

# REGULATION OF HORMONE – MIH MOULT INHIBITING HORMONE IN BARYTELPHUSA GUERINI

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## ABSTRACT

*Barytelphusa guerini* is a fresh water crab,moulting in these animals is controlled by the moulting hormone ecdysone secreted by the Y-organ present beneath the eye stalks .The activation and inhibition of Y-organ varies inversely with moulting hormone.The extirpation and implantation of the Y-organ revealed that the crustaceans eyestalks is the primary source of moulting .Moulting inhibiting hormone is a peptide secreted by certain neurosecretory neurons collectively called X-organs,present in the eyestalks.

**KeyWords:** *Barytelphusa guerini*,Moulting inhibiting hormone,Eyestalks,Haemolymph

## INTRODUCTION

Peptide hormones play an important role in regulating various physiological processes in crustaceans.The X-organs in the eyestalk of the crustaceans produces a family of crustacean hyperglycemic hormone ( CHH ). The CHH family members in crustaceans ( CHH , Moulting inhibiting hormone , MIH ), Mandibular inhibiting hormone, and Gonad/Vitellogenesis inhibiting hormone GIH / VIH, are involved in the regulation of variety of physiological processes (Keller , 1992 ; Klein and Van- Herp , 1995 ; Chung *et al .* , 1999 ; Spanings- Pierrot *et al .* , 2000 ; Chan , 2003 ; Spanings- Pierrot *et al .* , 2005 ). CHHs are multi -functional , having specific binding sites in multiple target tissues ( Bocking *et al .* , 2002 and Hsu *et al .* , 2006 ).

MIH is produced by neurosecretory neurons in the eyestalks , and is secreted from axon terminalis in the sinus gland ,inhibits synthesis of ecdysteroids by the Y-organ during intermoult.

Reduction of synthesis and release of MIH , releases Y-organ from repressive control, allowing increased ecdysteroids synthesis associated with premoult induction ( Watson *et al .* ,1989 ;Smith and Sedlmeier, 1990; Chang , 1993 ).

Peptide hormones serve as an intermediates between neurological signaling and terminal hormone signaling ( steroid hormones ).These peptide hormones are typically released from tissues in response to neurological stimulation , and are transported by the circulatory system to target sites , and bind receptors on the cell surface , Where they stimulate intracellular signal transduction pathways leading to the release of the next hormone in a cascade or directly regulating some physiological action ( Le- Blanc , 2007 ).

Ablation of the eyestalk leads to enhanced ecdysteroid secretion by Y-organs, and increased in the ecdysteroid titre and precocious moulting ( Keller and Schmid , 1979 ; Chang and Bruce 1980 , Hopkins ,1983 ). While injection of eyestalk extract or synthetic MIH into eyestalk

ablated animals lowers ecdysteroid titer and delays moulting ( Bruce and Chang , 1984 ; Chang *et al.* , 1987 ; Nakatsuji and Sonobe, 2004; Nakatsuji *et al.*, 2009 ).

The surgical extirpation of eyestalks results in a shortened moult cycle interval while the implantation of eyestalk contents restores this interval . A factor that normally inhibits the moulting process , and it has been named the MIH . Purification , characterization and amino acid composition of MIH is well established for several crustaceans . The major function of MIH is to restrict the production of moulting hormones which are ecdysteroids . If the production of MIH is reduced or eliminated by eyestalk extirpation , the result is usually precocious moulting , with elevated ecdysteroid levels (Reddy and Ramamurthi ,1999 ) .

In general ,the level of ecdysteroids in the haemolymph of *Penaeus clarkii* is low throughout intermoult stage,rises during premoult then falls prior to moulting resulting in a low level of ecdysteroids during ecdysis and postmoult stage (Skinner,1985) .

Several studies suggest that ecdysteroid production may be regulated not only by the level of circulating MIH,but also by the responsiveness by Y-organ to MIH . Stage specific changes in the responsiveness of Y-organs to MIH play an important role in the regulation of ecdysteroid production . Changes in glandular responsiveness to MIH are due to changes in glandular phosphodiesterase (PDE) activity (Sefiani *et al.*,1996; Chung and Webster,2003;Nakatsuji and Sonobe, 2004; Nakatsuji *et al.*,2006 and Nakatsuji *et al.*,2009).

The dual activity of these peptides makes the physiological actions of these hormones much more complex . It is possible that these molecules might have had their origin from the same ancestral molecule , still possessing significant homology with overlapping biological functions (Reddy and Rmamarthi ,1999 ) .

