDNA sequence (4477 bp) of *Of*int β1 was cloned from hemocytes. Phylogenetic analysis showed that *Of*int β1 belonged to the integrin βPS family of *Drosophila melanogaster* with highest sequence identity (78.7%) to the β-integrin of *Pseudoplusia includens*. Structural analysis of the deduced amino acid sequence indicated that *Of*int β1 had similar functional domains to known β-integrins in other lepidopteran insects. RT-PCR, Northern blotting, Western blotting and immunohistochemical analyses showed that *Of*INT β1 was expressed mainly in hemocytes, especially in plasmatocytes, and weakly in fat body, Malpighian tubes and epidermis. After hemocytes had spread onto slides, fewer antibodies to *Of*INT β1 bound to the surface of plasmatocytes. Furthermore, anti-*Of*INT β1 serum clearly inhibited the spreading of plasmatocytes. Together these results indicate that *Of*INT β1 may play an important role in regulating the spreading of plasmatocytes.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Encapsulation is a kind of rapid immune response of insect hemocytes, which results in the formation of capsules around foreign surfaces. In lepidopteran insects, capsule formation requires plasmatocytes to change from spherical cells circulating freely in the hemolymph to adhesive, flattened cells with numerous extending filopodia (Pech and Strand, 1996; Clark and Pech, 1997; Clark et al., 2001). However, it is unclear how the changes of plasmatocytes are regulated. In recent years, a cellular adhesive protein, integrin, has been demonstrated to be involved in cellular immune reactions in insects (Lavine and Strand, 2003; Levin et al., 2005; Zhuang et al., 2007, 2008). Integrins are a class of heterodimeric transmembrane glycoproteins composed of noncovalently attached α and β subunits, which have been found in many animal species, ranging from sponges to mammals. Integrins may recognize the RGD (Arg-Gly-Asp) motif and bind to several extracellular matrix proteins such as fibronectin, laminin and collagen (Ruoslahti, 1996; Hynes, 1992; Miranti and Brugge, 2002; Giancotti and Ruoslahti, 1999). In *Drosophila melanogaster*, integrins play a key role in regulating cell adhesion, migration, proliferation, and apoptosis (Yee and Hynes, 1993; O'Reilly et al., 2008; Dinkins et al., 2008).

In insects, α-integrins are distributed in fat bodies, midgut, Malpighian tubules, epidermis and hemocytes, and they have diverse functions. For example, αPi2 may regulate hemocyte adhesion in *Pseudoplusia includens* (Lavine and Strand, 2003). Three α-integrins from *Manduca sexta* were involved in encapsulation, and when their expressions in hemocytes were decreased by RNA interference (RNAi), the cell-mediated encapsulation response to foreign surfaces was abolished (Zhuang et al., 2008). αPS2 in *D. melanogaster* is essential for muscle attachment. Mutations in αPS2 abolished the ability of integrin to recruit laminin to the muscle attachment sites in the embryo and caused the detachment of integrins and talin from the extracellular matrix (Devenport et al., 2007).

Integrin βPS from *D. melanogaster* was the first insect integrin β subunit identified (MacKrell et al., 1988). β-Integrins were later discovered in several dipteran and lepidopteran insects including *Anopheles gambiae*, *Aedes aegypti*, *P. includens*, *M. sexta* and *Spodoptera frugiperda* (Mahairaki et al., 2001; Lavine and Strand, 2003; Levin et al., 2005; Moita et al., 2006). However, only β1-integrins from *M.*

* Corresponding author. Tel.: +86 20 84113612; fax: +86 20 84112297.
E-mail addresses: lsszwq@mail.sysu.edu.cn, tbzm611@yahoo.com (W. Zhang).

0022-1910/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2010.08.001

sexta and *P. includens* (Lepidoptera) were cloned and proved to be associated with encapsulation and adhesion of hemocytes (Lavine and Strand, 2003; Levin et al., 2005). The function of β-integrins in cellular immune reactions of insects has been closely examined than that of α-integrins. It was shown that the expression level of integrin β1 increased in granular cells and plasmatocytes in *P. includens* when hemocytes bound to a foreign surface or formed a capsule (Lavine and Strand, 2003). In *M. sexta*, RNAi of integrin β1 in larval hemocytes could significantly suppress the encapsulation of DEAE Sephadex beads (Levin et al., 2005). An integrin β ligand, tetraspanin, was identified in *M. sexta*, and it was mainly expressed in hemocytes and functioned in the cell-mediated immune responses (Zhuang et al., 2007). Moreover, the integrin β subunit also participates in phagocytosis. The expression of an integrin β on the surface of medfly hemocytes could trigger the engulfment of pathogen (Mamali et al., 2009). In insects, another integrin β family, βν, may also regulate phagocytosis. In *A. gambiae*, phagocytic activity of hemocytes was inhibited by treatment with integrin βν double-stranded RNA (dsRNA), but not by integrin β1 dsRNA (Moita et al., 2006).

