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Research Paper

# On the Proteins of Neuroendocrine Hormones in the Fresh Water Crab, *Barytelphusa guerini* (H. Milne Edwards) (Decapoda, Potamidea)

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**Abstract:** The protein profiling in the neuroendocrine structures carried out by the SDS-page electrophoresis in the freshwater crab, *Barytelphusa guerini*. Eyestalk showed over all more proteins as compared to brain and thoracic ganglion. Most abundant single protein resolved in between 60 kd and 150 kd. Two proteins resolved in the range of 150 kd to 200 kd. Remaining four proteins were observed in the range of 29 kd to 66 kd. As such, the eyestalk hormone contains total seven proteins. Brain showed only two proteins in the range of 66 kd to 150 kd. Thoracic ganglion resolved two prominent proteins in between 66 kd and 100 kd and one less abundant protein in the range of 150 kd and one very less abundant protein in the range of 29 kd to 66 kd. The data obtained in the present study will be a prelude to our understanding for further studies in order to determine the specific nature of each hormone acting on the various physiological activities. The studies on these lines yielding encouraging results.

## INTRODUCTION

Hyperglycemic hormone (HGH) from eyestalk is a polypeptide, its molecular weight about 7,000 daltons, it may activate phosphorylase and inhibit glycogen synthase (Kleinholz, 1976). Isolation of neurosecretory hyperglycemic hormone from the eyestalks of fresh water crab, *Barytelphusa cuniculanis* also suggested that the factor is polypeptide (Nagabhushanam, *et al.*, 1989). The experiments have demonstrated that the existence of two dissimilar substances with hormonal activity in crustacean in *Limulus Polyphemus* CNS extracts. One of these substances, the previously unreported LHGF apparently has a moleculer weight of about 6,400 daltons. In addition, it appears to be heat stable inactivated by hydrogen peroxide and sensitive to proteolytic enzymes (Paul D. Pezalla, *et al.*, 1978). Chang (2001) presented an overview of recent research on the isolation and characterization of members of the crustacean hyperglycemic

hormone neuropeptide family. Crustacean hyperglycemic hormone in crustacea is a pleiotropic neuropeptide that regulates a diverse array of physiological processes in decapod crustaceans. (Vasanta, et al 1979; Gangotri, et al 1987; Gangotri, et al 1989; Chang, 1993; Webster, 1998; Fangul-Moles, 2006; Tyler P., Zarubin *et al*, 2009; Gangotri and Gangotri, 2011) Encoded proteins of olfactory organ in lobsters was also reported by (Ruben Stepanyan, *et al.*, 2006).

Two iso-forms of crustacean hyperglycemic hormone designated as CHH-B and CHH-C in order of elusion were isolated and the amino acid sequence were investigated in pure form in the Mexican Crayfish *Procambarus bouvieri* (Alberto Huberman and Manuel; Anguilar, 1988, Alberto Huberman *et al*, 1993). The complementary DNA encoding a peptide related to the CHH family of the black tiger prawn *Penaeus Monodon* was successfully expressed. (Supattra *et al*, 2003)

Initial characterization and fractionation of the hormones from the eyestalks and CNS of, *Barytelphusa guerini* was studied and postulated that these hormones may be polypeptide. (Gangotri *et al*, 1986; Gangotri, 2011) The hormonal activity of brain and thoracic ganglion in *Barytelphusa guerini* was also increased after eyestalk ablation as these were also act antagonistic to each other in various physiological activities. (Gangotri, 2011) Therefore the efforts were made to detect the proteins in the neuroendocrine structures of this crab in the present study.

## MATERIALS AND METHODS

Collection, maintenance and choice of the animals were described as earlier (Gangotri, 2011). Total number of 50 crabs were used for experiment and eyestalk ablation was carried out by making deep incision at the base of eyestalks with a sharp scissor. The total number of eyestalk were collected and the outer cuticular part was removed and the inner X-organ sinus gland complex dissected out and freeze dried. Similarly, the brain and thoracic ganglionic mass were also dissected and freeze dried separately for the extraction of proteins. The proteins in all these three neuroendocrine structures were extracted by the Trizol method (Amanda, *et al*., 2007).

