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# CRUSTACEAN HYPERGLYCEMIC HORMONE: STRUCTURAL VARIANTS, PHYSIOLOGICAL FUNCTION, AND CELLULAR MECHANISM OF ACTION

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# CRUSTACEAN HYPERGLYCEMIC HORMONE: STRUCTURAL VARIANTS, PHYSIOLOGICAL FUNCTION, AND CELLULAR MECHANISM OF ACTION

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# CRUSTACEAN HYPERGLYCEMIC HORMONE: STRUCTURAL VARIANTS, PHYSIOLOGICAL FUNCTION, AND CELLULAR MECHANISM OF ACTION

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Key words: crustacean hyperglycemic hormone, gene expression, molecular polymorphism, mechanism of action.

## ABSTRACT

Crustacean hyperglycemic hormone (CHH) is a peptide hormone originally identified in the X-organ/sinus gland (XO/SG) complex of the eyestalks. It belongs to the CHH family which also includes molt-inhibiting hormone (MIH), vitellogenesis-inhibiting hormone (VIH), and mandibular organ-inhibiting hormone (MOIH), and ion transport peptide (ITP). Multiple molecular variants of CHH are generated by both post-transcriptional and post-translational mechanisms. In addition to the XO/SG complex, CHH gene is widely expressed in many extra-eyestalk tissues. Functionally, available data indicate that CHH is involved with the regulation of carbohydrate metabolism and stress-induced hyperglycemia. Several other physiological processes, including molting, ion and water balance, reproduction, and immunity, are likely also regulated by CHH. The functional role(s) of the alternatively splice form CHH-like peptide (CHH-L) are different from those of CHH and remain unknown. Combined results showed that cyclic guanosine 3',5'-monophosphate (cGMP) plays important roles in mediating the effects of CHH on carbohydrate metabolism. Recent studies of receptor guanylyl cyclase (rGC) may lead to characterization and identification of CHH receptor(s). Future efforts are needed to fully understand the functional roles of the different CHH variants; identification of CHH receptor(s) hold the promise of revealing in greater depth the structure/functional relationships of the various structural variants.

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## I. INTRODUCTION

Crustacean hyperglycemic hormone (CHH) is a peptide hormone originally identified in a crustacean neurosecretory complex, the X-organ/sinus gland complex (XO/SG complex), located within the eyestalks of decapod crustaceans [16, 60]. Historically, biological significance of the eyestalk XO/SG complex had been inferred by ablation-replacement experiments that suggested the presence of several hormonal factors. These included hyperglycemic hormone, gonad-inhibiting hormone, gonad-stimulating hormone, molt-inhibiting hormone, chromatotrophic hormones, etc. [39, 40]. The wide array of physiological processes that appear to be under the hormonal control of the eyestalk factors underscores the importance of the XO/SG complex; it has also spawned ever since research interests in understanding the molecular, biochemical, and physiological aspects of these peptide hormones [see 16, 42].

Earlier biochemical and physiological studies [e.g., 7, 29, 30, 35, 41, 52, 62, 65, 78], soon followed by a surge of research effort in molecular cloning of the encoding genes [see 4, 20], have established that CHH, being a prototypical member, belong to a family of polypeptide hormones, the CHH family [34, 36, 60]. The family also includes molt-inhibiting hormone (MIH), vitellogenesis-inhibiting hormone (VIH), and mandibular organ-inhibiting hormone (MOIH), and insect ion transport peptide (ITP) [10, 20, 36]. Recent studies have expanded the existence of the CHH family peptides beyond arthropods to ecdysozoans [11, 48].

This paper summaries studies of CHH and CHH-related peptides of decapod crustaceans, including aspects relating to structure of CHH peptide and gene, spatial pattern of CHH gene expression, physiological roles, and cellular mechanism of actions.

