

CRIAL-TRIO protein-mediated endocytosis of vicilin in larval *Callosobruchus maculatus* midgut epithelial cells

Kunz, D.¹; Oliveira, G.B.¹; Samules, R.I.²; Macedo, M. L. R.³; Silva, C.P¹.

¹ Departamento de Bioquímica, Centro de Ciências Biológicas, UFSC, Florianópolis, SC, Brazil; ² Laboratório de Entomologia e Fitopatologia, Centro de Ciências e Tecnologia Agropecuárias, UENF, Campos dos Goytacazes, RJ, Brazil; ³ Departamento de Tecnologia de Alimentos e Saúde Pública, UFMS, Campo Grande, MS, Brazil.

INTRODUCTION. The transport of proteins across the intestinal epithelium of insects is still little known. There is evidence that vicilin, a major storage protein of cowpea seeds (*Vigna unguiculata*), is internalized in larvae of the bruchid *C. maculatus*. It has been reported that this storage globulin interacts with proteins present in the microvillar membranes along the digestive tract of the larvae. In the present work, we studied the cellular routes involved in the endocytosis of vicilin in larval *C. maculatus*.

MATERIAL and METHODS: FITC-labeled vicilin (purified from *C. maculatus* susceptible seeds of cowpea) were incorporated into the diet of the larvae at physiological concentration (0.5% m/m). The fate of labeled or non-labeled globulins was monitored by confocal microscopy, immunohistochemistry and western blotting. The microvillar vicilin-binding protein was purified by using affinity chromatography on a vicilin-Sepharose column followed by MALDI-TOF mass spectrometry. **RESULTS**

and DISCUSSION: The absorption of vicilins is a case of receptor-mediated endocytosis. The putative vicilin receptor was purified and showed high homology with proteins from the SEC14 family. These proteins are present in the luminal surface of the midgut cell microvilli and inside these epithelial cells, associated to endocytic vesicles. Endocytic vesicles and cisternae were found throughout the extent of midgut epithelial cells. The type of transcytosis of these macromolecules was confirmed through the use of specific inhibitors of clathrin or caveolin-mediated pathways. The inhibitors filipin, nystatin and wortmannin significantly inhibited the endocytosis of vicilin, suggesting that the endocytic pathway is mediate by caveolin.

CONCLUSION: In this study we showed that the transcytosis of vicilin through the midgut cells of larval *C. maculatus* is mediated by a member of the SEC14 family. It is possible to suggest that vicilin is internalized by endocytosis dependent on caveolin.

Keywords: Endocytosis; insect midgut; SEC14 protein family.

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