Research Paper

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# Extration And Qualitative Analysis of Peptide Hormone in A Crustacean

Biotechnology

**KEYWORDS :** Macro brachium rosenbergii, Salt precipitation, protein extraction, MIH.

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## ABSTRACT

*Eye stalk ablation is the most common procedure used to induce crustacean reproduction in commercial hatcheries. How ever, other physiological and metabolic process is affected by the removal of the X- organ sinus gland complex located in the eyestalk. Macro brachium rosenbergii is a commercially important species of crustacean cultured extensively throughout Southeast Asia. The main objective of this experiment was extraction and qualitative estimation of MIH hormones. The easiest way of extraction of proteins can be done by "Salt Precipitation method". Fraction-I, II and III of the samples have shown the presence of proteins. The samples are having proteins with different molecular weight ranging from 7-14 kd. Salt precipitate and MIH specific-antibody clearly showed that MIH is thoroughly extracted from the eye stalks through salt-precipitate without the necessity of cost devices such as HPLC at least at the preliminary phases of this investigation.*

## INTRODUCTION

Since 1970, eyestalk ablation (EA) has been used to improve the production of Macrobrachium rosenbergii larvae in a aquaculture. The X organ Sinus Gland (XOSG), located in the eyestalks, is the principal neuroendocrine gland in crustaceans. Synchrony among these different metabolic processes is essential to ensure normal gonad maturation, copulation, fecundity and larvae development. Subramoniam T (2011). They are regulated by secretions from the eye stalk gland complex.Petidergic neurons of the crustacean X-organ, located in the medulla terminalis of the eyestalk (MT-XO) synthesize a family of neuropeptides hormones essential for the regulation of the physiology and metabolism of these organisms Hopkins PM (2012). Vitellogenin synthesis is under the inhibitory regulation of the neuroendocrine system, which is centered in the X-organ/sinus gland complex in the paired eyestalks in crustaceans. T sukimura B (2001) The X-organs/sinus gland complex secrets several neuropeptides hormones, such as vitellogenesis inhibiting hormone or gonad inhibiting hormone (VIH or GIH), molt-inhibiting hormone (MIH), and crustacean hyperglycemic hormone (CHH), that are included in CHH/MIH/VIH family (reviewed by Huberman and Hopkins) .

The molting process is a complex one; the molting cycle may be divided into four periods.

- Premolt (or) Proecdysis: premolt or proecdysis is a period of active preparation for molt, including gradual thinning of the cuctle, storage in the gastroliths or hepatopancrease of inorganic constitute for a new exoskeleton.
- Molt (or) Ecdysis: The splitting and shedding of the old, partially resorbed cuticle and an abrupt size increase due to immediate absorption of water.
- Post molt: A period of rapid redeposition of chitin and inorganic salts to produce a new cuticle and of tissue growth.
- Inter molt (or) Interecdysis: A period of relative quiescence during which physiological processes normally associated with the active molting process.

The present study is carried out to demonstrate the extraction of peptide hormones through salt precipitation method and to test the presence of molt inhibiting hormone in the purified extract through specific antigen antibody binding technique.

## MATERIAL AND METHODS

**Source, animal handling, and initial preparation:**

Adult female (20-30g) fresh water prawns (M. rosenbergii) were obtained from seashore coast of Chennai.The prawns were anaesthetized and microdessection was performed under ice cold sterile condition. The sinus glands were collected into mortar. Crustacean saline is added homogenized and protein was extracted by the ammonium chloride precipitation method. Lee FY, Chang CF (1997)

**Isolation of protein through Salt precipitation:**

Reagent Preparation: Phosphate buffer saline pH (7)

- Solution –A: 3.12 gm of monobasic sodium phosphate is dissolved in 100ml of distilled water.
- Solution-B : 2.81 gm of dibasic sodium phosphate is dissolved in 100ml of distilled water.
- 19 ml of Sol A and 81 ml of Sol B are mixed and diluted to 200ml with distilled water.

**Salt precipitation method:**

Eye stalks were taken in to a mortar and I ml of crustacean saline was added and homogenized 1ml of 0.1N Hcl was added and heated at 80$^{o}$ Cand centrifuged at 1600 rpm for 15 min. 10 ml of cold PBS was added and mixed well to form a paste sediments are allowed to settle down and supernatant was poured in centrifuge tube. It is centrifuged at 5000rpm for 15min. Chang ES(1992). The pellet was separated and supernatant was consider as Fraction -I. again the saturated ammonium sulphate was added such that its concentration is 33% and centrifuged at 1600rpm for 10 min, this supernatant is considered as Fraction –II. it was repeated up to Fraction –III. Known volumes of respective Fractions mixtures were considered for analysis of protein.

Estimation of Protein by Lowry's Method: standard concentration of protein ranging from 20-30mg is taken in different test tubes and the volume is made up to 4ml with PSB buffer. To each tube 5.5ml of alkaine copper reagent was added. This is mixed well and allowed to stand to room temperature for 10-15 min. 0.5 ml of folin phenol reagent is added so each tube and kept for incubation at 37$^{o}$C for 30 min. The color is measured at 650 nm with calorimeter. Gr`eve P, Sorokine(1999).

