Isolation of Vicilin-containing Vesicles from the Fat Body Cells of the Seed-feeding Beetle *Callosobruchus maculatus*

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Vicilins (7S globulins) are important storage proteins found in legume seeds and other plants. We have demonstrated that vicilins from cowpea (*Vigna unguiculata*) once ingested by the larvae of the cowpea seed-feeding beetle *Callosobruchus maculatus* are absorbed through the midgut epithelium and circulate in their trimeric form in the haemolymph and are deposited in the fat body. In fat body cells, vicilins are deposited in membrane-containing vesicles and are partially hydrolyzed. In order to characterize the proteolytic activity responsible for the vicilin hydrolysis, the fat body cells were submitted to subcellular fractionation by differential centrifugation followed by ultracentrifugation in a discontinuous sucrose gradient. The localization of the vesicle fractions was performed through labeling of vicilin with FITC (fluorescein isothiocyanate). The labeled vesicles were recovered from the interfaces between 30-40% and 40-50% sucrose in 2 mM tris/HCl 215 mM KCl, pH 7.0, after ultra-centrifugation (96,000 g for 3 h at 4°C). Proteolytic assays have demonstrated that the vesicle fractions were capable of hydrolyzing suc-Ala-Ala-Pro-Phe-pNa and purified vicilins. The hydrolysis of purified vicilins was also verified by electrophoresis. Peptides derived from the *in vitro* hydrolysis seem to be similar with the peptides deposited in the eggs. This study provides additional insights into the partial hydrolysis of vicilins culminating with the release of anti-microbial peptides that are eventually deposited in the eggs.

Keywords: Ultracentrifugation, fat body, *Callosobruchus maculatus*, *Vigna unguiculata*, vicilin, sucrose gradient.

Supported by: FAPESC, CNPq, PROCAD/CAPES and FAPERJ.