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A repertoire of the dominant transcripts from the salivary glands of the blood-sucking bug, *Triatoma dimidiata*, a vector of Chagas disease

Hirotomo Kato[a,*], **Ryan C. Jochim**[b], **Eduardo A. Gomez**[c], **Ryo Sakoda**[a], **Hiroyuki Iwata**[a], **Jesus G. Valenzuela**[b], and **Yoshihisa Hashiguchi**[d]

[a]Department of Veterinary Hygiene, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan

[b]Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, NIH, USA

[c]Departamento de Oncocercosis, Servicio Nacional de Erradicacion de la Malaria, Ministerio de Salud Publica, Ecuador

[d]Department of Parasitology, Kochi Medical School, Kochi University, Kochi, Japan

Abstract

Triatoma (*T.*) *dimidiata* is a hematophagous Hemiptera and a main vector of Chagas disease. The saliva of this and other blood-sucking insects contains potent pharmacologically active components that assist them in counteracting the host hemostatic and inflammatory systems during blood feeding. To describe the repertoire of potential bioactive salivary molecules from this insect, a number of randomly selected transcripts from the salivary gland cDNA library of *T. dimidiata* were sequenced and analyzed. This analysis showed that 77.5% of the isolated transcripts coded for putative secreted proteins, and 89.9% of these coded for variants of the lipocalin family proteins. The most abundant transcript was a homologue of procalin, the major allergen of *T. protracta* saliva, and contributed more than 50% of the transcripts coding for putative secreted proteins, suggesting that it may play an important role in the blood-feeding process. Other salivary transcripts encoding lipocalin family proteins had homology to triabin (a thrombin inhibitor), triafestin (an inhibitor of kallikrein–kinin system), pallidipin (an inhibitor of collagen-induced platelet aggregation) and others with unknown function.

Keywords

Triatoma dimidiata; Saliva; Transcriptome; Bioinformatics; cDNA library

1. Introduction

Triatoma (*T.*) *dimidiata* is found throughout Central America and in the northern part of South America and is a main vector species of Chagas disease in these areas (Ramírez et al., 2005; Dorn et al., 2007). It feeds exclusively on blood for nutrition and for egg production throughout

*Corresponding author. Tel.: +81 83 933 5900; fax: +81 83 933 5900. katoh@yamaguchi-u.ac.jp..

Appendix A. Supplementary data Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2009.10.012.

its entire developmental cycle (Beard, 2005). Although the adult *T. dimidiata* are large insects, approximately 30 mm in body length, and feed for 20–30 min, they take blood efficiently despite the host hemostatic response (Martínez-Ibarra et al., 2001).

Hematophagous insects have evolved a wide set of pharmacologically active molecules to counteract host hemostatic processes (Ribeiro, 1995). When probing in the host skin for blood feeding, they inject saliva containing various physiologically active substances including anticoagulants, vasodilators, and inhibitors of platelet aggregation induced by collagen, adenosine diphosphate (ADP), arachidonic acid or thrombin (Ribeiro, 1995; Valenzuela, 2004, 2005; Ribeiro and Francischetti, 2003; Champagne, 2005; Andersen et al., 2005). Other salivary molecules almost certainly involved in the feeding process include anti-histamine, a sialidase, a serine protease, a sodium channel blocker, immunosuppressants and pore-forming molecules (Ribeiro, 1995; Valenzuela, 2004, 2005; Ribeiro and Francischetti, 2003; Champagne, 2005; Andersen et al., 2005). Since hematophagous arthropods have evolved their feeding strategy independently, different types of molecules have developed to overcome host hemostatic defenses in different species (Ribeiro, 1995; Champagne, 2005). Thus, insect salivary components have been extensively studied in various species to understand their unique physiological activities and have attracted attention as novel candidates for natural pharmacological agents (Champagne, 2005).

Salivary proteins of triatomine bugs have been explored, especially in *Rhodnius* (*R.*) *prolixus*, and their functional analyses have discovered novel pharmacologically active components (Montfort et al., 2000; Andersen et al., 2005). Furthermore, massive transcriptome and proteome analyses of the salivary components of *R. prolixus* identified abundant nitrophorins, which are nitric oxide (NO)-binding heme proteins belonging to lipocalin family and function as both vasodilators and inhibitors of platelet aggregation (Ribeiro et al., 2004a). Recently, in order to advance the knowledge of the salivary biomolecules of Triatominae, sialotranscriptome analyses were performed in two *Triatoma* species, *Triatoma brasiliensis* (Santos et al., 2007) and *Triatoma infestans* (Assumpção et al., 2008), both of which belong to the Infestans group that includes South American species (Schofield and Galvão, 2009). Although Infestans group salivary components were rich in lipocalins, they lacked nitrophorins, which differs from reports in *R. prolixus* (Santos et al., 2007; Assumpção et al., 2008). These results indicate that the strategy to overcome host hemostatic defenses while blood feeding is quite different between the two tribes. In the present study, to obtain further insight into the salivary biochemical and pharmacological complexity of the triatomine bug, the salivary gland transcripts were analyzed in *T. dimidiata*, which is a member of the Rubrofasciata group that includes North American species (Schofield and Galvão, 2009).

