



# Alkaline phosphatase activity in salivary gland cells of *Rhodnius neglectus* and *R. prolixus* (Hemiptera, Triatominae)

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**ABSTRACT.** Alkaline phosphatase activity was detected in salivary gland cells of the *Rhodnius neglectus* Lent, 1954, and *R. prolixus* Stal, 1859, vectors of *Trypanosoma cruzi* Chagas, 1909 (etiological agent of Chagas disease) and *T. rangeli* Tejera, 1920 (pathogenic to insect). The Gomori technique was used to demonstrate alkaline phosphatase activity. Alkaline phosphatase activity was observed throughout the entire gland, with an increased activity in the posterior region of the principal gland. In particular, phosphatase activity was found in the nucleolar corpuscles, suggesting a relationship with the rRNA

transcription and ribosomal biogenesis. Alkaline phosphatase was also detected in the nuclear membrane and nuclear matrix, suggesting an association with the nucleo-cytoplasmic transport of ribonucleoproteins and the mechanisms of cell cycle and DNA replication, respectively. This study highlights the importance of alkaline phosphatase in the salivary gland of *R. prolixus* and *R. neglectus* and emphasizes its importance in secretory activity. Secretory activity is directly involved in hematophagy and, consequently, in development during metamorphosis. The observed presence of alkaline phosphatase suggests its involvement in the production of saliva allowing feeding of these insects that are important vectors of Chagas disease.

**Key words:** Cytochemistry; Hematophagy; Triatominae subfamily

## INTRODUCTION

Triatomines (Hemiptera, Heteroptera, Reduviidae, Triatominae) are vectors of two protozoa: *Trypanosoma cruzi* Chagas, 1909, and *T. rangeli* Tejera, 1920. *T. cruzi* is an etiological agent of Chagas disease, a disease endemic to Latin America, which affects about 7 to 8 million people in the world (WHO, 2014). *T. rangeli* is considered pathogenic only to the insect itself and can prejudice the gut, Malpighian tubules, cuticle, trachea, salivary glands, and nervous system (Vallejo et al., 2009).

Triatominae is comprised of six tribes, namely, Alberproseniini, Bolboderini, Cavernicolini, Linshcosteini, Rhodniini, and Triatomini (Alevi et al., 2013). Rhodniini comprises two genera (*Rhodnius* and *Psammolestes*) and 22 species (19 of *Rhodnius* and 3 of *Psammolestes*) (Abad-Franch et al., 2013; Alevi et al., 2013).

Triatomines are hematophagous insects. They have a gland that produces saliva, which is constantly released during the blood meal. This saliva is necessary for localization of the blood within the blood vessel, as well as the ingestion of blood to maintain the flux (Ribeiro, 1987; Law et al., 1992; Soares et al., 2006). It is secreted in order to antagonize hemostatic, inflammatory, and immunologic systems imposed by the host (Ribeiro and Francischetti, 2003).

In *Rhodnius*, the salivary gland is composed of two units: the principal (reddish, elongated, and bigger) and accessory (translucent and rounded) units. These structures have been studied anatomically, histologically (Lacombe, 1999), cytogenetically (Anhê and Azeredo-Oliveira, 2008; Anhê et al., 2014), and biochemically (Anhê et al., 2007).

These insects are hematophagous and strict repast is fundamental for the molts occur. Therefore, this paper aimed to describe the activity of alkaline phosphatase in the cells of the *R. neglectus* Lent, 1954, and *R. prolixus* Stal, 1859, salivary glands that are so important for the feeding and development of these vectors.

