Available online at www.sciencedirect.com
SCIENCE DIRECT
Insect Biochemistry and Molecular Biology 36 (2006) 547–560
Insect Biochemistry and Molecular Biology
www.elsevier.com/locate/ibmb

Sequence characterization and expression patterns of defensin and lysozyme encoding genes from the gut of the reduviid bug *Triatoma brasiliensis*

C.A.C. Araújo[a,b], P.J. Waniek[a], P. Stock[a], C. Mayer[a], A.M. Jansen[b], G.A. Schaub[a,*]

[a]*Department of Special Zoology, Ruhr-University, D-44780 Bochum, Germany*
[b]*Department of Protozoology, Fundação Oswaldo Cruz-FIOCRUZ, Av. Brasil, 4365, Manguinhos, CEP: 21045-900, Rio de Janeiro, Brazil*

Received 20 February 2006; received in revised form 8 April 2006; accepted 10 April 2006

Abstract

The cDNAs encoding an intestinal defensin (*def1*) and lysozyme (*lys1*) of the reduviid bug *Triatoma brasiliensis* have been amplified by PCR using specific oligonucleotide primers and 5′- and 3′-RACE, cloned and sequenced. The 576 bp clone has an open reading frame of 282 bp and encodes a pre-prodefensin with 94 amino acid residues, containing a putative signal and activation peptide cleavage site at Ser19 and Arg51, respectively. The genomic DNA contains a second defensin gene with similar characteristics, 88.3% identity and also one intron of 107 nucleotides. The 538 bp clone has an open reading frame of 417 bp, encoding a pre-lysozyme with 139 amino acid residues. The putative signal peptide is cleaved at alanine 18. Using whole mount in situ hybridization, high expression of both genes has been found, distributed uniformly throughout the entire cardia and the blood-storing stomach and to a much lower extent in the digesting small intestine. Using quantitative real-time PCR, the expression level of *def1* was also shown to be very low in small intestine, rectum and salivary glands; in the stomach, expression was 500–2500 times higher than in the cardia and fat body. No expression of *lys1* could be detected in the salivary glands and rarely a very low expression in the small intestine, rectum and fat body. *Lys1* expression in the stomach was 60–300 times higher than in the cardia. Comparing the levels in unfed fifth instars and up to 15 days after feeding, a strong *def1* induction was evident in the fat body at 15 days after feeding and in the stomach a maximum level of *def1* and *lys1* at 5 days after feeding.

Keywords: *Triatoma brasiliensis*; Defensin; Expression patterns; Gut; In situ hybridization; Lysozyme

1. Introduction

Triatoma brasiliensis is an important vector of *Trypanosoma cruzi*, the aetiologic agent of Chagas disease, in the northeast region of Brazil where these bugs colonize sylvatic, peridomestic and domestic areas (Dias et al., 2000; Herrera et al., 2003). Whereas dipteran blood-sucking insects, e.g. mosquitoes, take up nectar, honeydew and blood (Lehane, 1991), all post-embryonic stages of triatomines ingest only blood. Since this blood is sterile the presence of intestinal antibacterial compounds would seem unnecessary. However, like all terrestrial insects triatomines swallow air before moulting. Thereby airborne bacteria get access to the intestine. In addition, the development of the triatomines strongly depends on intestinal symbionts (Wigglesworth, 1952), which they obtain via coprophagy from the faeces of other bugs (Schaub, 1988; Schaub et al., 1989). After the blood meal the symbionts grow intensively in the two anterior regions of the midgut, the short cardia and the large, distensible, blood-storing stomach, and are lysed in the digestive posterior midgut, the small intestine (Eichler and Schaub, 2002).

Insects synthesize a battery of antifungal/antibacterial peptides in response to an infection of the haemocoel by bacteria, fungi or parasites (Hetru et al., 1998; Lamberty et al., 1999). Most of the antimicrobial peptides are produced in the fat body or haemocytes and then released

*Corresponding author. Tel.: +49 234 3224587; fax: +49 234 3214787.
E-mail address: guenter.schaub@rub.de (G.A. Schaub).

0965-1748/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibmb.2006.04.003

into the haemolymph of insects (Dimarcq et al., 1998; Lamberty et al., 1999; Lopez et al., 2003). Despite structural differences, they can be grouped into four families: cecropins and three families of peptides/polypeptides which are rich in proline, glycine or cysteine, respectively (Hoffmann et al., 1996). The family of cysteine-rich peptides is widely distributed, being considered as the first line of defense against microbial infections and containing peptides with molecular weights from 2 to 6 kDa and 1–4 disulphide bridges (Lamberty et al., 1999). These peptides act strongly against a broad spectrum of Gram-positive bacteria, less against Gram-negative bacteria and occasionally against fungi (Dimarcq et al., 1998; Lamberty et al., 1999). Within this family, defensins are the most widespread antimicrobial peptides in invertebrates (Bulet et al., 2004).