2. Materials and methods

2.1. T. dimidiata salivary glands

T. dimidiata was reared in an insectary room at National Institute of Health and Tropical Medicine in Ecuador and maintained at 28 ± 2 °C with uncontrolled humidity. They were fed at 2-week intervals on chickens. Salivary glands were dissected from fourth- and fifth-instar nymphs from the same colony 2-weeks after feeding, transferred to RNA*later* reagent (Ambion, Austin, TX) and stored at 4 °C for 4 days and then at −20 °C for 10 weeks until mRNA extraction.

2.2. Construction of salivary gland cDNA library

T. dimidiata salivary gland mRNA was isolated from 22 sets of the salivary glands using the Micro FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). The PCR-based cDNA library was prepared following the instructions for the SMART cDNA library construction kit

(BD-Clontech, Palo Alto, CA) with some modifications (Valenzuela et al., 2004). The quality of the cDNA was checked by agarose gel electrophoresis and the absence of smaller fragments derived from degraded mRNA was confirmed. The obtained cDNA library was fractionated using a Chromaspin 1000 column (BD-Clontech) into small (approximately 400–800 bp), medium (approximately 800–1200 bp) and large (>1200 bp) transcripts based on their electrophoresis profiles on a 1.1% agarose gel. Pooled fractions were ligated into Lambda TriplEx2 vector (BD-Clontech) and packaged into lambda phage (Stratagene, La Jolla, CA).

2.3. Sequence analysis of cDNA library

Single, isolated phage plaques were picked from the plate using sterile wooden sticks and placed into 50 μl of water. Amplification of cDNA was performed in a volume of 20 μl using a pair of primers, PT2F1 (5′-AAG TAC TCT AGC AAT TGT GAG C-3′) and PT2R1 (5′-CTC TTC GCT ATT ACG CCA GCT G-3′), Premix Taq (Takara Bio, Shiga, Japan) and 4 μl of phage suspension as templates. After an initial denaturation at 75 °C for 3 min and following 95 °C for 4 min, PCR amplification was performed with 33 cycles of denaturation (95 °C, 1 min), annealing (49 °C, 1 min) and polymerization (72 °C, 1 min 30 s). PCR products were cleaned using Multiscreen PCR cleaning plates (Millipore Corporation, Bedford, MA), and the cleaned PCR product was resuspended in 50 μl of water. The products were submitted to Macrogen Inc. (Seoul, Korea) for sequence analyses with PT2F3 primer (5′-TCT CGG GAA GCG CGC CAT TGT-3′).

2.4. Bioinformatics

Bioinformatics analysis was as Ribeiro et al. (2004a); however, a brief description follows. Expressed sequence tags (ESTs) were trimmed of primer and vector sequences and clustered. The ESTs were grouped based on nucleotide homology of 95% identity over 100 residues using the BLASTn algorithm (Altschul et al., 1997). The assembly of the ESTs into transcript contigs was done using the CAP3 algorithm, generating a consensus sequence (Huang, 1992). Contigs and singletons (contig containing only one sequence) were compared using BLASTx or BLASTn (Altschul et al., 1997) of the non-redundant (NR) protein database of the National Center of Biological Information (NCBI), the gene ontology database (GO) (Ashburner et al., 2000), and the Conserved Domains Database (CDD) that includes all Pfam (Bateman and Birney, 2000), SMART (Schultz et al., 1998) and COG protein domains in the NCBI (Marchler-Bauer et al., 2002). Additionally, contigs were compared using BLASTn (Altschul et al., 1997) to custom databases of mitochondrial (mit-pla) and rRNA (rrna) nucleotide sequences. Identification of putative secreted proteins was conducted using SignalP server (Bendtsen et al., 2004).

2.5. Phylogenetic analysis

The sequences that had homologies with secreted proteins by BLASTx analyses were aligned with CLUSTAL W software (Thompson et al., 1994) and examined using the Molecular Evolutionary Genetics Analysis (MEGA) version 3.1 program (Kumar et al., 2004). Phylogenetic trees by the neighbor-joining method were constructed with the distance algorithms available in the MEGA package. Bootstrap values were determined on 1000 replicates of the data sets.

3. Results and discussion

3.1. Sequence analysis of T. dimidiata salivary gland cDNA library

T. dimidiata salivary gland cDNA library was constructed and sequence analysis was performed on 576 randomly selected clones. As a result, 458 high-quality sequences with an average size of 612 bp were obtained. Three categories of expressed genes were derived from

the manual annotation of the contigs: secreted, housekeeping and unknown. The putative secreted category comprised 48.5% of the clusters and 77.5% of the total sequences. The high ratios of transcripts encoding secreted proteins were reported in other triatomine bugs: 64.0% in *R. prolixus* (Ribeiro et al., 2004a) and 68.2% in *T. brasiliensis* (Santos et al., 2007) aswellasother hematophagus insects (Valenzuela et al., 2003, 2004; Kato et al., 2006). The housekeeping category had 33.9% of the clusters and 16.2% of the total sequences. Finally, the category of "unknowns" comprised 17.6% of the clusters and 6.3% of the sequences.

