摘要

甲殼類升血糖荷爾蒙 (Crustacean Hyperglycemic Hormone, CHH) 是甲殼類眼柄神經節中 X-organ/sinus gland complex (XO/SG) 所製造與分泌的神經荷爾蒙。近年來的研究發現，CHH 具有分子多型態、多重組織性及多功能性。目前在克氏螯蝦 (*Procambarus clarkii*) 中，已發現至少具有 6 種 *CHHs* 轉錄子，分別為 *CHH1*、*CHH1-L*、*t-CHH1*、*CHH2*、*CHH2-L* 及 *t-CHH2*，經序列分析認為此六個分子主要是由兩個 *CHH* 基因經由替代性剪接而產生。此外，在眼柄神經節中與胸神經節中，分別成功的純化出 CHH1 與 CHH1-L 蛋白，此兩種蛋白的特徵為前 40 個胺基酸序列完全相同，而在第 41 個胺基酸之後的序列則有差異性。另外，經生理活性測試得知 CHH1 具有升血糖活性，而 CHH1-L 則無。利用大腸桿菌表現系統，表現克氏螯蝦的 CHH1 與 CHH1-L 重組蛋白 (rCHH1 和 rCHH1-L) ，並企圖藉由改變核甘酸序列中的稀有密碼子 (rare codons) 數量、mRNA 穩定性 (mRNA stability) 與在 N 端加上不同類型的融合蛋白 (fusion protein) 等方法，加以嘗試改善 CHH1 與 CHH1-L 於大腸桿菌中的重組蛋白表現量。由實驗結果中可知，可分別利用在 N 端加上 pelB-leader 融合蛋白和減少稀有密碼子數量等方法，改善 rCHH1 與 rCHH1-L 的表現量。接著經由再折疊 (refold) 後，利用高效能液相色層分析儀 (High Performance Liquid Chromatography, HPLC) 純化，且使用西方墨漬法 (Western Blot) 與質譜儀 (Mass Spectrometry) 確認所製備的重組蛋白。同時以圓二色光譜 (Circular Dichroism, CD) 分析後，顯示所製備的 rCHH1 與 rCHH1-L 皆屬於高比例的 α-helix 為主的二級結構 (分別為 30.5 與 41 %)。此外，將進行胺化反應 (amidation) 後的 rCHH1 (rCHH1amide)，進行升血實驗後發現，注射 10 pmol rCHH1amide 所引起的升血糖反應與注射相同劑量由血竇腺中所純化到的 CHH1，所引起的升血糖反應相同。除此之外，在白點症病毒 (White Spot Syndrome Virus, WSSV) 感染螯蝦後，病毒增生與組織中 CHH1/CHH1-L 蛋白質含量的實驗中，發現在注射白斑病毒後 12 小時可偵測到病毒，在 48 與 72 小時含量會極顯著的上升，並且促使眼柄神經節中 CHH1 的，於感染後 24 小時迅速的降低，但在胸腺神經節中，則無法觀察到 CHH1-L 的含量變化。因此，初步推測 CHH 家族蛋白，可能與螯蝦體內的免疫調節有關。

Abstract

Six transcripts, each encoding a distinct CHH (crustacean hyperglycemic hormone) or CHH-like (CHH-L) peptides, *Procambarus clarkii-CHH1*, *CHH1-L*, *truncated-CHH1* (*t-CHH1*) , *CHH2*, *CHH2-L*, and *t-CHH2*, were identified in the crayfish. These transcripts were generated by alternative splicing of RNA precursors of two CHH genes (*chh1* and *chh2*). In addition, can purified the native CHH1 and CHH1-L from neurosecretory tissues, tissue extracts (sinus gland: SG; thoracic ganglia: TG) were chromatographically separated. CHH1 and CHH1-L, two structural variants of the crustacean hyperglycemic hormone family identified in the crayfish (*Procambarus clarkii*), are identical up to the 40^{th} residue, but different from each other in the remaining sequence. In this study, recombinant proteins (rCHH1 and rCHH1-L) were produced by an *E. coil* expression system, and attempt to increases rCHH1 and rCHH1-L yields by decreases amount of rare codons, change either mRNA stability or tag fusion protein in the N-terminal. In results, expression with pelB-leader protein or decreases amount of rare codons, that can increase the rCHH1 and rCHH1-L yields, respectively. And then refolded, purified, and confirmed by western blotting and mass spectrometric analyses. In addition, Circular dichromatic spectra of rCHH1 and rCHH1-L indicate they are rich in α-helixes (30.5 % and 41 %, respectively). Purifying rCHH1 was C-terminally amidated (rCHH1amide) in accordance with native counterpart. Functionally, rCHH1amide at 10 pmole/animal elicited significant hyperglycemic responses, that likes native CHH1 response. In the other hand, present study examined the effects of white spot syndrome virus (WSSV) on the CHH and CHH-like peptide system, that *P. clarkii* challenged by the white spot syndrome virus (WSSV) , WSSV genomic DNA was first detected at 12 hr. At 48 and 72 hours of post challenge, the viral load increased substantially. The CHH1 protein levels in the eyestalk ganglia were significantly decreased 24 and 48 hr after challenge, but CHH1-L protein levels in the thoracic ganglia did not change significantly after challenge. The WSSV-activated peptide systems might be involved in mediating adaptive processes (e.g., hyperglycemic response) in response to pathogen-related stresses or in the processes of immune defense against invading pathogens.