Blood digestion of triatomines and the body louse *Pediculus humanus corporis* – a review

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Zusammenfassung: Die mehr als 140 Arten der Unterfamilie Triatominae (Reduviidae, Hemiptera) leben fast ausschließlich in Lateinamerika. Alle postembryonalen Stadien saugen in relativ kurzer Zeit viel Blut, lagern es im sehr dehnbaren Magen und transportieren die wässrigen Anteile sofort in die Hämolymphe und dann in die Malpighischen Gefäße. Das aufkonzentrierte Blut wird im Dünndarm bis zur Häutung in das nächste Nymphenstadium langsam verdaut. Triatominen weisen einen sauren pH im Darmlumen auf und verdauen die Proteine mit Cystein- und / oder Aspartat-Proteasen und dann mit Amino- und Carboxypeptidasen. Bei aufgereinigten Aspartat-Proteasen ist die Aktivität bei pH 2 bis pH 4 am stärksten, aber der pH-Wert im Darm sinkt nach der Blutaufnahme nicht unter 5,2. Die Amino- and Carboxypeptidasen sind wohl eher im Bereich der perimikrovillären Membranen aktiv.
Kleiderläuse (*Pediculus humanus corporis,* Pediculidae, Phthiraptera) sind kosmopolitische Ektoparasiten und leben auf dem Menschen oder in seiner Kleidung. Alle postembryonalen Stadien saugen regelmäßig Blut, mindestens einmal am Tag und verdauen es rasch. Sie setzen für die Proteinverdauung die Serin-Proteasen Trypsin und Chymotrypsin ein, die bei alkalischem pH aktiv sind, und dann Amino- und Carboxypeptidasen. Die Konzentration der mRNA der Serin-Proteasen ist hoch, aber nur im dehnbaren anterioren Mitteldarm und einem kurzen Abschnitt des sich anschließenden dünnen Mitteldarms, in dem dann die Exoproteasen aktiv sind.

Key words: Triatominae, aspartate protease, cysteine protease, blood digestion, body lice, chymotrypsin, trypsin, aminopeptidase, carboxypeptidase

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Introduction

Both triatomines and body lice are temporary ectoparasites, but differ in many aspects. Of the > 150 species of Triatominae, the majority lives in Latin America, whereas *Pediculus humanus corporis* is a cosmopolitan (Schaub & al. 2016, Schaub & Mehlhorn 2016). Triatomines transmit *Trypanosoma cruzi* (Trypanosomatidae, Kinetoplastida), the aetiologic agent of Chagas disease. According to the association to houses, *Triatoma infestans* is the most important vector. Body lice are vectors of *Rickettsia prowazekii*, *Borrelia recurrentis* and *Rochalimaea quintana*, causing classical epidemic typhus, louse-borne relapsing fever and trench or 5-day fever, respectively. The sizes of triatomines and lice differ strongly, ranging up to 4.2 cm and 0.5 cm, respectively. All these species have a hemimetabolous development, covering five or three nymphal stages in triatomines and lice, respectively. Therefore, all postembryonic stages suck blood. While triatomines attack all warm-blooded animals, body lice infest only humans. In our searching for targets to control these vectors, we investigate the activities of digestive enzymes, identify genes encoding these enzymes and analyse the expression of these genes at different times after feeding and in different tissues.

Blood ingestion and digestion of triatomines

Triatomines infest the host only for a relatively short period of time. Within up to 20 minutes, they take up a lot of blood in the distensible stomach, a part of the anterior midgut, about 6–12-times their own body weight. Females of *Dipetalogaster maxima* ingest up to 4.3 ml (Stadler & al. 2011). This is sufficient to starve long periods of time, in some larval instars for up to about 1 year. Already during blood

ingestion, the blood is concentrated by withdrawal of ions and water that are excreted very rapidly via the Malpighian tubules. In the stomach, anticoagulants from the saliva and the stomach inhibit a clotting of the blood that is only processed by lysis of erythrocytes and resorption of sugar (Azambuja & al. 1983, Meiser & al. 2010). Directly after blood ingestion, acidification begins, reducing the pH of blood (naturally pH 7.4). This will continue up to eight days after blood ingestion until pH 5.2 is reached, followed by a slight increase (Balczun & al. 2012a). Small portions of stomach content are gradually passed to the following narrow posterior midgut, the small intestine, and enveloped by developing perimicrovillar membranes. In nymphs, nearly all stomach contents are digested before the moult to the next instar. In the last nymphal stage of *T. infestans*, the whole period of digestion lasts more than 5 weeks.
Haemoglobin is digested in the small intestine, starting immediately after the passage and indicated by a change of colour of the intestinal contents from red to brown. Triatomines digest the blood via cysteine and/or aspartate proteases (intracellularly located in lysosomes in other insects). In a quantification of hydrolytic activities of the intestines of the triatomine *Rhodnius prolixus*, activities of cathepsin B are stronger than those of cathepsin D (Terra & al. 1988). Later on, cDNAs encoding cathepsin L-like enzymes were identified from the intestines of *R. prolixus* and *T. infestans* (Lopez-Ordoñez & al. 2001, Kollien & al. 2004a). The hydrolytic activity of the cysteine proteases cathepsin B and L in small intestine extracts of fifth instar nymphs of unfed and fed *T. infestans* is optimal at pH 5, decreases within the first two days after feeding and is much higher 5 and 10 days after feeding (Kollien & al. 2004a). Sequencing of mRNA of *T. infestans* indicates the presence of two cathepsins B and one cathepsin L. The cathepsin B1 gene is expressed constitutively at a very low level in the gut tissues of unfed and fed *T. infestans*. According to in situ hybridizations, a high level of cathepsin B1 mRNA is present in the small intestine of fifth instar nymphs 5 days after feeding, but not in the stomach and intestines of unfed nymphs as well as 8 and 20 days after feeding (Balczun & al. 2012b). The primary structures of both cysteine proteases differ considerably, indicating a predominant exopeptidase and endopeptidase activity of cathepsin B and L, respectively (Balczun & al. 2012b). Therefore, the initial hydrolysis of peptide bonds within the polypeptide chain seems to be carried out by cathepsin L.