3.2. Housekeeping genes

The clusters of sequences attributed to housekeeping genes (56 gene clusters with 74 sequences in total) were further divided into 16 subgroups, according to their possible function (Table 1). The two largest subgroups were associated with "translation, ribosomal structure and biogenesis" (13 sequences in 11 clusters) and "energy production and conversion" (10 sequences in 8 clusters). Forty-one sequences in 27 clusters, which represent conserved proteins with unknown function were classified as "unknown conserved". Other sequences were identified with homology to housekeeping genes and were associated with transport, metabolism, signal transduction, and cell structures, among other potential activities.

3.3. Putative secreted proteins

The transcripts coding for secreted proteins were further analyzed using the BLASTx algorithm for comparison to the NCBI non-redundant protein database. Table 2 shows the classification of transcripts coding for putative secreted proteins in *T. dimidiata* salivary glands. Remarkably, out of 355 transcripts associated with putative secreted proteins, 319 transcripts (89.9%) code for the lipocalin family of proteins. Other transcripts encoding for secreted proteins are homologous to antigen 5-related protein, hemolysin, inositol polyphosphate 5-phosphatase, serpin, apyrase, trypsin, heme-binding protein and others (Table 2). Lipocalins, the most abundant transcripts in this cDNA library were also reported to be present in the salivary glands of ticks (Mans, 2005;Mans and Ribeiro, 2008;Mans et al., 2008), but not in the saliva of mosquitoes and sand flies (Valenzuela et al., 2003,2004; Ribeiro et al., 2004b,2007; Arcà et al., 2005,2007;Anderson et al., 2006;Kato et al., 2006). A high percentage of secreted lipocalins were also reported in other *Triatoma* species: 55.0% in *T. infestans* (Assumpção et al., 2008) and 93.8% in *T. brasiliensis* (Santos et al., 2007).

3.3.1. Lipocalins—Of the transcripts coding for lipocalin-like molecules from this cDNA library, homologues of procalin, previously identified in *T. protracta* saliva (Paddock et al., 2001), were the most abundant transcripts accounting for 59.5% from the transcripts coding for this family of proteins (Table 3 and S1). The second most abundant transcript (14.4%) was a triatin-like salivary lipocalin previously identified in the saliva of *T. infestans* (Feijo and Teixeira, unpublished). Other transcripts had homologies to pallidipin-like salivary lipocalin from *T. infestans* (5.0%) and pallidipin 2 from *T. pallidipennis* (3.8%) (Table S1). Phylogenetic analysis of *T. dimidiata* salivary lipocalins together with other representative triatomine lipocalin resulted in the formation of seven different divergent and separated clades (Fig. 1 and Table S1). Further characteristics of *T. dimidiata* lipocalin-like clades are described below.

3.3.1.1. Clade I: procalin-like protein: Procalin was identified as the major allergen of *T. protracta* saliva (Paddock et al., 2001); however the role of procalin during blood feeding is not known. This group was the most abundant transcript in *T. dimidiata* salivary glands, and ten transcripts of the procalin-like protein were identified in the *T. dimidiata* cDNA library, Td01–08, Td12 and Td110. Alignment of the encoding proteins with the procalin of *T. protracta* showed a high level of homology (Fig. S1). The GXW motif, which is often observed in lipocalins, was conserved, and all six cysteine residues were located at the corresponding

positions in all molecules (Fig. S1). Phylogenetic analysis resulted in the formation of two distinct subclades (Fig. S1).

3.3.1.2. Clade II: triatin-like protein: Triatin was originally identified in the salivary glands of *T. infestans* (Feijo and Teixeira, unpublished) and its function remains unknown. Four contigs from the *T. dimidiata* cDNA library (Td14, Td15, Td22 and Td29) were homologous to triatin. Alignment of these triatin-like molecules shows a high level of homology among them (Fig. S2). The GXW motif and the six cysteine residues were highly conserved in these molecules (Fig. S2). Phylogenetic analysis resulted in the formation of two distinct subclades: one containing Td14 and Td15, and the other containing Td22 and Td29 (Fig. S2).

3.3.1.3. Clade III: triabin-like protein: Two contigs (Td60 and Td101) from this cDNA library were homologous to the salivary molecule triabin. Triabin is a thrombin inhibitor from the saliva of *T. pallidipennis* (Noeske-Jungblut et al., 1995). Alignment of *T. dimidiata* triabin-like proteins and triabin from *T. infestans* shows a high level of homology, the presence of the GXW motif and the presence of five of the six conserved cysteines (Fig. S3). Thus, proteins derived from Td60 and Td101 may function as thrombin inhibitors in *T. dimidiata* saliva.

