

Original papers

Comparative 2-D electrophoresis of salivary proteins in *Triatoma dimidiata* and *Rhodnius prolixus* (Hemiptera: Reduviidae) and major cross-reactive antigens

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ABSTRACT. An immune response to triatomine's saliva is an immunological marker of exposure to triatomine bites. However, considerable variability in salivary protein profiles did exist among species. In the present work, we compare salivary proteins from Mexican *Triatoma dimidiata* and *Rhodnius prolixus* using 2-D electrophoresis. A clear differential saliva profile was found to exist between these two triatomine species. Fewer protein spots were detected in *R. prolixus* than in *T. dimidiata*. More than half of the proteins had an isoelectric point between 5 and 7 and a molecular weight between 10 and 30 kDa in *T. dimidiata*. Mice exposed to *T. dimidiata* saliva mount an immune response to three major cross-reacting antigens in *R. prolixus* saliva with weights of 10 kDa and 55 kDa. Our findings may alert for the presence of cross-reacting antigens between triatomine species in regions where two or more species are overlapping in the same geographical area.

Key words: *Triatoma dimidiata*, *Rhodnius prolixus*, salivary proteins, 2-D analysis, immunogens

Introduction

Triatoma dimidiata vector of Chagas disease shows a widespread distribution in Mexico. There are three haplogroups of *T. dimidiata*: haplogroup 1 in the Yucatan Peninsula [1], haplogroup 2 collected along the Gulf of Mexico and Pacific coast [2] and haplogroup 3 in Chiapas and the Pacific coast [3]. Whereas *Rhodnius prolixus* had showed a restricted area distribution, mainly in regions connected to Guatemala by the Pan American highway – Chiapas and Oaxaca States in the Pacific coast – with cases reported since 1938 [4–5]. In this area can be find more than two different species. Although, Mexico was certified free of *R. prolixus* since 2009 and endemic countries of Central America by 2011 [6], the risk of re-introduction of *R. prolixus* from South America is a latent threat.

One strategy to detect re-emerging vector population in a controlled area is to identify immunogenic salivary proteins as immunological markers of exposure to triatomine bites [7]. However, the saliva of triatomines can differ in its composition between genus and species, even between populations of the same specie [8–10]. In this context, it is indispensable characterize salivary antigen and saliva proteins in triatomines that share same geographical area.

In previous work using animal models, such as mice, chickens and guinea pigs exposed to bites of *Triatoma infestans* a diversity of antigens was demonstrated. Immunized mice recognized six antigens between 80–120 kDa [11] chickens recognized five antigens between 12–59 kDa and guinea pigs reacted with four antigens between 15–83 kDa [7]. In contrast, humans exposed to

Triatoma protracta saliva mount an immune response preferentially to low molecular weight proteins 17–25 kDa [12]. Above data demonstrated, that antigenicity depends on kind of host species and triatomine.

Studies on *T. dimidiata* saliva demonstrated considerable variability in salivary protein profiles within the *T. dimidiata* Guatemalan population using SDS-PAGE [13], but no information is available yet of salivary protein profile in Mexican *T. dimidiata* neither to *R. prolixus* cross-reacting antigens. In this work, we analyzed *T. dimidiata* and *R. prolixus* saliva by 2-D means and Western blotting.

Materials and Methods

T. dimidiata adult triatomines collected from peridomestic rural and urban areas of Campeche State in the Yucatán peninsula were maintained in the laboratory. The identification of triatomines was performed using the dicotomic key proposed by Lent and Wygodzonsky [14]. *Rhodnius prolixus* specimens were from a laboratory-reared colony at Nideyo Noguchi Institute in Merida Yucatan, Mexico. Ten to fifteen μL of saliva was obtained from ten triatomines fasted for two weeks. Saliva was manually obtained directly from the proboscides helping with a tip. The protein concentration of the pooled saliva was 12.05 mg/mL, it was kept at -70°C until use. A group of five Balb/c mice were exposed to triatomine bugs bite for 10 min every two weeks for a period of three months. After the last natural immunization, the mice were bled to obtain anti-saliva immune serum.