In this study, we report the cloning, characterization and functional study of an integrin β1 subunit (*Of*int β1) from the Asian corn borer *Ostrinia furnacalis*. Phylogenetic analysis indicated that *Of*int β1 belonged to the integrin βPS family of *D. melanogaster*. *Of*INT β1 was mainly expressed in hemocytes, particularly in plasmatocytes. After hemocyte spreading onto the slides, fewer *Of*INT β1 antibodies bound to the surface of plasmatocytes. Furthermore, anti-*Of*INT β1 serum clearly inhibited the spreading of plasmatocytes. These results indicate that *Of*INT β1 may play an important role in regulating the spreading of plasmatocytes.

2. Materials and methods

2.1. Insects

O. furnacalis were originally collected from cornfields in Jiangsu province, China, and reared as described previously (Hu et al., 2003). Larvae on the third day of the fifth instar were used in this study.

2.2. Collection of hemocytes and other tissues for total RNA and protein extraction

Hemocytes were collected from *O. furnacalis* larvae according to the procedure of Pech et al. (1994) with slight modifications. Larvae were first bled onto parafilm by cutting away a caudal disc. About 100 μl hemolymph was transferred into an eppendorf tube containing 1 ml Pringle's saline (Pringle, 1938), and then centrifuged at 480 × *g* for 5 min to collect hemocytes. Other tissues, including epidermis, fat body, midgut and Malpighian tubules, were collected by dissecting *O. furnacalis* larvae under ice-cold Pringle's saline, and stored at −80 °C for further use.

2.3. cDNA cloning, sequence alignment and structural analysis

Total RNA was isolated from hemocytes using TRIzol reagent (Invitrogen) and then the first strand cDNA was prepared using reverse transcriptase AMV (Takara). Degenerate primers 1-F and 1-R (Table 1) were designed based on the conserved regions of known integrin β1 subunits from several insects to clone a fragment of *O. furnacalis* integrin β1 (*Of*int β1-1). Polymerase Chain Reaction (PCR) was performed in 25 μl reactions for 30 cycles using the following conditions: denaturing at 94 °C for 30 s, annealing at 46 °C for 30 s and extension at 72 °C for 1 min. The PCR product was subsequently cloned into the pMD18-T vector (Takara) and sequenced by the dideoxynucleotide method with gene-specific primers designed from the partial cDNA sequence. Other degenerate primers (2-F, 2-R, 3-F and 3-R in Table 1) were also designed based on the known sequences of integrin β1 subunits to obtain overlapping PCR products, *Of*int β1-2 and *Of*int β1-3. The 5′ and 3′ ends of the cDNA were obtained by 5′- and 3′-RACE (Rapid Amplification of cDNA Ends) with two gene-specific primers (Table 1) according to the manufacturer's protocol (BD SMART^{TM} RACE cDNA Amplification Kit, Clontech). Finally, the full length cDNA of *Of*int β1 was obtained by assembling the five overlapped fragments.

Multiple amino acid sequence alignments were analyzed using the Clustal W program (www.clustalw.genome.ad.jp). Phylogenetic trees were constructed by the neighbor-joining (NJ) method (Saitou and Nei, 1987) with the basis of three amino acid distances. The protein pattern and profile of *Of*INT β1 were obtained from the Prosite database using InterPro Scan (http://www.ebi.ac.uk/InterProScan/). The transmembrane helix and the signal peptide were analyzed using TMHMM v.2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) and SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/). The potential N-glycosylation and O-glycosylation sites were predicted using NetNGlyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/) and NetOGlyc 3.1 (http://www.cbs.dtu.dk/services/Net-OGlyc/).

Table 1
The primers used in the cDNA cloning of *Of*int β1 and RT-PCR and Northern blotting analysis.

PCR fragment	Name of primer	Type of primer[a]	Primer sequence (5′–3′)
*Of*int β1-1	1-F	F, D	CCNBTCGAYBTKTACTATCTSATGG
	1-R	R, D	TTCADGTGRCAYTVCHYRTCRTT
*Of*int β1-2	2-F	F, G	AACGACAAGGAGAAGCTCAGTACC
	2-R	R, D	GTCACACTCGCAGWAHBGWCC
*Of*int β1-3	3-F	F, G	TTCAACCAAACGACGAGCAGTGTC
	3-R	R, D	TCYTYCTCRAAVYKSGCRWACTCBCT
*Of*int β1-4	Universal primer A mix (UPM)	F, G	Long: 5′-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCATCGCAGAGT-3′ Short: 5′-CTAATACGACTCACTATAGGGC-3′
	4-R	R, G	GCCGCCTTTCATGCTGGACTCTTCG
*Of*int β1-5	5-F	F, G	GCAGAAGGAACGAGGATGTCCAAAGAAG
	Universal primer A mix (UPM)	R, G	Long: 5′-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3′ Short: 5′-CTAATACGACTCACTATAGGGC-3′
Probe	*Of*int-F	F, G	TCGAAGAGTCCAGCATGAAAGGCGG
	*Of*int-R	R, G	TCCGCTACTGCCCTGTCAAAACTAT

The PCR fragment ("Probe") was used as the probe for Northern blotting.
[a] F, forward; R, reverse; D, degenerate primer; G, gene-specific primer.

Download English Version:

https://daneshyari.com/en/article/5922076

Download Persian Version:

https://daneshyari.com/article/5922076

Daneshyari.com