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Thioredoxin fused CHH1 protein as antigen for polyclonal antisera: Application to regulate glycemia in *Penaeus monodon*

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Crustacean hyperglycemic hormone (CHH) family neuropeptides have been in research limelight for the past two decades due to their importance in the regulation of glycemia, moulting and gonad development in crustaceans. Under natural conditions, low levels of CHH neuropeptide and the structural similarity of the three CHH family neuropeptides limit their purification directly from the animal. In this study, we isolated the mature region of the CHH1 gene, constructed the recombinant translation expression vector (pET32a+ - PmCHH1) and produced thioredoxin fused protein in *Escherichia coli* (BL21 (DE3) pLysS). The translation expression vector construct (pET32a+ - PmCHH1) was successfully built for production of thioredoxin fused mature CHH1 protein (mf-PmCHH1-29.47 kDa). Mf-PmCHH1 produced a hyperglycemic effect similar to that of the eyestalk extract when experimentally injected into adult eyestalk ablated *Penaeus monodon*. The polyclonal antiserum (anti-mf-PmCHH1) was developed in mice against the purified thioredoxin fused mf-PmCHH1 protein. A hypoglycemic effect was induced in adult *P. monodon* by the polyclonal antiserum which was raised against thioredoxin fused CHH1 protein. Immunolocalization of CHH1 producing neurosecretory cells in the eyestalk of *P. monodon* was a practical result obtained with the polyclonal antiserum anti-mf-PmCHH1. Therefore, mf-PmCHH1 and its antiserum (anti-mf-PmCHH1) are added to the list of tools to better understand the endocrine mechanisms regulating glycemia and reproduction in *P. monodon*.

Keywords: CHH neuropeptides, Crustacean hyperglycemic hormone, Diabetogenic factor, Immunolocalization,