3.3.1.4. Clade IV: salivary lipocalin-like protein: The proteins in this clade had homology with salivary lipocalin of *T. brasiliensis* and *T. infestans* with unknown function (Table S1) (Santos et al., 2007;Assumpção et al., 2008). This clade contained four contigs (Td19, Td27, Td28 and Td47), and the alignment of the encoding proteins with salivary lipocalin of *T. brasiliensis* showed a high level of homology among them (Fig. S4). The GXW motif was not found in these members except for Td19 and four of the six cysteine residues were located at conserved positions (Fig. S4). Phylogenetic analysis resulted in three distinct subclades (Fig. S4).

3.3.1.5. Clade V: triafestin-like protein: Triafestin-1 and -2 were identified in *T. infestans* saliva and were shown to be the inhibitors of the activation of the kallikrein–kinin system (Isawa et al., 2007). In the *T. dimidiata* cDNA library, contigs Td25, Td26 and Td40 are homologous to triafestin-1 and -2. Alignment of the encoded proteins with triafestin-1 showed that the GXW motif and six cysteine residues were located at conserved positions (Fig. S5). Phylogenetic analysis resulted in the formation of two distinct subclades; the first contained Td25 and Td26, and the second contained Td40 (Fig. S5).

3.3.1.6. Clade VI: pallidipin 2-like protein: Pallidipin 2 from *T. pallidipennis* and triplatin-1 and -2 from *T. infestans* saliva were characterized as inhibitors of collagen-induced platelet aggregation (Noeske-Jungblut et al., 1994; Haendler et al., 1995; Morita et al., 2006). Ten contigs from the *T. dimidiata* cDNA library (Td17, Td30, Td31, Td33, Td34, Td38, Td41, Td42, Td59 and Td153) had homology with pallidipin 2 and triplatin-1 and -2. Of the ten contigs, Td17, Td41 and Td42 had the highest homology with pallidipin 2, while the rest (Td30, Td31, Td33, Td34, Td38, Td59 and Td153) were closer to pallidipin-like salivary lipocalin from *T. infestans* with unknown function (Table S1)(Assumpção et al., 2008). When the encoded proteins were aligned with pallidipin 2, four of the six cysteine residues were conserved; possibly generating a similar tertiary protein structure based on the disulfide bonds (Fig. S6). Phylogenetic analysis resulted in the formation of three distinct subclades; the first containing Td17, Td41 and Td42 that had the highest similarity to pallidipin 2, the second containing Td33, Td34, Td38, Td59 and Td153, and the third containing Td30 and Td31 (Fig. S6).

3.3.1.7. Clade VII: pallidipin-like lipocalin-like protein: Five contigs from the *T. dimidiata* cDNA library (Td09, Td10, Td11, Td18 and Td45) were closely related to a pallidipin-like lipocalin with unknown function (Table S1)(Assumpção et al., 2008).

Alignment of the encoded proteins showed a high level of homology with pallidipin-like lipocalin from *T. infestans* (Fig. S7). The GXW motif was conserved in three of the five members, and five of the six cysteine residues were conserved in these sequences (Fig. S7). Phylogenetic analysis resulted in two distinct subclades: the first contained Td9, Td10, Td11 and Td45, and the second contained Td18 (Fig. S7).

3.3.1.8. Other lipocalins: One contig (Td99) had homology with biogenic amine-binding protein (ABP) identified from the saliva of *R. prolixus* (Andersen et al., 2003)(Fig. 1 and Table S1). ABP binds serotonin, epinephrine and norepinephrin, resulting in inhibition of platelet aggregation and smooth muscle contraction (Andersen et al., 2003). It may also act to inhibit inflammatory responses at the feeding site by binding these proinflammatory molecules. Two contigs, Td23 and Td24 showed homology with another salivary lipocalin of *T. infestans* with unknown function (Table S1). These transcripts were also relatively similar to triabin, a thrombin inhibitor from *T. pallidipennis* (Fig. 1). Td46 showed similarity to triabin-like lipocalin 4a precursor from *T. infestans* with unknown function (Table S1).

3.3.2. Antigen 5-related protein—This family of proteins belongs to the cysteine-rich secretory proteins (CRISPs) and is related to venom allergens in social wasps and ants (Lu et al., 1993; Hoffman, 1993; King and Spangfort, 2000). Transcripts coding for the members of this protein family have been identified in the salivary glands of blood-sucking insects such as mosquitoes (Francischetti et al., 2002; Arcà et al., 2007), sand flies (Valenzuela et al., 2004; Anderson et al., 2006; Kato et al., 2006) and triatomine bugs (Ribeiro et al., 2004a; Santos et al., 2007; Assumpção et al., 2008). The ratio of the transcripts coding for this molecule was higher in *T. dimidiata* (4.2%) when compared to *T. infestans* (2.0%) (Assumpção et al., 2008) and *T. brasiliensis* (0.1%) (Santos et al., 2007)(Table 2). Although the antigen 5 family of protein has been widely identified in blood-sucking arthropods, the function in their saliva has yet to be determined. Four contigs (Td16, Td36, Td48 and Td143) are included in this family. Alignment and phylogenetic analysis of antigen 5-related proteins from various species indicated that *T. dimidiata* salivary antigen 5-related proteins had closer relationships with those from triatomine bugs than those from saliva of other blood feeders (Fig. S8).