Defensins are cationic peptides with molecular weights of about 4 kDa, three disulphide bridges, formed by six cysteine residues and three characteristic domains, an amino terminal flexible loop, followed by an α-helix and a carboxy-terminal anti-parallel β-sheet (Bonmantin et al., 1992; Bulet et al., 1999). This peptide can be found in different organisms, such as plants, fungi, molluscs, scorpions, insects and birds, and also in different cells of various mammals, e.g. epithelial cells, neutrophils, macrophages (Lehrer et al., 1991; Cociancich et al., 1993; Charlet et al., 1996; Ehret-Sabatier et al., 1996; Thevissen et al., 1999; Zhao et al., 2001; Ganz, 2003; Bulet et al., 2004; Mygind et al., 2005). Defensins have been isolated and characterized as part of the innate immune response from the haemolymph of all the insect species so far investigated, e.g. Odonata, Diptera, Coleoptera, Lepidoptera and Hemiptera (Bulet et al., 1992; Ishibashi et al., 1999; Lamberty et al., 1999; Lopez et al., 2003; Bartholomay et al., 2004). The expression of genes encoding defensins has been described in *Drosophila melanogaster*, *Stomoxys calcitrans* and *Aedes aegypti* (Dimarcq et al., 1994; Cho et al., 1997; Munks et al., 2001; Bulet et al., 2004). Whereas the expression of only some mammalian defensin genes seems to be induced by bacterial infections (Ganz, 2003), all insect defensin genes described so far are induced by bacteria (Lowenberger et al., 1999; Hoffmann and Hetru, 1992) and, in the yellow fever mosquito *Ae. aegypti*, defensins are the predominant inducible host defense peptide (Lowenberger et al., 1999; Bartholomay et al., 2004). Insect defensins not only act against bacteria, but also interfere with the development of eukaryotic parasites in the vector, if a haemolymphal stage is a necessary part in the respective life cycle, e.g. in *Plasmodium* and filarial helminths (summarized by Lopez et al., 2003). As with mammals (Ganz, 2003), defensins are also present in the guts of insects. This has been intensively investigated in dipterans feeding on decomposing fruits (e.g. fruit flies) or bacteria-contaminated nectar, honeydew and blood (e.g. mosquitoes, sand flies and stable flies) (Munks et al., 2001; Vizioli et al., 2001). In sand flies, an injection of bacteria into the haemocoel, and also infection with the protozoan parasite *Leishmania major*, induces an intestinal production of defensins (Boulanger et al., 2004).

In triatomines, defensins have only been investigated in *Rhodnius prolixus* (Lopez et al., 2003). After an injection of bacteria into the haemocoel, the haemolymph contained several antimicrobial peptides. One of these was a defensin, which has been isolated, purified and sequenced (Lopez et al., 2003). After sequence characterization of the encoding cDNA, Northern blot autoradiography and quantitative real-time PCR, an induction of transcription was not only found in the fat body of immunized insects, but also in the gut. However, only the whole gut had been considered, no specific localization has been reported and, in addition, only interactions of the defensin with *T. cruzi* were discussed, not those with intestinal bacteria or symbionts.

A widespread antibacterial protein is lysozyme, which is produced in plants and potentially in all multicellular animals (Hultmark, 1996). In the animal phyla, the lysozymes are grouped in three types: chicken-type (*c*), goose-type (*g*) and invertebrate-type (*i*) (Jollès and Jollès, 1984; Grunclová et al., 2003). Insect lysozymes belong to the *c*-type lysozymes (Daffre et al., 1994; Lee and Brey, 1995; Hultmark, 1996; Dimarcq et al., 1998). Lysozymes hydrolyse the β-(1,4)-glycosidic bonds of the peptidoglycan layer in the wall membrane of the Gram-positive bacteria and cause cell rupture (Russell and Dunn, 1991; Daffre et al., 1994). In addition to this direct effect, lysozymes seem to contribute to the degradation of the bacterial cell wall after the bacteria have been killed by other factors (Boman et al., 1991).

The role of lysozyme as a part of the haemolymphal immune defense in insects against Gram-positive bacteria has been extensively described, also in triatomines (e.g. Azambuja and Garcia, 1987; Hultmark, 1996). However, in insects which ingest large amounts of bacteria from decomposing matter, e.g. the cyclorrhaphan flies *Musca domestica*, *Anastrepha fraterculus* and *D. melanogaster*, lysozyme also seems to be involved in the digestion of these bacteria (Lemos and Terra, 1991; Regel et al., 1998). This digestive function seems to be correlated to an acidic pH in the respective midgut region. The importance of this function is indicated by the high number of genes encoding lysozymes—in *D. melanogaster* there are at least eight lysozyme-encoding genes (Kylsten et al., 1992; Daffre et al., 1994). The major lysozyme genes *lysA*, *B*, *C*, *D* and *E* are expressed at high levels in the anterior midgut of larvae and/or adults, but not in late pupae, while *lysS* is mainly expressed in the larval gastric caeca. The expression of *lysX* starts primarily very late in the larval midgut and ends in early pupa (Kylsten et al., 1992; Daffre et al., 1994). *LysP* is expressed in the adult salivary glands and has a pH optimum around pH 5 while the digestive lysozymes have a pH optimum around pH 3.5 (Daffre et al., 1994; Regel et al., 1998).

In the haemolymph of the triatomine *R. prolixus*, antibacterial activity was induced by the inoculation of