At least three different forms of aspartate proteases are present in *T. infestans*, and the genes of two forms are expressed both in the walls of the stomach and the small intestine, but not in the tissues of the rectum, salivary glands, Malpighian tubules and haemocytes (Balczun & al. 2012b). One of the genes is most strongly expressed 2 days after blood sucking and then less and less up to 20 days after feeding of fifth instar nymphs, while the other is hardly expressed initially and only slightly stronger in the following. Purified aspartate proteases are very active from pH 2 to pH 4, but the pH values in the intestine do not decrease below pH 5.2. In the triatomine *Triatoma brasiliensis*, western blot analysis confirms the presence of cathepsin L in the lumen of the small intestine (Waniek & al. 2012).

The amino- and carboxypeptidases, of which several isoforms occur, are active in extracts of the wall of the stomach and the small intestine and in the content of the small intestine (Balczun & al. 2012b). In *R. prolixus*, these enzymes seem to be located between the perimicrovillar membranes (Billingsley & Downe 1985). In *T. brasiliensis*, transcript abundances of two serine carboxypeptidases are high in the small intestine and lower in the stomach (Waniek & al. 2014).

Blood ingestion and digestion of lice

Lice always live near or on the host and suck blood regularly, usually several times but at least once per day. Similar to triatomines, they store the blood in the distensible part of the anterior midgut, but they digest it rapidly, also in this part. Like most bloodsucking insects, lice use serine proteases for blood digestion that are active at alkaline pH and then amino- and carboxypeptidases. When investigating a strain of body lice adapted to feed on rabbits or via membranes in daily intervals, three activity bands of serine proteases become apparent three hours after feeding in native-polyacrylamide gel electrophoresis and subsequent azocasein zymography (Waniek & al. 2002). According to sequencing, one trypsin1 gene, five isoforms/variants of the trypsin2 gene and six isoforms/variants of the chymotrypsin gene are present (Kollien & al. 2004b, Waniek & al. 2005). While trypsins2 and chymotrypsins are activated by a trypsin, the activation sequence in trypsin1 is cut off by a chymotrypsin, a previously unknown

phenomenon. According to in situ hybridization, 1 – 24 hours after feeding, the mRNA of trypsin1, trypsin2 and chymotrypsin is present in the wide anterior midgut and the beginning of the narrow posterior region. Real time PCR shows a constitutive expression of these genes. The mRNA concentration of chymotrypsin is highest, that of trypsin1 similarly high or slightly lower and that of trypsin2 low (Waniek & al. 2005). While genes encoding these serine endopeptidases are highly expressed in the anterior midgut, the expression level of genes encoding exopeptidases is high in the narrow posterior region (Waniek 2009). Body lice possess a leucine aminopeptidase (Ochanda & al. 2000). A glutamyl aminopeptidase is expressed constitutively, mainly in the narrow posterior region of the midgut (Kollien & al. 2007). However, according to a screening of a body lice library (Pedra & al. 2003), both aminopeptidases are not present, indicating a low-level expression of these genes.

Conclusions

This comparison demonstrates the strong differences of blood ingestion and digestion by triatomines and lice. The GenBank contains sequences of several isoforms of triatomine proteases and the transcriptome of *R. prolixus* many transcript variants of the corresponding genes (Ribeiro & al. 2014). Therefore, the putative regulatory network of proteases remains to be clarified as well as the effect of blood of different host species on the expression of theses genes. In addition, unknown aspects are the nature of enzymes digesting mutualistic symbionts in the small intestine, the relative impact of cathepsins B and L on blood digestion and differences between enzymes present intracellularly in lysosomes and active in the gut. The number of investigations of human lice is relatively low. The expression of genes coding for digestive enzymes in different regions of the digestive tract should be examined separately and not in the context of whole body homogenates. Such investigations should consider data of genome sequencing (Kirkness & al. 2010).

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