**Data Analysis:** Protein migrate at rates inversely proportional to the log of their molecular weights. The molecular weights of proteins were deter-mined by using the standard marker lane.

## RESULT

**Eyestalk:** Eyestalk shows overall more proteins as compared to central nervous structures. Most abundant single protein resolved in between 66 kd and 150 kd. Two proteins were resolved in the range of 150 kd to 200 kd. Remaining four proteins were observed in the range of 29 kd to 66 kd. As such, the eyestalk hormone contains total seven proteins. Further investigation needs to analyze the different functional properties of these proteins (Figure 1 and 2).

**Thoracic Ganglionic Mass:** Thoracic ganglia shows two prominent proteins in between 66 kd and 100 kd and one less abundant protein in the range of 150 kd to 200 kd and one very less abundant protein in the range of 29 kd to 66 kd (Figure 1 and 2).

**Brain:** As per isolation protocol, brain shows only two proteins in the range of 66 kd to 150 kd (Figure 3).

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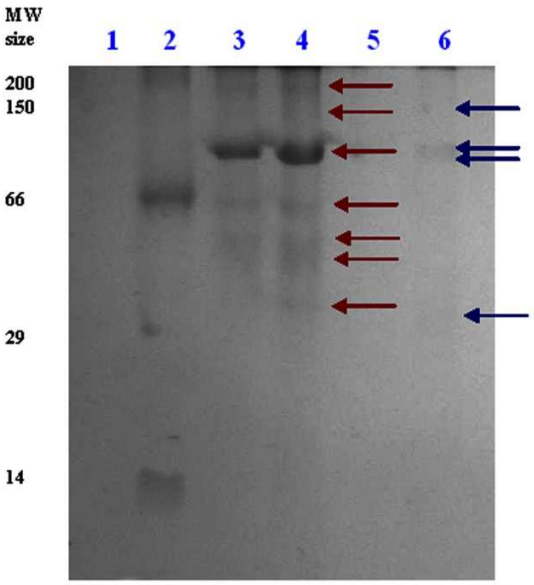


**Fig. 1 : SDS PAGE 10% resolving gel showing proteins isolated from Eyestalk and thoracic ganglionic mass:** Lane 2: Protein molecular weight marker (200kd, 150kd, 66kd, 29kd and 14kd) Lane 3 & 4 (i.e. duplicate) shows seven protein bands ranging (indicated by red arrows) from 29 kd to 200 kd; Lane 5 empty; Lane 6: Thoracic ganglionic mass (indicated by blue arrows) shows four proteins in the range of 29 kd to 150 kd

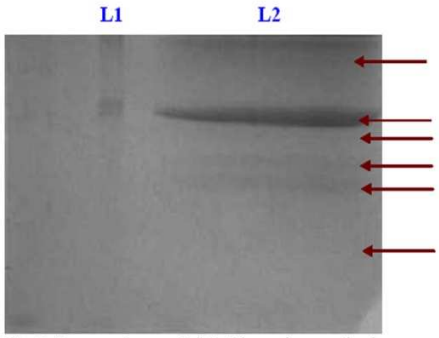


**Fig.2: Preparatory gel:** L1: Thoracic ganglionic mass and L2: Eyestalk

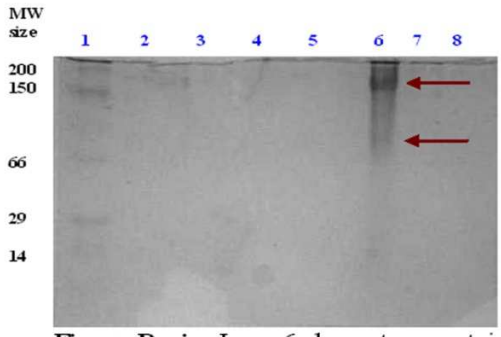


**Fig. 3: Brain**: Lane 6 shows two protein bands 66 kd to 150 kd

## DISCUSSION

These experiments have demonstrated the existence of seven dissimilar proteins in the eyestalks, four proteins in the thoracic ganglionic mass and two proteins in brain of freshwater crab, *Barytelphusa guerini*. Gangotri, *et al.*, (1986) studied the initial characterization and fractionation of the hyperglycenic hormone of the eyestalks in this crab and suggested that the hormone is a polypeptide or a protein molecule.