Analysis of protein by SDS PAGE: Electrophoresis for protein peptide in polyacrylamide gel has proved to be one of the most widely used techniques in protein chemistry. SDS PAGE is a powerful tool for protein separation and characterization this technique is followed widely because of its high resolution. Chang CF, Shih TW, Hong HH (1993)

**Stock solutions**

- Acrylamide (30%)- Dissolve 30g of acrylamide and 0.8g bis acrylamide in 80 ml of distilled water and make it upto 100ml with water.
- Separating gel buffer- Dissolve 18.1g of tris and SDS of 0.3g in 80 ml distilled water adjust pH with low concentrated Hcl final volume made to 100 ml with distilled water.

- Stacking gel buffer- 6.06 of tris and 0.47g SDS are dissolved in 80ml distilled water and adjust pH with 6.8 with 10N Hcl final volume is made to 100ml with distilled water.
- Running buffer- Dissolve 120 g of trisbase, 560g lycine and 40g SDS in 800ml distilled water and make up to 1000ml.
- Sample buffer- Mix 2% SDS, 10%glycerol, 0.005% bromophenol blue, 0.0625m trisHcl, 5% mercapto ethanol and stored at 4°C.

The plates were cleaned and dried at 150°C for 3hrs and then finely wiped with 0.1% SDS solution. The spacer strips were coated with vaccum grease between plates and held with clmps.Comb was placed and stacking gel was poured and allowed for polymetization for 30min then sample buffer is poured. Sample was boiled in water bath for about 2 min. The electrophoresis is run at a constant volt at 80-150 untill the bromophenol blue marlcer dye reaches the bottom of the gel. Destaining of the gel is carried with methanol:acetic acid:water (25:1:24) until gel back ground become clear.

**Double Immuno Diffusion:**

This assay is frequently used for comparing different antigen preparation, in these test different antigen preparations each containing single antigenic species are allowed to diffuse from separate wells against the antiserum. The pattern of lines that form can be interpreted to determine whether antigens are same or different.4 cm square slide was cleaned and placed on a uniform table molten agarose was poured on to the bottom of a petridish to form a uniform thickness of 3mm and allowed to solidify. 20micro liters of MIH concentration of 1/32 was taken in the centre well and 20ml of protein extract containing MIH was taken in side wells. The gels were stained with coomassie blue and it was distained. Nakatsuji T, Lee CY, Watson RD (2009).

Estimation of protein: protein contents of the tissues were estimated by the method of lowrys et al.(1951).

**Alkaline copper reagent:**

- O.2% solution of Na2CO3 in 0.1N NaOH.
- 0.5% solution of CuSO4.5H2O in 1% solution of potassium sodium tartarate.
- 50 ml of reagent A was mixed with 1 ml of reagent B.
- Commercially available Folin-ciocaltcau reagent diluted twice was used

**Standard:** 100mg of Bovine albumin serum (BSA) was dissolved in distilled water and made up to 100ml. This gave a standard of 1mg/ml.

Procedure: 0.1 ml of Three Fraction solutions were taken to this 5ml of alkaline copper reagent was mixed and allowed to stand to room temperature for 10 min. Later 0.5 ml of folin –phenol reagent was added shake well and kept for 45 min. The blue complex formed was measure at 660 nm against blank.

| S.No | Vol of standard | Vol of buffer | Vol of copper reagent | Incubate at room temp for 10-15 min | Vol of folin phenol reagent | Incubation at 37°C for 30 min | O.D at 660nm | Amount of protein in 100µl |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.2 | 3.8 | 5.5 | | 5.5 | | 0.376 | 140 µg |
| 2 | 0.2 | 3.8 | 5.5 | | 5.5 | | 0.092 | 30 µg |
| 3 | 0.2 | 3.8 | 5.5 | | 5.5 | | 0.108 | 32 µg |
| 4 | 0.2 | 3.8 | 5.5 | | 5.5 | | 0.081 | 28 µg |
| 5 | 0.2 | 3.8 | 5.5 | | 5.5 | | 0.068 | 24 µg |
| 6 | 0.2 | 3.8 | 5.5 | | 5.5 | | 0.16 | 40 µg |
| 7 | 0.2 | 3.8 | 5.5 | | 5.5 | | 0.043 | 21 µg |
| 8 | 0.2 | 3.3 | 5.5 | | 5.5 | | 0.559 | 204.5µg |

**DISCUSSION**

The crustacean eyestalk is the source of one or more hormones which controls the growth and blood glucose levels. One these hormones called the hyperglycemic hormone (HCG, diabetagenic hormone) are able to elevate the level of blood sugar, resembling thus glucagon in vertebrates. Abramowitz et al (1944) for the first time reported the relation between the eyestalk principle and carbohydrate metabolism in crustaceans. So this experiment was done in laboratory on prawn's eyestalk to for the quantitative estimation of growth hormones which were produced in X-organ and Y- organ. The present result confirms that salt precipitation is the easy method which we can use in the lab for estimating and purification of the growth hormones. The study has shown that salt precipitation method support for precipitating protein which is the easiest method adapted for laboratory and it purification was done by using SDA PAGE and Immuno diffusion method was done to study antibody and antigen activity respectively.

**CONCLUSION**

The main objective of this experiment was extraction and qualitative estimation of MIH hormones.. Different proteins can be extracted from the eyestalks by many methods in which HPLC is an important one. But is very expensive and needs skilled persons to operate the systems. The easiest way of extraction of proteins can be done by "Salt Precipitation method".

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