3.3.3. Hemolysin-like protein—One contig (Td21), containing 6 sequences, shared homology with a hemolysin-like secreted salivary protein 3 from *T. infestans* (Assumpção et al., 2008)(Table 2 and Fig. S9). Hemolysin is a pore-forming toxin and is often described in microorganisms (Gouaux, 1998; Melton et al., 2004; Wassenaar, 2005). The role of this protein in the saliva of triatomine bugs is not known; however, it is speculated that the protein may act as a cytolytic protein, causing erythrocyte lysis and helping the early steps of the digestion process (Assumpção et al., 2008). Alternatively, it may work as an antimicrobial peptide in the saliva.

3.3.4. Inositol polyphosphate 5-phosphatase-like protein—Inositol polyphosphate phosphatases (IPPs) regulate the pool of 5-phosphorylated inositol phosphates and phosphoinositides, thereby influencing cellular processes related to signal transduction, secretion and cytoskeletal structure (Erneux et al., 1998; Guo et al., 1999). Thus, IPPs in the saliva of blood feeders are speculated to act to reduce the bulk concentration of $PI(4,5)P_2$ and $PI(3,4,5)P_3$ in the plasma membrane of cells or platelets, resulting in the elimination of substrates for phospholipase C and PI3-kinase and causing changes in cytoskeletal architecture, membrane trafficking and vesicle secretion (Andersen and Ribeiro, 2006). In this study, two singletons (Td61 and Td95) were best matched with IPPs of *T. infestans* (Assumpção et al., 2008)(Table 2).

3.3.5. Serpin-like protein—Serpins are a large family of structurally related proteins found in animals, plants, and viruses, and generally function as inhibitors of a number of fundamental biological processes such as blood coagulation, complement activation, fibrinolysis, angiogenesis and inflammation (Hekman and Loskutoff, 1987; Rubin, 1996; Prevot et al., 2006). Serpins in the saliva of blood-sucking arthropods affect hemostatic activities in several phases including platelet adhesion, coagulation pathway and fibrinolysis to facilitate blood feeding (Brandt et al., 2004; Prevot et al., 2006). A singleton (Td66) had homology with serpin 3 of *Ctenocephalides felis* (Brandt et al., 2004)(Table 2), but the sequence was truncated in the 5′ region.

3.3.6. Apyrase-like protein—Apyrases are nucleoside triphosphate-diphosphohydrolases present in a variety of organisms. In the saliva of blood-sucking arthropods, apyrases function to hydrolyze ADP in a Ca^{2+}- dependent manner and inhibit ADP-induced platelet aggregation to facilitate the blood-feeding process (Ribeiro and Francischetti, 2003; Faudry et al., 2004). A singleton (Td64) had homology with the 79 kDa salivary apyrase of *T. infestans* (Assumpção et al., 2008) (Table 2), but the sequence was truncated in the 5′ region.

3.3.7. Trypsin-like protein—A singleton (Td70) showed 43.2% identity to a trypsin-like serine protease LlSgP4 identified in the salivary glands of the tarnished plant bug *Lygus lineolaris* (Table 2). Extra-intestinal digestion through injection of saliva containing trypsin and other enzymes into plant tissue is considered to facilitate the ingestion of plant juice (Zhu et al., 2003). The enzyme is predicted to play an important function for protein digestion and absorption in blood-sucking arthropods as well. Similar transcripts have been identified in the salivary glands of *T. brasiliensis* (Santos et al., 2007).

3.3.8. Heme-binding protein—A singleton (Td76) shared 43.6% identity to heme-binding protein from *T. infestans* (Assumpção et al., 2008)(Table 2). The transcripts were also identified in the salivary glands of *T. brasiliensis* (Santos et al., 2007) and in the hemolymph and oocytes of *R. prolixus* (Paiva-Silva et al., 2002). The heme-binding proteins contain conserved pheromone/odorant binding protein domains. The role of these salivary proteins for blood feeding is unknown in *Triatoma*, but it is speculated that they could be acting as partners in gustatory perception as reported in *Drosophila* (Galindo and Smith, 2001).

3.3.9. Other putative secreted proteins—Two singletons (Td78 and Td117) had homology with *Apis mellifera* proteins similar to CG5059-PA (Genbank accession number: XP_393165.2) and CG5885-PA (XP_624674.1), respectively, both of which functions are unknown. Singletons, Td119 and Td154, were similar to a *Tribolium castaneum* hypothetical protein (XP_976325.1) and an *Anopheles gambiae* protein AGAP002087-PA (XP_550715.2), respectively. Two singletons (Td67 and Td68) had homology with rhamnose-binding lectin identified from a genomic sequence of *Strongylocentrotus purpuratus* (XP_001189047). Two singletons (Td69 and Td114) did not have marked similarity to known proteins.

