

Detergent-resistant Domains in the Midgut Microvillar Membrane of *Spodoptera frugiperda* Larvae

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Introduction: Lipid rafts are detergent-resistant membrane (DRM) domains which are not solubilized by non-ionic detergent treatments at 4 °C. They are present in a variety of cells and organisms involved in many processes such as receptor-mediated signaling and microbial invasion. **Objectives:** Identify the presence of DRM in the midgut microvillar membrane (MM) of *Spodoptera frugiperda* larvae. **Methods:** Isolated MM were submitted to 1% (w/v) Triton X-100 treatment for 30 or 120 min at 4 °C, or for 30 min at 25 or 37 °C. Whenever needed 30 mM methyl- β -cyclodextrin (MBCD) was used for 30 min incubation at 37 °C prior to Triton X-100 treatment. Samples were layered in the bottom of a sucrose gradient (25-60%, w/v) and centrifuged for 18h at 100,000 x g. Cholesterol, aminopeptidase, alkaline phosphatase (AP), trypsin and gamma-glutamyl transferase (GGT) were determined along the gradient. Major activity peaks were further investigated by shotgun proteomics. **Results and discussion:** Detergent-treated and detergent-free samples presented distinct activity distribution, suggesting the presence of DRM in the latter. Solubilization kinetics at 4 °C showed that after 120 min incubation DRMs are still present. At 37 °C, solubilization of DRMs was almost complete, in contrast to incubation at 25 °C which had DRM profiles similar to the ones obtained at 4 °C. MBCD apparently does not affect DRM stability. Aminopeptidase, AP and trypsin are present in DRMs whereas GGT is not. Proteomic analyses of activity peaks confirmed the presence of assayed enzymes plus other proteins such as carboxypeptidases and dipeptidase IV. **Conclusions:** DRM seems to exist in MM of *Spodoptera frugiperda* at physiological temperature and it is not affected by MBCD treatment. Proteins like AP, trypsin and aminopeptidase are located in these domains.

Keywords: lipid rafts, *Spodoptera frugiperda*, proteomics.

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