In the present investigation the molecular weight of MIH were determined using SDS-PAGE gel electrophoresis, and the fluctuating levels of MIH were also observed during the probable moult cycle.

#### **Material and Method:**

In the present investigation the molecular weight of MIH , Moult Inhibiting Hormone is determined using SDS-Page gel electrophoresis by 12% separating gel, according to the method described by Lammeli (1970).

The fluctuating levels of MIH were also determined during the probable moult cycle by comparing the eyestalk and the haemolymph samples because the MIH is stored in and release from the sinus gland into the haemolymph.

The eyestalk and haemolymph samples were taken for the experiment .The experiment were carried out during the 1<sup>st</sup> , 4<sup>th</sup> , 8<sup>th</sup> and 12<sup>th</sup> day of the probable moult cycle. The 1<sup>st</sup> day of the experiment corresponds to the moult stage,the 4<sup>th</sup> day corresponds to the postmoult stage, the 8<sup>th</sup> day corresponds to the premoult stage and the 12th day corresponds to the subsequent moult stage.

**Standard** - The protein ladder with range of 14 - 95 KD were taken as reference protein from Sisco Research Laboratories Pvt Ltd.

The eyestalks were homogenated with Tris Buffer 1.12gm in 100 ml distilled water and Tris Hcl 1.4 gm in 100 ml distilled water .

The haemolymph samples were prepared by adding Tris Buffer 1.12gm in 100 ml distilled water and Tris Hcl 1.4 gm in 100 ml distilled water .The electrophoresis were carried out at constant 250 volts for 3-hours . Following electrophoresis,the protein bands were visualized by staining with Coomassie Brilliant Blue R-250.

The fluctuating levels of MIH during the probable moult cycle were determined by observing the width of the bands in the eyestalk and the haemolymph samples.

### **Results**

The results shows the estimated molecular weight of MIH is about 51 KD as compared with the standard reference protein . The results indicates 9 fractions in the electropherogram which includes .

Fraction 1 – Indicates marker ( standard reference protein ranges between 14 - 95 KD ).

Fraction 2- Indicates the level of MIH in the eyestalk sample at about 51KD on the 1<sup>st</sup> day of the experiment which corresponds to the moult stage . A thin band is observed .

Fraction 3- Indicates the level of MIH in the haemolymph sample at about 51KD on the 1<sup>st</sup> day of the experiment which corresponds to the moult stage . A thick band is observed compared to the eyestalk sample .

Fraction 4- Indicates the level of MIH in the eyestalk sample at about 51KD on the 4<sup>th</sup> day of the experiment which corresponds to the postmoult stage . A thin band is observed .

Fraction 5- Indicates the level of MIH in the haemolymph sample at about 51KD on the 4<sup>th</sup> day of the experiment which corresponds to the postmoult stage . A thick band is observed compared to the eyestalk samples .

Fraction 6- Indicates the level of MIH in the eyestalk sample at about 51KDa. A thin band is observed on the 8<sup>th</sup> day of the experiment which corresponds to the premoult stage .

Fraction 7- Indicates the level of MIH in the haemolymph sample at about 51KD on the 8<sup>th</sup> day of the experiment which corresponds to the premoult stage .

Fraction 8- Indicates the level of MIH in the eyestalk sample at about 51KD on the 12<sup>th</sup> day of the experiment which corresponds to the subsequent moult stage .

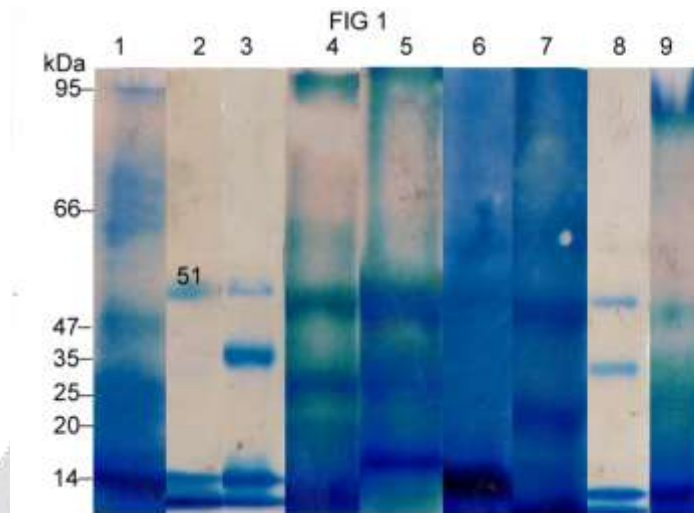
Fraction 9- Indicates the level of MIH in the haemolymph sample at about 51KD on the 12<sup>th</sup> day of the experiment which corresponds to the subsequent moult stage.