3.4. Concluding remarks

The present study shows a high number and variety of lipocalin transcripts in the salivary glands of *T. dimidiata*, a member of the Rubrofasciata group. Overall, *T. dimidiata* salivary components were similar to those of other *Triatoma* species (Santos et al., 2007; Assumpção et al., 2008), importantly, more than 50% of the transcripts coding for secreted proteins were homologues of procalin. Although procalin was identified as the major allergen of *T. protracta* saliva, the function in the blood-feeding process is not determined. Furthermore, this salivary protein has the potential to be used as marker for *T. dimidiata* exposure for epidemiological studies. Procalin-homologues are also similar to triabin, a thrombin inhibitor identified in *T. pallidipennis* saliva (Noeske-Jungblut et al., 1995) and triafestins, an inhibitor

of the activation of the kallikrein–kinin system in *T. infestans* saliva (Isawa et al., 2007). Thus, further functional analysis of the transcript-derived protein may lead to interesting findings in the role of procalin-like proteins as potential bioactive molecules and modulators of blood feeding. Although salivary transcripts with homology to triabin, triatin, pallidipin and triafestin were identified in *T. dimidiata* as well as *T. infestans* and *T. brasiliensis*, other transcripts such as those coding for Kazal domain containing peptides found in both *T. infestans* (2.6% of the transcripts coding for the putative secreted proteins) (Assumpção et al., 2008) and *T. brasiliensis* (2.2%) (Santos et al., 2007), trialysin (11.5%), defensin and immunity-related peptide (5.0%), and triatox (1.2%) in *T. infestans* (Assumpção et al., 2008), were not detectable in *T. dimidiata* salivary glands. The lower complexity of the transcripts in *T. dimidiata* salivary glands may in part be due to the smaller number of cDNA clones analyzed or the actual differences in the salivary components between these species. A more comprehensive analysis may reveal the presence of these molecules in *T. dimidiata* salivary glands.

In conclusion, the most abundant proteins of *T. dimidiata* saliva were identified in this study. These results will provide further insights into the evolution of salivary components in blood-sucking arthropods. In addition, the cDNAs and future recombinant proteins prepared from these transcripts will result in the discovery of novel pharmacologically active compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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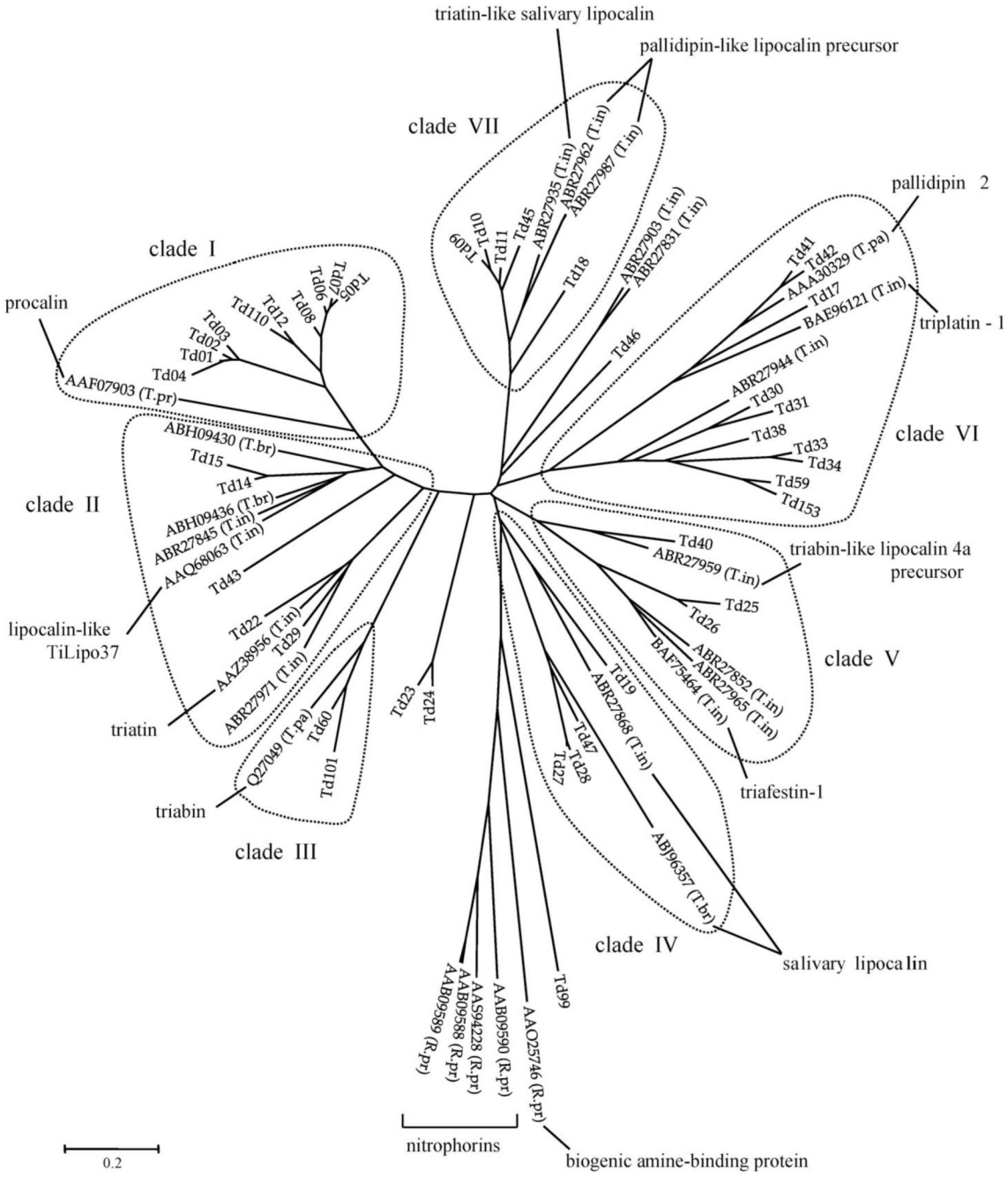