## MATERIAL AND METHODS

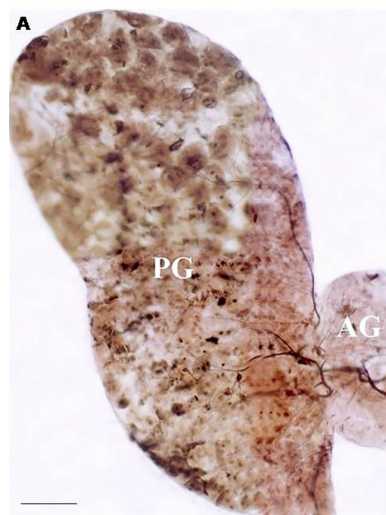
The Gomori technique (Gomori, 1952) was used to identify alkaline phosphatase, whose activity is at pH between 9.0 and 9.6. Unfixed whole salivary glands of adult, male and female *R. neglectus* and *R. prolixus* were studied. The salivary glands of 30 insects (15 males and 15 females) of each species were analyzed. They had been assigned by the

“Triatominae Insectarium” at the Departamento de Ciências Biológicas na Faculdade de Ciências Farmacêuticas (FCFAR/UNESP), Câmpus Araraquara (Insetário de Triatominae, UNESP - Araraquara, São Paulo). The material was incubated in a medium containing sodium  $\beta$ -glycerophosphate substrate. Calcium chloride and magnesium sulfate were used to activate the enzyme. The enzyme released phosphate from the substrate, which reacts with the calcium ions resulting in calcium phosphate. This, treated with cobalt nitrate, was converted to cobalt phosphate. Subsequently, the material was incubated in a 1% ammonium sulfide solution and was converted to cobalt sulfide, which was observed through a light microscope as a dense and dark granular accumulation.

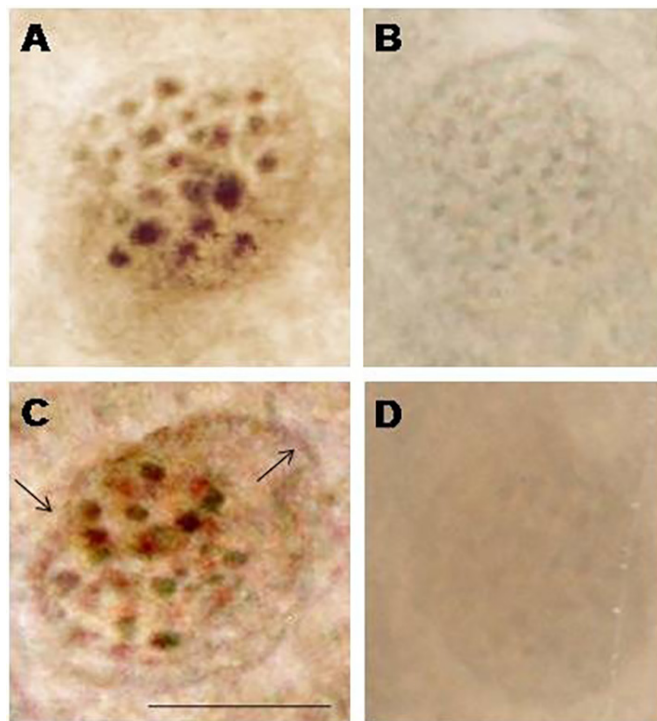
At the same time, a control reaction was performed using two incubation media: 1) without the sodium  $\beta$ -glycerophosphate substrate and 2) with substrate but without magnesium sulfate. The preparations were examined with a Zeiss Jenaval photomicroscope and photographed. The negatives were developed and printed in specialized laboratories.

## RESULTS

Males and females of both species showed similar alkaline phosphatase activity in their salivary gland cells. Figure 1 shows a general view of the salivary gland of *Rhodnius* subjected to the alkaline phosphatase technique. The cobalt sulfide precipitation, the final product of the reaction, was more evident in the posterior region of the principal gland. In both species and sexes, the nuclei showed evident enzymatic activity on the nucleolar corpuscles (as granules) as well as in the nuclear matrix (Figure 2A and C). In addition, the nuclear and plasmatic membranes showed intense alkaline phosphatase activity (indicated by arrows in Figure 2C). When the sodium  $\beta$ -glycerophosphate substrate (Figure 2B) or magnesium sulfate (Figure 2D) were absent, the glands showed a total absence of enzyme activity.