2-D electrophoresis was performed in a Isoelectric focusing (IEF) cell equipped with cooling system; 7 cm long strip of polyacrylamide and a linear pH gradient between 3–10 was allowed to rehydrate for 12 h in the presence of 100 μg of saliva. The second dimension was carried out in a Mini cell vertical electrophoresis system; the polyacrylamide gel concentration was 12%. Images are representative three experiments. For immunoblotting, proteins were transferred to a PVDF membrane using a Mini transfer-Blot cell at 40 V and 225 mA for 16 h. The membrane was blocked with PBS containing Tween 20 at 0.01% and 5% dried skimmed milk (PBS-T-M) for 40 min. After five washes with PBS-T-M, the membrane was incubated for 1 h with the immune sera from

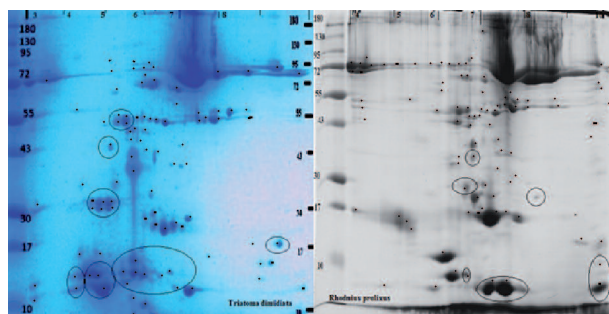


Fig. 1. Left panel: 2-D electrophoretic profile of *Triatoma dimidiata* saliva. Right panel: 2-D electrophoretic profile of *Rhodnius prolixus* saliva. Explanations: protein spots considered in the analysis are marked with a small dot. The enclosed circles represent the proteins that are exclusive to a given triatomine species. One hundred micrograms of saliva protein was electrophoresed in a strip 7 cm long with a linear pH gradient between 3 and 10.

mice diluted 1:100 in PBS-T-M. After extensive washes in PBS-T-M, the membrane was incubated for 1 h with peroxidase-conjugated rabbit anti-mouse IgG diluted 1:1000 in PBS-T-M. The reaction was developed with Tris buffer pH 7.6, diaminobenzidine and 0.01% H_2O_2 .

For the comparison of the isoelectric point and molecular weight of salivary proteins between both triatomine species, we used the Mann-Whitney test ($p < 0.05$). The software used was GraphPad Prism.

Results

The 2-D electrophoresis profile of saliva revealed 97 spots in *T. dimidiata* whereas 82 were observed in *R. prolixus*. There were 24 protein spots exclusive to *T. dimidiata* and 8 protein spots exclusive to *R. prolixus*; the enclosed circles contain the protein dots that are exclusive to a given triatomine species; we observed a set of proteins differentially present in *T. dimidiata* and *R. prolixus* (Fig. 1). The protein spots are indicated by a small dot beside each spot. It is known that 2-D pattern is strongly influenced by the protein concentration in the sample. In our study, we tested different saliva concentration, but finally we decided to used 100 μg of saliva protein; this concentration allowed us to identify proteins even at low concentrations. It is clear that pI and MW solely cannot give us enough information for a given protein, because glycosylation, phosphorylation, sulfation, and deamidation can influence the pI, as well two different protein with different amino-acid sequence

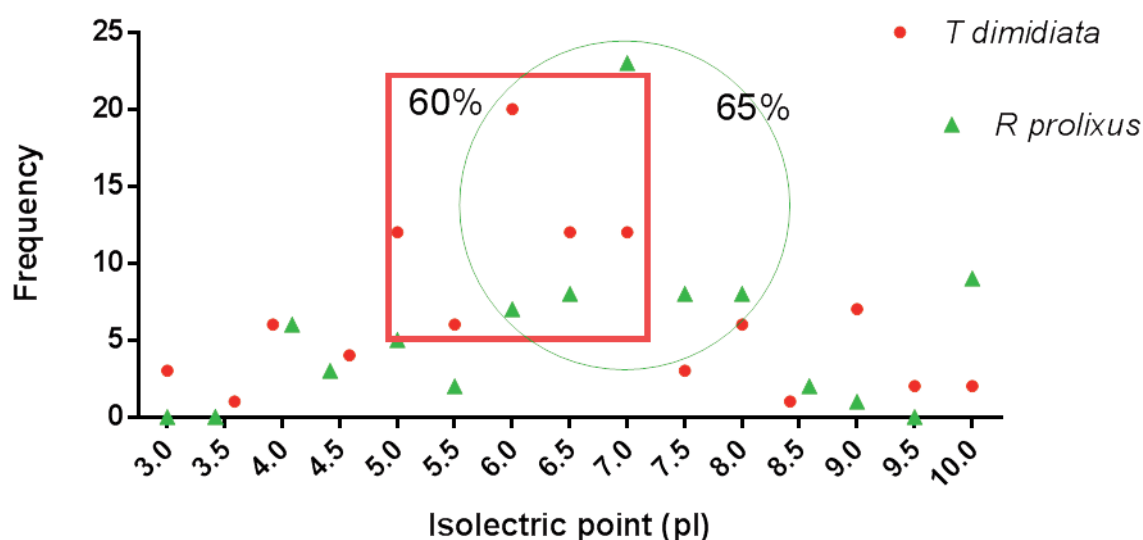


Fig. 2. Representation of the frequency distribution of the isoelectric points of saliva proteins from *Triatoma dimidiata* and *Rhodnius prolixus*

Explanations: the square contains 60% of *T. dimidiata* saliva proteins. The circle contains 65% of *Rhodnius prolixus* saliva proteins.

may have same pI and vice-versa. However, these two features can give us an approach to characterized the complexity of a given mixture.