Fig. 1.

Phylogenetic tree of the *T. dimidiata* salivary lipocalins with those from other triatomine bugs. The sequences of *T. dimidiata* lipocalin obtained in the present study were aligned with representative triatomine lipocalin sequences obtained from the non-redundant protein database, and a phylogenetic tree was constructed. The sequences from the database are represented by "GenBank accession number (abbreviation of the species name)". T.in, *T. infestans*; T.br, *T. brasiliensis*; T.pa, *T. pallidipennis*; T.pr, *T. protracta*; R.pr, *R. prolixus*. The scale bar represents 0.2% divergence.

Table 1

Functional classification of the housekeeping genes expressed in *Triatoma dimidiata* salivary glands.

Types of transcripts	Clusters	%	Sequences	%
Translation, ribosomal structure and biogenesis	11	19.6	13	17.5
Energy production and conversion	8	14.2	10	13.5
Secondary metabolites biosynthesis, transport and catabolism	4	7.1	4	5.4
Carbohydrate transport and metabolism	3	5.3	4	5.4
Lipid transport and metabolism	2	3.6	4	5.4
Intracellular trafficking, secretion, and vesicular transport	2	3.6	2	2.7
Posttranslational modification, protein turnover, chaperones	2	3.6	2	2.7
RNA processing and modification	2	3.6	2	2.7
Signal transduction mechanisms	2	3.6	2	2.7
Extracellular structures	1	1.8	12	16.1
Amino acid transport and metabolism	1	1.8	1	1.4
Cell wall/membrane/envelope biogenesis	1	1.8	1	1.4
Cytoskeleton	1	1.8	1	1.4
Inorganic ion transport and metabolism	1	1.8	1	1.4
Nuclear structure, intracellular trafficking, and vesicular transport	1	1.8	1	1.4
Unknown conserved	14	25.0	14	18.9
Total	56	100.0	74	100.0

Table 2

Classification of transcripts coding for putative secreted proteins in *Triatoma dimidiata* salivary glands.

Types of transcripts	Clusters	Sequences	% sequences
Lipocalin family	58	319	89.9
Antigen 5-related	6	15	4.2
Hemolysin	1	6	1.7
Inositol polyphosphate 5-phosphatase	2	2	0.5
Serpin	1	1	0.3
Apyrase	1	1	0.3
Trypsin	1	1	0.3
Heme-binding protein	1	1	0.3
Others	9	9	2.5
Total	80	355	100.0

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Table 3

Transcripts coding for lipocalin family proteins in *Triatoma dimidiata* salivary glands.

