Characterization of long-chain acyl-CoA synthetases 1 and 2 and its physiological role in the insect Rhodnius prolixus

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Long-chain acyl-CoenzymeA (-CoA) esters serve as important intermediates in lipid biosynthesis and fatty acid degradation. Besides this basal function, a large body of evidence has accumulated indicating that long-chain acyl-CoA esters also have an important function in the regulation of intermediary metabolism and gene expression. Long chain fatty acids are activated to CoA thioesters by long-chain acyl-CoA synthetase (ACSL) activity. In mammals, five ACSL isoforms are differentially involved in anabolic or catabolic routes in a tissue-specific manner. In Rhodnius prolixus, a blood sucking insect vector of Chagas’ disease, we identified two genes that encode putative ACSL enzymes (RpAcsl1 and RpAcsl2). Gene expression measurements showed that transcription of these genes is tissue-specifically modulated. RpAcsl1 gene expression increased in the midgut and ovary (20- and 5-fold increase, respectively) at times of high glycerolipid synthesis. In the fat body, the major form, RpAcsl2, was highly transcribed (3-fold increase) when TAG was mobilized. In order to confirm the actual ACS activity from predicted genes and evaluate their specific functions, both enzymes were cloned and expressed in Escherichia coli as Flag-fusion proteins. Purified RpACSL1 and RpACSL2 isoforms were strongly inhibited by Triacsin C. Both isoforms showed similar apparent Kg values for palmitic acid and oleic acid substrates (2-5 μM). However, the Vmax value measured for RpACSL2 was 6-fold higher than observed for RpACSL1 (90 and 15 μmol fatty acid · min⁻¹ · mg protein⁻¹, respectively). To further evaluate the physiological relevance of ACSL isoforms, we performed dsRNA-mediated knockdown of these genes. Obtained results indicate that the ablation of RpACSL2 enzyme leads to accumulation of triacylglicerol in the fat body, suggesting that this enzyme may be relevant for lipid mobilization and/or beta-oxidation of fatty acids. Moreover, RpAcsl2-silenced insects showed reduced egg production (~30% decrease compared to control animals) and impairment of nymph eclosion. The mechanisms by which RpACSL2 affects the insect metabolism are now under investigation. In summary, this work provides the first insights regarding the physiological function of ACSL enzymes in arthropods and may contribute to the understanding of key aspects of lipid metabolism in vectors of diseases.

Supported by: NIH Grants DK59935 and DK090141, CNPq, FAPERJ and INCT-EM.

Key words: Lipid metabolism; Long-chain acyl-CoA synthetases (ACSL); Rhodnius prolixus.