## II. STRUCTURE OF CHH PEPTIDE AND GENE

Molecular characterizations of CHH precursors indicated that the precursor consists of a signal peptide, a CHH

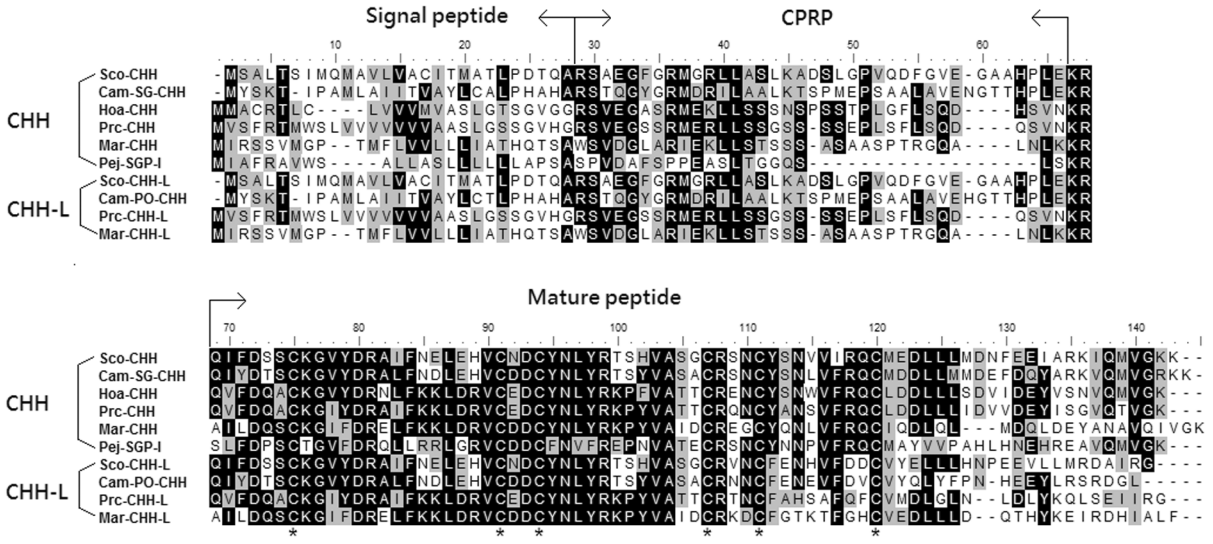


**Fig. 1. Multiple alignments of decapod CHH and CHH-L precursor sequences. Conserved cysteine residues characteristic of CHH family peptides are indicated by an asterisk (*). CPRP: crustacean hyperglycemic hormone precursor-related peptide. CHH sequences are from the following sources, Sco-CHH: *Scylla olivacea*, AY372181 [69]; Cam-SG-CHH: *Carcinus maenas*, X17596 [74]; Hoa-CHH: *Homarus americanus*, S76846 [18]; Prc-CHH: *Procambarus clarkii*, AB027291 [78]; Mar-CHH: *Macrobrachium rosenbergii*, AF219382 [9]; Pej-SGP-I: *Marsupenaeus japonicus*, AB007507. CHH-L sequences are from the following sources, Sco-CHH-L: *S. olivacea*, EF530127 [69]; Cam-PO-CHH: *C. maenas*, AF286084 [21]; Prc-TG-CHH: *P. clarkii*, AF474408; Mar-CHH-L: *M. rosenbergii*, AF372657 [9].**

precursor-related peptide (CPRP), and a mature CHH peptide. Based on this and other sequence characteristics, it was proposed that CHH peptides to be categorized as members of the type I subgroup of the CHH family (Fig. 1), whereas MIH, VIH/GIH, and MOIH (the precursors of which lack CPRP) as members of the type II subgroup of the CHH family [10, 20, 43]. A third subgroup, Type III subgroup, containing insect, collembolan, and branchiopod ITPs, was later created based on phylogenetic analysis of pancrustacean CHH family peptides [48].

Multiple CHH variants presumably encoded by separate genes were reported mainly for astacideans and penaeids. Thus, there are two CHH variants for *Homarus americanus* and *Cherax destructor* [3, 67], five for *Penaeus monodon* [17], and six for *P. japonicus* [38, 77] that are different to varying extent in the primary sequences. Direct evidence showing the presence of multiple CHH genes is provided by studies of *Metapenaeus ensis* [26, 27]. In *M. ensis*, there are at least 6 copies of CHH-A genes that share 98-100% of the encoded amino acid sequence identity [26], and 2 copies of identical CHH-B genes [27]. Genomic analysis revealed that most *CHH* genes are characterized by containing 4 exons and 3 introns, excepting CHH genes of *M. ensis*, which like other type II peptide genes, contain 3 exons and 2 introns [10].