Name	Length	No. of seq in cluster	Mature MW (kDa)	Cleavage position	Best match to NR protein database	*E* value	% identity	Accession no.
Td08	622	39	17.9	18–19	Procalin precursor (*T. pr rotracta*): AAF07903	2.00E–34	45	AB470365
Td03	628	32	17.5	18–19	Procalin precursor (*T. pr rotracta*): AAF07903	1.00E–32	41	AB470360
Td05	615	31	17.9	18–19	Procalin precursor (*T. pr rotracta*): AAF07903	2.00E–34	44	AB470362
Td01	615	27	17.6	18–19	Procalin precursor (*T. pr rotracta*): AAF07903	1.00E–28	39	AB470358
Td02	627	25	17.5	18–19	Procalin precursor (*T. pr rotracta*): AAF07903	6.00E–35	45	AB470359
Td12	629	23	17.9	18–19	Procalin precursor (*T. pr rotracta*): AAF07903	5.00E–36	44	AB470369
Td11	681	16	20.2	18–19	Triatin-like salivary lipo ocalin (*T. infestans*): ABR27935	1.00E–50	53	AB470368
Td10	690	14	20.4	18–19	Triatin-like salivary lipo ocalin (*T. infestans*): ABR27935	2.00E–53	55	AB470367
Td09	678	9	20.6	18–19	Triatin-like salivary lipo ocalin (*T. infestans*): ABR27935	2.00E–48	52	AB470366
Td04	628	8	17.5	18–19	Procalin precursor (*T. pr rotracta*): AAF07903	3.00E–32	41	AB470361
Td17	711	8	19.7	18–19	Pallidipin 2 (*T. pallidipe ennis*): AAA30329	1.00E–49	52	AB470373
Td14	595	8	16.9	18–19	Salivary lipocalin (*T. in nfestans*): ABR27845	5.00E–47	58	AB470370
Td18	691	7	20.2	18–19	Triatin-like salivary lipo ocalin (*T. infestans*): ABR27935	1.00E–47	49	AB470374
Td22	647	5	20.6	16–17	Triatin (*T. infestans*): AA AZ38956	3.00E–63	57	AB470377
Td29	684	5	20.4	18–19	Triatin-like salivary lipo ocalin (*T. infestans*): ABR27971	2.00E–65	65	AB470384
Td19	771	4	22.3	18–19	Salivary lipocalin (*T. infestans*): ABR27868	1.00E–46	50	AB470375
Td06	639	4	18.0	18–19	Lipocalin-like TiLipo37 7 (*T. infestans*): AAQ68063	9.00E–35	43	AB470363
Td30	719	4	22.7	18–19	Pallidipin-like salivary lipocalin (*T. infestans*): ABR27944	7.00E–32	38	AB470385
Td23	792	3	21.2	18–19	Salivary lipocalin (*T. infestans*): ABR27831	1.00E–28	38	AB470378
Td25	671	3	17.3	18–19	Salivary lipocalin (*T. infestans*): ABR27852	2.00E–49	57	AB470380
Td43	624	3	18.4	18–19	Lipocalin-like TiLipo37 7 (*T. infestans*): AAQ68063	2.00E–34	46	AB470393
Td31	709	3	22.8	18–19	Pallidipin-like salivary lipocalin (*T. infestans*): ABR27944	2.00E–24	34	AB470386
Td33	787	3	21.7	18–19	Pallidipin-like salivary lipocalin (*T. infestans*): ABR27944	2.00E–37	37	AB470387
Td38	724	3	21.3	18–19	Pallidipin-like salivary lipocalin (*T. infestans*): ABR27944	5.00E–42	43	AB470389
Td40	710	3	21.3	18–19	Triabin-like lipocalin 4a a precursor (*T. infestans*): ABR27959	2.00E–75	68	AB470390
Td27	701	2	18.8	15–16	Salivary lipocalin (*T. brasiliensis*): ABJ96357	3.00E–38	47	AB470382
Td28	717	2	20.0	18–19	Salivary lipocalin (*T. brasiliensis*): ABJ96357	1.00E–39	48	AB470383
Td47	716	2	19.8	22–23	Salivary lipocalin (*T. brasiliensis*): ABJ96357	4.00E–41	50	AB470396

Name	Length	No. of seq in cluster	Mature MW (kDa)	Cleavage position	Best match to NR protein database	*E* value	% identity	Accession no.
Td26	673	2	20.7	18–19	Salivary lipocalin (*T. infestans*): ABR27965	7.00E–57	56	AB470381
Td07	616	2	18.0	18–19	Procalin precursor (*T. pr rotracta*): AAF07903	2.00E–34	44	AB470364
Td15	596	2	18.2	18–19	Lipocalin-like TiLipo37 7 (*T. infestans*): AAQ68063	3.00E–36	52	AB470371
Td41	567	2	17.8	18–19	Pallidipin 2 (*T. pallidipe ennis*): AAA30329	3.00E–61	67	AB470391
Td42	648	2	18.6	18–19	Pallidipin 2 (*T. pallidipe ennis*): AAA30329	4.00E–63	65	AB470392
Td45	613	2	20.1	18–19	Pallidipin-like lipocalin precursor (*T. infestans*): ABR27987	1.00E–50	52	AB470394
Td46	728	2	20.9	16–17	Triabin-like lipocalin 4a a precursor (*T. infestans*): ABR27959	2.00E–27	40	AB470395
Td24	799	1	21.2	18–19	Salivary lipocalin (*T. infestans*): ABR27903	2.00E–30	41	AB470379
Td110	636	1	17.8	18–19	Procalin precursor (*T. pr rotracta*): AAF07903	1.00E–32	42	AB470402
Td59	663	1	20.8	18–19	Pallidipin-like salivary lipocalin (*T. infestans*): ABR27944	6.00E–30	35	AB470397
Td124	707	1	19.3	18–19	Pallidipin-like salivary lipocalin (*T. infestans*): ABR27944	9.00E–40	41	AB470403
Td153	757	1	21.9	18–19	Pallidipin-like salivary lipocalin (*T. infestans*): ABR27944	7.00E–30	37	AB470405
Td60	618	1	20.9	21–22	Triabin precursor (*T. pallidipennis*): Q27049	3.00E–47	62	AB470398
Td101	645	1	20.0	21–22	Triabin precursor (*T. pallidipennis*): Q27049	5.00E–42	57	AB470401
Td99	692	1	21.4	18–19	Biogenic amine-binding protein (*R. prolixus*): AA025746	1.00E–11	28	AB506673

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