The data obtained in the present study confirms the view of earlier studies. The eyestalk shows overall more proteins as compared to central nervous structures. Most abundant single protein resolved in between 66 kd and 150 kd. Two proteins were resolved in the range of 150 kd to 200 kd. Remaining four proteins were observed in the range of 29 kd to 66 kd. As such, the eyestalk contains total seven proteins.

The brain and thoracic ganglionic contains an array of hormones which act antagonistic to eyestalk hormone in various physiological activities (Vasantha, *et.al.*, 1979; Gangotri, *et.al.*, 1986, 1989; Gangotri and Gangotri,

2011). Initial characterization and fractionation of the hypoglycemic hormones from brain and thoracic ganglionic mass concluded that the hypoglycemic hormone is highly thermolabile, resistant to freezing and its solubility in fat solvents like acetone and alcohol. Therefore, it is suggested that the factor may be a protein molecule of smaller size (Gangotri, 2011).

The results obtained in the present study clearly supports the assumption made by Gangotri (2011). The thoracic ganglion shows two prominant protein in between 66 kd and 100 kd and one less abdundant protein in the range of 150 kd to 200 kd and a very less abdundant protein in the range of 29 kd to 66 kd. Brain shows only two proteins in the range of 66 kd to 150 kd.

TRIZOL reagent can be effectively used to isolate not only RNA and DNA, but also proteins. This property is extremely useful when the sample for nucleic acid and protein extraction is not feasible. Protein can be recovered from phenolethanol supernatants by either precipitation or by dialysis, although, the dialyzol pellets are significantly easier to solubilize and thereby result in better recovery efficiency. A combination of 0.05% SDS and 4 M urea in Tris- HCl, pH 8.0 allows the most complete solubilization of dialyzed pellets without apparant decrease in the integrity of the isolated proteins (Amanda, *et al.*, 2007).

Earlier studies on *Limulus Polyphemus* suggested that CNS extracts contain at least two distinct peptides. LHGF is clearly hyperglycemic in *orconectes* (Mol. Wt. about 6,400 daltons) and another LUC previously known as Chromatopharotropins (Mol. Wt. about 1,850 daltons) (Paul D Pezalla, *et.al.*, 1978). Studies on *Procambarus bouvier*, Alberto Humberman, *et al.*, (1993), investigated that the hyperglycemic hormone is a 72 residue peptide (Mol. Wt. 8,388 daltons). Ernest S. Chang (2001), presented an overview of recent research on the isolation and characterization of members of Crustacean Hyperglycemic Hormone (CHH) neuropeptide family. Members of this arthropod specific family include CHH, Multi Inhibiting Hormone (MIH), Vitellogenesis Inhibiting Hormone (VIH) and Mandibular Organ Inhibiting Hormone (MOIH). The CHH is a pleiotrophic neuropeptide that regulates a diverse array of physiological processes in decapod crustaceans (Webster, 1998; Chang, *et.al.*, 1998; Fangul-Moles, 2006). The structural and mutational analyses have identified functionally important regions of CHH mature peptide in tropical land crab, *Gecarcinus lateralis* (Tyler P. Zarubin, *et al.*, 2009).

The total profiling of proteins of the neuroendocrine structures in the *Barytelphusa guerini* is a prelude to our understanding for further studies to analyse the particular nature of hormones acting on the various physiological activities. The further efforts are being made to detect the specific nature of polypeptide in these neuroendocrine hormones acting on various physiological activities. The studies on these lines are yielding encouraging results.

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