A CHH-like variant was initially purified from the pericardial organ (PO) of *Carcinus maenas* [21]. This novel CHH-like peptide (CHH-L) and the sinus gland-derived CHH share an identical N-terminal sequence (residues 1-40), but differ considerably in the remaining sequence; they are alternatively spliced products [21]. CHH/CHH-L variants showing similar differences in sequence characteristics have recently been reported in *Macrobrachium rosenbergii*, *Pachygrapsus marmoratus*, *Potamon ibericum*, *Scylla olivacea*, and *Procambarus clarkii* [5, 9, 15, 68, 69, 75]; the native peptides have been purified and characterized for *C. maenas*, *S. olivacea*, and *P. clarkii* [5, 21, 75].

CHH and CHH-L undergo post-translational modifications. Both peptides contain 3 disulfide bonds formed by 6 highly conserved cysteine residues [5, 21], which is a distinguishing feature of the family members. In addition, the N-terminal end of both peptides is pyroglutamated, whereas C-terminal amidation only occurs in CHH [5, 21]. While N-terminal pyroglutamination appears not necessary for the hyperglycemic activity of CHH [13], C-terminal amidation significantly affects its biological activity [5, 33, 49].

Structural comparison of the crab (*S. olivacea*) native CHH and CHH-L shows that the 2 peptides have similar spectral profiles of circular dichroism and thermo-stability, indicating they are composed mainly of α-helices (41% and 36%, respectively) and have a very similar melting temperature at around 75°C [5]. No experimentally resolved structure has been reported for any CHH peptides. Three-dimensional structure for brachyuran CHH and CHH-L were modeled using Pej-MIH [32] as the template structure. Though still need experimental verification, the modeled structures for CHH and CHH-L revealed that they are sterically folded in similar manners (the root mean square deviation is 0.43), with each modeled structure contains an N-terminal tail region and 4 α-helices (Fig. 2), consistent with the data of a similarly modeled structure of *P. japonicus* CHH [32], and in general with data showing that CHH and CHH-L have the same disulfide bond pattern [5, 21]. These data suggested that, despite

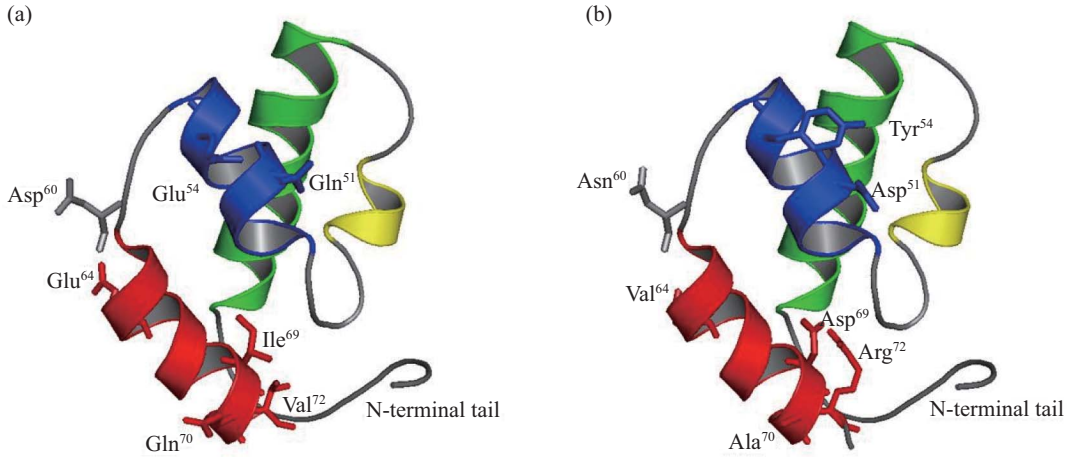


**Fig. 2. Ribbon model of CHH and CHH-L. Three-dimensional structure of CHH and CHH-L was modeled using the SWISS model server with Pej-MIH structure as the template structure. Modeled structure of the mud crab (*Scylla olivecea*) (a) CHH or (b) CHH-L is composed of an N-terminal tail region and 4 α-helices (green: helix 1, yellow: α-helix 2, blue: α-helix 3, red: α-helix 4). Residues located in corresponding positions of CHH and CHH-L but with side chains of different properties are indicated by 3-letter code with superscript number showing residue number; only side chains of these residues are depicted.**

significant sequence variations in the C-terminus, CHH and CHH-L are much similar to structure at higher level [5].

