Chitin is a component of *Rhodnius prolixus* midgut

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Chitin is an essential peritrophic matrix (PM) component, a structure that limits the insects’ gut responsible by protection against mechanical damage and pathogens. The *Rhodnius prolixus* doesn’t have PM but it has an analogous structure called perimicrovillar membrane which, until this moment, chitin hasn’t been described. To investigate chitin in midgut, we performed luminal contents collection from *R. prolixus* females at 9th day after blood meal for chitin purification. These bolus were submitted to papain treatment in acetate buffer, lipid extraction, treated with 1M KOH at 65°C, added with HCl: acetone (2.5:97.5) to remove heme group. The material obtained was washed, dried and submitted to FTIR and 1H-NMR spectroscopy analysis. The chitin was also purified from 20 females injected with 1µCi of N-acetyl-D-[1-3H]glucosamine/♀. We also elucidated the presence of chitin synthase gene (*CHS*) in *R. prolixus* sequencing cDNA synthesized from purified 5 midguts RNA pool. The 516pb fragment coding the *CHS* catalytic domain was cloned and used as targeted for specific dsRNA synthesis to *CHS* knock-down (dsRNA*CHS*) using MEGAscript-RNAi kit. In order to localize chitin in midgut, *R. prolixus* females were plasma fed and, 4hs after, injected with 1µg of dsRNACHS/♀ or water (control). Nine days after meal, midguts from dsRNACHS treated and controls females were fixed in 4%paraphormaldehyde. Cryosections were analyzed after treatment or not with chitinase. Chitin was evidenced in the epithelium after staining using Rhodamine-CBD (Rh-CBD), specific probe to detect chitin, by fluorescence microscopy. The *CHS* silencing in midguts was also evidenced by qPCR. FTIR spectrum from midgut-material showed peaks characteristic of chitin molecule, in 3500, 1675 and 1085 cm-1 similar chitin standard spectrum. The midgut-material and chitin standard 1H-NMR spectra showed a 1.88ppm peak (methyl protons of the acetamide group). The radioactivity associated with midgut-material was 184,5 cpm of N-acetyl-D-[1-3H] glucosamine/mg chitin by liquid scintillation technique. The *CHS* cDNA translated sequence into protein showed CHSs conserved domains. Phenotypical alterations were observed in midguts from dsRNACHS treated females like retardation of meal digestion. These tissues also showed chitin signal, Rh-CBD labeling, decreased when compared with control in fluorescence microscopy. The chitinase pre-treatment decreased Rh-CBD labeling on the control midgut. This indicated that this material is susceptible to chitinase digestion and is either chitin or a partially acetylated chitosan polymer. The relative reduction of *CHS* transcripts of approximately 80% achieved by RNAi assay justifies the phenotypical alterations found in midgut. These data strongly suggest that the chitin is indeed a component of *R. prolixus* midgut.

Word Keys: chitin, midgut, *Rhodnius prolixus*

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