**Figure 1.** Salivary gland of a female *Rhodnius neglectus* subjected to a cytochemical reaction for alkaline phosphatase identification in the principal (PG) and accessory (AG) glands. Note that the alkaline phosphatase activity is observed in the whole gland, with a more intense activity in the posterior region of the principal gland. Bar: 10  $\mu$ m.



**Figure 2.** Salivary gland of *Rhodnius neglectus* and *R. prolixus* subjected to cytochemical reaction for alkaline phosphatase (A. and C.) and controls (B. and D.). Note the alkaline phosphatase activity in the nucleolar corpuscles, nuclear matrix (A and C) and nuclear membranes (C, arrows). With the control techniques without sodium  $\beta$ -glycerophosphate substrate (B) or without magnesium sulfate (D), there was a total absence of enzyme activity. Bar: 10  $\mu\text{m}$ .

## DISCUSSION

Alkaline phosphatase activity was found in the salivary gland of both sexes of *R. prolixus* and *R. neglectus*. This phosphatase, whose activity has been studied in many insects, is involved in many vital biochemical pathways (Eguchi, 1995). Alkaline phosphatase has been found to be widely distributed in the gastrointestinal tract, salivary glands, and Malpighian tubules of both nymphs and adult insects (Srivastava and Saxena, 1967). The most intense response of this cytochemical technique was detected in the cell nuclei of the *Rhodnius* salivary gland. This agrees with the results obtained by Azeredo-Oliveira and Mello (1986) in the Malpighian tubules of *Triatoma infestans* Klug, 1843. According to Azeredo-Oliveira and Mello (1986), alkaline phosphatase activity found in the nuclei might be related to transcription phenomena and cell metabolism. This hypothesis can also be applied to the present study, since alkaline phosphatase activity was found in nucleolar corpuscles. Furthermore acid phosphatase is another enzyme too described in nucleolus (Anhê et al., 2007), which suggests that this enzyme is active during rRNA molecule transcription (Anhê et al., 2007). Alkaline phosphatase has also been found to be associated with cell membranes in the medium intestine of *R. prolixus* (Ferreira et al., 1988). Wolfersberger (1984) suggested that alkaline phosphatase

is a typical marker enzyme, and it was detected mainly in the intestinal microvillousities based on the markings. In the present study, alkaline phosphatase activity was also observed in the plasmatic and nuclear membranes. This may be related with intercellular and intracellular transports. This activity in the transport of substances, through biological membranes, has been observed in several other studies (Haussler et al., 1970; Cutler et al., 1974; Ferreira and Terra, 1980; Azeredo-Oliveira and Mello, 1986). Hence, the finding of alkaline phosphatase in the nuclear membrane of the triatomine salivary gland cells was expected, since this structure is a place for nucleo-cytoplasmic exchange (Feldherr, 1965). Cutler et al. (1974) demonstrated that, ultrastructurally, the alkaline phosphatase activity in the nuclear membrane was located in the nuclear pores.

Alkaline phosphatase activity was found in the nucleoli, nuclear membrane, and nuclear matrix of salivary gland cells, suggesting relationships with the rRNA transcription and ribosomal biogenesis, nucleo-cytoplasmic transport of ribonucleoproteins, and the mechanisms of cell cycle and DNA replication, respectively. The presence of alkaline phosphatase in the plasmatic membranes suggests a role in the intercellular transport of molecules. These processes are directly involved with the activity of gland secretion.

The difference in reactivity found between the anterior and posterior regions of the gland may be associated with differences in metabolism and physiology. It may also be related to the biosynthesis of different secretions that assist in hematophagy and, as such, in the development of these insects of epidemiological importance.

This study highlights the importance of alkaline phosphatase in the salivary gland of *R. prolixus* and *R. neglectus* and emphasizes its importance in secretory activity. Alkaline phosphatase activity is directly involved in hematophagy and consequently in insect development during metamorphosis. The presence of alkaline phosphatase may be related to the production of saliva that allow feeding of these insects that are important vectors of Chagas disease.

### Conflicts of interest

The authors declare no conflict of interest.

### ACKNOWLEDGMENTS

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