A rare type of post-translation modification occurs in CHH [63]. CHH variants differing from each other in the stereo configuration of the third residue (a phenylalanine) were isolated from the sinus glands of various astacideans [2, 3, 61, 65, 77]. It was proposed that D-Phe$^3$ CHH, which contains a D-phenylalanyl residue at the third position, is derived from the all-L CHH by the action of putative peptide isomerase in a late step during the maturation process of the prohormone [54, 64]. On the other hand, CHH-L peptide appears not being subjected to the L-to-D isomerization. In *P. clarkii*, a CHH-L was present in the thoracic ganglia; immunoblotting analysis of the tissue extracts using an antibody specific for D-Phe$^3$-CHH did not detect any immunoreactive protein [75], despite the fact that the sequences of the first 40 residues from the N-terminus of CHH and CHH-L are identical. It is suggested that the putative isomerase activity involved in the post-translational L-to-D conversion of CHH [54, 64] is absent in the thoracic ganglia [75].

## III. SPATIAL PATTERN OF GENE EXPRESSION

The presence of CHH peptides in the extra-eyestalk tissues was proposed more than a decade ago [6, 18]. Subsequently, it was found that a CHH identical to that originally found in the XO/SG complex is also expressed in the gut of *C. maenas* [12]. As stated above, extra-eyestalk tissues (the pericardial organ and thoracic ganglia) expressed CHH-L peptides [5, 9, 21, 68, 69, 75]. The consensus appears to be that at the protein level the eyestalks predominantly express CHH, whereas the extra-eyestalk tissues express CHH-L [5, 21, 69, 75]. However, reverse transcription-polymerase chain reaction (RT-PCR)

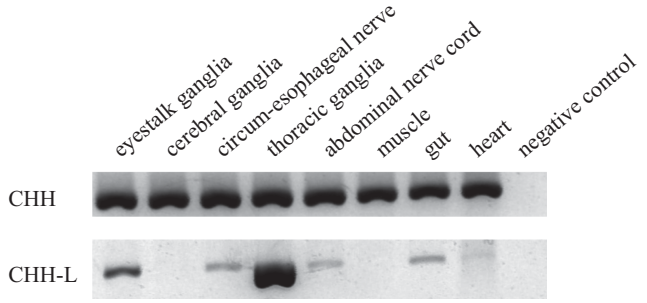


**Fig. 3. Tissues distribution of *CHH* and *CHH-L* transcripts. Total RNA prepared from the tissues as indicated was reverse transcribed and amplified with specific primers for *CHH* or *CHH-L*. Negative control reaction was performed using water in stead of tissue cDNA sample as the template.**

analysis of tissues transcripts revealed that both CHH and CHH-L transcripts are widely expressed in tissues [31, 69]. In *P. clarkii*, CHH transcript is clearly and equally detectable by RT-PCR in the eyestalk ganglia, cerebral ganglia, thoracic ganglia, circumesophageal nerves, abdominal ganglia, muscle, gut, and heart; whereas CHH-L transcript is most predominant in the thoracic ganglia but also detectable in the eyestalk ganglia, circumesophageal nerves, abdominal ganglia, gut, and heart (Fig. 3). One possibility is that certain transcripts are probably not translated into proteins or translated into proteins at extremely low levels. Using a sensitive sandwich type enzyme-linked immunosorbent assay, it has been shown that indeed, while the CHH is predominantly present in the sinus gland, it is also detectable though at much lower levels in the extra-eyestalk tissues [76].

## IV. PHYSIOLOGICAL ROLES

A hyperglycemic factor residing in crustacean eyestalks

was first revealed by Abramowitz *et al*. [1]. Accumulated studies indicated that CHH is involved in regulating blood glucose levels mainly through mobilization of glucose from glycogen depots, in particular the muscle and hepatopancreas [56]; it acts on the target tissues promoting glycogenolysis while inhibiting glycogen synthesis [37, 57]. A recent study showed that eyestalk ablation significantly increased glycogen synthase (GS) transcript levels and decreased glycogen phosphorylase (GP) transcript levels in the hepatopancreas of *M. japonicus* [51]. CHH-mediated hyperglycemia in response to various environmental stressors (extreme temperature, hypoxia, organic and inorganic pollutants, bacterial infection, etc.) has been demonstrated [6, 15, 23, 47, 55, 72, 80].