### **LEGEND FOR FIGURE**

The electropherogram shows 1 to 9 fractions of Moulting Inhibiting Hormone with a molecular weight about 51 KDa .

- 1- Marker protein(14-95 KDa)
- 2- Eyestalk sample at moult stage
- 3- Haemolymph sample at moult stage
- 4- Eyestalk sample at postmoult stage
- 5- Haemolymph sample at postmoult stage
- 6- Eyestalk sample at premoult stage

- 7- Haemolymph sample at premoult stage
- 8- Eyestalk sample at moult stage
- 9- Haemolymph sample at moult stage
- 10-



### Discussion

The crustacean hyperglycemic hormones are produced in the X-organ of malacostracan crustaceans and are stored in and secreted from the sinus gland (Keller and Sedlmeier, 1988). Secretion of CHH hormones occurs in response to monoamine neurotransmitter stimulation (Chen *et al.*, 2003). The CHH family of peptide hormones consists of ~ 80 amino acids that share significant sequence similarity (Chen *et al.*, 2005).

In crustaceans, the moulting cycle is hormonally controlled and associated with environmental cues, including light and temperature. Moulting is triggered by progressive increase in the titre of the circulating moulting hormone, 20-hydroxyecdysone, which is a metabolite of ecdysone (Spindler *et al.*, 1980). Neurosignaling of CHH release occurs in response to both environmental and endogenous cues (Beltz, 1988).

The existence of a Moulting Inhibiting Hormone (MIH) was established by the observations that eyestalk ablation results in a significant acceleration of the moulting cycle and the injection of the eyestalk extracts neurosecretory X-organ sinus gland complex delays the moulting of eyestalk ablated animals (Skinner and Klienholz, 1985; Chaigneau, 1983). MIH acts by inhibiting ecdysterogenesis in the Y-organ. A decrease in the biosynthesis of ecdysone by the Y-organ would reduce the body fluid concentration of the active hormone, 20-hydroxyecdysone (Naya *et al.*, 1989).

The mechanism of MIH signaling revealed changes in Y-organ responsiveness throughout the moulting cycle (Zmora *et al.*, 2009). The level of MIH in haemolymph was high during intermoult, dropped significantly during premoult and remained elevated through the remaining of the moulting cycle. The drop in MIH seen during early premoult coincided with a statistically significant increase in the level of ecdysteroids. The results are consistent with the hypothesis that MIH suppresses



ecdysteroid production during intermoult and postmoult, and that a drop in the level of MIH during the early premoult permits the increase in ecdysteroid production seen during the same stage (Nakatsuji and Sonobe, 2004). Whereas in *Carcinus maenas* there is no change in the level of MIH during premoult (Chung and Webster, 2005).

The rate of ecdysteroid secretion are influenced not only by MIH, but also by stage-specific changes in the responsiveness of the Y-organs to MIH. In *Penaeus vannamei* a decline in the responsiveness of Y-organs to MIH was observed during middle and late premoult (Sefiani *et al.*, 1996).

In the present investigation the concentration of MIH is less in the eyestalks which is represented by thin band and more in the haemolymph which is represented by thick band shows that MIH is stored in the eyestalks and released into the haemolymph subsequently

The electropherogram results suggests that the level of MIH in haemolymph samples were more than compared to eyestalks. On the 1<sup>st</sup> day of the experiment (the moult stage), the concentration of MIH is very less, reaches to its maximum on the 4<sup>th</sup> day i.e., postmoult stage, on 8<sup>th</sup> day i.e., premoult stage it is less and on the 12<sup>th</sup> day i.e., subsequent moult stage again it is least.

The suppressed ecdysteroid secretion by Y-organs suggest that intracellular PDE activity may be involved in regulation of Y-organ responsiveness (Mattson and Spaziani, 1985 b). To access the possible involvement of PDE in determining the responsiveness of Y-organs to MIH, Y-organs were removed from crayfish, *Procambarus clarkii*. The responsiveness of Y-organs to MIH was high during the intermoult stage, decline in the early premoult, and was low during middle and late premoult, and then increased during postmoult. The results suggest that stage-specific changes in the responsiveness of Y-organs to MIH may be a result of changes in glandular PDE activity (Nakatsuji *et al.*, 2006 a).

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