When we compared the isoelectric points of saliva proteins between both triatomine species, the analysis (Mann-Whitney test) indicated that these protein populations were significantly different ($p < 0.05$). In the case of *T. dimidiata*, 59/97 (60%) of saliva dot proteins had an isoelectric point between 5 and 7, whereas in *R. prolixus*, 54/82 (65%) of proteins had a higher isoelectric point, situated between 6 and 8 (Fig. 2).

On the other hand, when we compared the molecular weights of these salivary proteins between species using the same analysis (Mann-Whitney test), there was no statistically significant difference (data not shown). However, in *T.*

dimidiata, 44/97 (45%) of saliva proteins had a molecular weight between 10 and 30 kDa, whereas in *R. prolixus*, 37/82 (45%) of proteins had a molecular weight between 40 and 60 kDa (Data not shown).

Thus, the saliva presents a diverse protein composition able to function as immunogens in the host. We found that immune sera of mice exposed to *T. dimidiata* bites recognized three major antigens and four minor acidic antigens in *R. prolixus* saliva. Two major antigens had a low molecular weight of 10 kDa and the third protein 55 kDa. The four minor acidic antigens showed molecular weights between 30 and 80 kDa (Fig. 3).

Discussion

Our data are in agreement with a recently published paper, in which a differentiable 2-D pattern was observed in the saliva of *R. prolixus*, *T. lecticularia* and *P. herreri* [10]. Actually, the amount of spots were similar for *Rhodnius* and *Triatoma* genera. In our work we detected 97 spots in *T. dimidiata* and 82 in *R. prolixus*, whereas Montadon et al. [10] detected approximately between 84 and 115 depending on triatomine species. However, in *R. prolixus* a difference was observed in the region of molecular weight below 20kDa. In regard to *T. dimidiata* saliva, Pineda et al. [13] were able to detect 61 bands; the majority of them were in the range of 10–50 and 74–100 kDa. In our work, we detected 97 spots and forty five

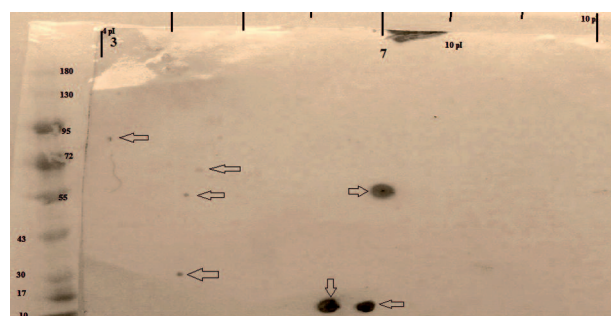


Fig. 3. Representative image of Western blotting of *Rhodnius prolixus* saliva against immune sera of mice exposed to *Triatoma dimidiata* bites

Explanations: three major antigens and four minor antigens are indicated with arrows

percent were in the range of 10–30 kDa. These findings are congruent with previous works, where it was demonstrated that the saliva of triatomines can differ in its composition between genus and species, even between populations of the same specie [8–9]. In similar way, the pattern distribution of saliva proteins based on pI is different between species, in *T. dimidiata* (60%) of saliva dot proteins had an isoelectric point between 5 and 7, whereas in *R. prolixus* (65%) of proteins had a higher isoelectric point, situated between 6 and 8 (Fig. 1 and 2).

In the same context, Kato et al. [15] reported 355 transcripts in the sialotranscriptome of *T. dimidiata* associated with putative secreted proteins with an average size between 16–22 kDa. Where Lipocalin family represented 89.9% of proteins. Among these transcripts, a high percentage were also reported in other *Triatomas* species: 55.0% in *T. infestans* 64% in *R. prolixus* and 68.2 % in *T. brasiliensis*.

Immune sera of mice exposed to *T. dimidiata* bites recognized in *R. prolixus* saliva. Two major antigens had a low molecular weight of 10 kDa and the third protein 55 kDa. Our data are discordant to previous studies on *T. infestans* saliva. Volf et al. [11] reported six antigens with molecular weights between 80 and 120 kDa in mice exposed to *T. infestans* saliva. Whereas, humans exposed to *T. protracta* saliva recognized low molecular weight proteins (17–25 kDa) [12]. Above data confirm that immunogenicity depends on host and triatomine species. In this context should be important to consider the presence of cross-reacting antigens before to use immunogenic salivary proteins as immunological markers of exposure to triatomine bites. Particularly, where two or more species are overlapping in the same geographical area.

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