It has been recognized that CHH is a pleiotropic hormone. It was noted that CHH, in addition to MIH, also suppresses ecdysteroidogenesis by the Y-organs though at a higher dose than MIH [7, 14, 73, 78]. The physiological significance of the CHH-suppressed ecdysteroidogenesis especially with regard to molting remains to be ascertained.

Regarding the 2 functions mentioned above, it appears that CHH and CHH-L are functionally divergent in that CHH has significant activity in inducing hyperglycemia, suppressing ecdysteroid synthesis, or both, whereas CHH-L does not have either activity [5, 21, 53]; functional roles have yet to be determined for any identified CHH-L. It was shown that co-injection of CHH and CHH did not change the pattern of hyperglycemic responses seen in *C. maenas* injected with CHH alone. It is therefore unlikely that CHH-L functions as a negative regulator of CHH [21]. Differential responses to stress stimuli between CHH and CHH-L transcript levels were observed in the blue crab *Callinectes sapidus* [15].

Another physiological role of CHH involves regulation of ion and water balance. The discharge of the gut-derived CHH during pre-molt is suggested to be involved in regulating water and ion uptake, allowing the swelling necessary for successful ecdysis and the subsequent increase in size during post-molt [12]. It has also been demonstrated that CHH exerts ionoregulatory actions on the gill by increasing trans-epithelial potential and sodium influx [8, 59, 66]. The molecular entity directly affected by CHH has yet to be identified.

It is worth a note mentioning that data indicate that D-Phe$^3$ CHH appears to be more potent than its all-L counterpart in inhibition of ecdysteroid release from Y-organs and [78] and regulation of hemolymph osmolality [59]. Whether the L-to-D isomerization of CHH represents a post-translational mechanism that increases bioactivity of the hormone requires additional studies.

Other physiological processes that have been proposed to be regulated by CHH include vitellogenesis [19, 38, 70] and methyl farnesoate synthesis by the mandibular organ [46]. Notably, it was demonstrated that *in vivo* injection of recombinant CHH significantly elicited immune responses, increased pathogen clearance ability and survival rate of pathogen-infected shrimps [71] and that CHH is expressed in hemocytes [76]. These recent data suggested a role for CHH in immune regulation.

## V. CELLULAR MECHANISM OF ACTION

Previous studies on the cellular mechanism of action of CHH suggest that cyclic guanosine 3',5'-monophosphate (cGMP) plays important roles in mediating the effects of CHH on carbohydrate metabolism. Cyclic GMP levels in CHH target tissues are significantly increased shortly after injection of CHH [58], *in vitro* incubation of the target tissues with CHH elevates tissue cGMP levels in a dose- and time-dependent manner [24, 25, 50, 58], and the increase in intracellular cGMP precedes an increase in glucose release into incubation media [58]. The effect of CHH on cGMP levels is potentiated by phosphodiesterase inhibitors, suggesting that CHH acts primarily by stimulating GC [24]. Further, CHH stimulates GC activity in membrane (but not cytosolic) preparations of muscle (a CHH target tissue), indicating that increase in cGMP levels in response to CHH is due to the activation of membrane-bound GC [24].

cDNA encoding crustacean receptor type GCs (rGCs) were reported from the muscle of the crayfish, *P. clarkii*, the Y-organ of blue crab *C. sapidus*, and the tropical land crab, *Gecarcinus lateralis* [44, 45, 79]. Sequence analysis predicts that the encoded proteins contain the signature domains characteristic of rGCs, including an extracellular ligand-binding domain, a single transmembrane domain, and intracellular kinase-like and cyclase catalytic domains [44, 45, 79]. The PcGC-M2 transcript is widely expressed in many tissues [45], fitting the profile of the sites of action of CHH, a pleiotropic hormone with a wide range of target tissues [22, 28].

In summary, accumulated data generated from studies of CHH-related peptides revealed multiple molecular variants and sites of expression. More efforts are needed to correlate the hormone titers in the blood and tissues with the physiological status of the animals and to devise corresponding bioassays in order to gain a fuller understanding of the functional roles of the different molecular variants. Ultimately, identification of specific receptor(s) would reveal in greater depth the structure/functional relationships of the various structural CHH variants.

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