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SDS-PAGE ANALYSIS AND ELECTRON MICROSCOPY OF CORPUSCLES OF STANNIUS SECRETION IN THE FRESHWATER FISH, *NOTOPTERUS NOTOPTERUS*

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ABSTRACT

The SDS-PAGE analysis of corpuscles of Stannius extract was carried out in the freshwater fish, *Notopterus notopterus*. Pair of corpuscles of Stannius is present embedded in the posterior portion of the kidney. The results indicate that the SDS-PAGE application of CS tissue homogenate indicates that the products of CS of *Notopterus notopterus* is a protein having molecular weight 41 kDa which might be a hypocalcemic hormone reported in other fishes and can be called as hypocalcin. Stanniocalcin (STC) is present throughout vertebrates, including humans, but a structure for STC has not been identified in animals that evolved before bony fish. The origin of this pleiotropic hormone known to regulate calcium is not clear. In the present study,

KEYWORDS: Electron Microscopy, Hypocalcin. *Notopterus notopterus* SDS-PAGE

INTRODUCTION

The Corpuscles of Stannius protein of non-humans has been studied extensively. Corpuscles of Stannius protein has been purified in the kidneys of teleost fish have been found to contain secretory granules, the Corpuscles of Stannius. Electron microscopy indicates that the granules are of a proteinaceous nature and may represent hormones or enzymes of unrecognized physiological and biochemical function (Butkus, A. et al. Mol. Cell Endocrinol, 54:123-33 (1987).

The corpuscles of Stannius (CS) have been most consistently implicated in the control of plasma calcium metabolism (Wendelaar Bonga and Pang, 1991). Stanniocalcin (STC), (formerly known as both teleocalcin and hypocalcin) is an anti-hypercalcemic, glycoprotein hormone that is produced by the Corpuscles of Stannius, endocrine glands which are confined to bony fishes.

The polypeptide of the present invention has a high degree of homology at the amino acid level to the glycoprotein hormone from fish which is involved in the regulation of calcium level secreted by the CS, lowers plasma calcium levels by reducing gill calcium uptake (Fenwick 1974; So and Fenwick, 1979; Milet *et al*. 1979; Lafeber and Perry, 1988; Lafeber *et al*., 1988)Since role of CS in fish has not been still established however, there are some reports that CS secretes a glycoprotein hormone which regulates calcium homeostasis (Fontain, 1964; Lafeber *et al*., 1988; Agber and Renfro, 1994).

Hence, in the present investigation effect of CS extract on calcium regulation has been studied in the freshwater fish *Notopterus notopterus* Stanniocalcina are large (approximately 250 amino acids) glycoprotein hormones in vertebrates whose classicl function was established to be the inhibition of calcium uptake from the environment in teleost fish gills (Wagner *et al*.1986)

MATERIALS AND METHODS

SDS-Polyacrylamide Gel Electrophoresis

The corpuscles of Stannius (CS) were dissected out from the fish *Notopterus notopterus* maintained in the laboratory. The CS glands were homogenized in 0.05 M ammonium acetate (pH 7.4) using Patter homogenizer. The supernatant obtained after centrifugation (at 900 g for 5 min.) was lypholized and prepared for sodium decyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE. SDS-PAGE was performed according to (Laemmli, 1970) with 8% polyacrylamide slab gels. SDS-PAGE was carried out under reducing conditions with mercaptoethanol. After fixation of the proteins in the gels by methanol and glutaraldehyde. The gels were stained with coomassic brilliant blue R-250.

Collection of Corpuscles of Stannius and Fixation for Electron Microscopic Studies

Fish were killed with a blow to the head. Then a longitudinal incision was made through the ventral body wall to expose the kidneys. The CS or white bodies were clearly visible along most of the length of the kidney, each CS together with the surrounding kidney was flooded with ice-cold glutaraldehyde (icg) buffered to pH 7.3 with phosphate and fixed for 5 min. CS were excised from the adjacent kidney tissue and placed individually, in separate 2 ml glass vials containing additional ice-cold fixative for 2 hr. In the fish, *N. notopterus,* there are two white oval corpuscles, approximately 0.5 to 2.00 mm in diameter, which are embedded in the ventral surface of the anterior position of the posterior functional kidney at the point where the right post cardinal vein leaves it. Both CS were transferred to the Ice cold Glutaraldehyde (ICG) fixative for 2 min then removed, cut into about four pieces, and placed in a vial of ICG for 2 h.

Fixation: The corpuscles of Stannius (CS) were primarily fixed in Glutaraldehyde (because its capacity to stabilize most of the protein without coagulation) following fixation in the primary fixative, the tissues were washed in 0.1 M buffer. The tissues were post fixed in 1%, Osmium (OsO_4) for 1-2 hours at 4^0C. They are dehydrated using absolute alcohol (glass distilled) at 4^0C.

Clearing: The tissues are cleared with a clearing agent propylene oxide to facilitate infiltration. 2 changes – 15 min, each at room temperature

Infiltration and Embedding: The tissue is embedded in embedding medium (Epon 812/Araldite Cy212). Infiltration involves gradual replacement of the dehydrating agent with the embedding medium and embedding consists of complete impregnation of the interstices of the tissue specimen with the medium. It also attaches the tissue block sufficiently strongly, enabling it to be handled to obtain ultrathin sections.

Infilteration is carried out at room temperatures with a liquid resin with which embedding of tissues are carried out.

Semithin Sections for Light Microscopy

Before proceeding to ultrathin sectioning 1 µ thick section are cut for scanning the tissue under the light microscope. The semithin sections floated on the water are lifted with a thin glass rod on a clean glass slide. The slide is placed on a hot plate at about 80^0C and dried. The sections are stained using 1% toludine blue for 1 minute. Washed in running water, dried and mounted with dibutylpthalate plasticizer xylene (DPX). The slides are observed under light microscope.

Pieces of CS in each vial were washed several times with phosphate buffer and post-fixed for 2 hr in chilled 1% O_SO_4 buffered to pH 7.3 with phosphate followed by dehydration in a graded series of chilled ethanol, then propylene oxide and embedded in araldite. Ultrathin sections were cut at various levels through the tissue blocks using a diamond knife on a Reichert OM U3 ultra-microtome, the sections were mounted on copper grids, stained with uranyl acetate and lead citrate and examined using an electron microscope. Representative photomicrographs of these tissue samples were taken. All sections were viewed at a magnification of 5500. As noted above, sample areas (500 lm2) were selected randomly and photographed.

OBSERVATIONS

Identification of the Hypocalcemic Factor

Figure-1 shows the densitometric scans of coomassic brilliant blue stained products after SDS-PAGE under reducing conditions present in a crude tissue homogenate of CS of the fish, *Notopterus notopterus*. A product with an apparent molecular weight of approximately 41 kDa Protein analysis from the tissue homogenate of 100 mg dry weight of CS with a protein content of 6 mg. Electron microscopy indicates that the cell type-I cell with secretary granules are of a proteinaceous nature and may represent hormones Figure-2 and vacuoles also seen

SDS-PAGE: (SDS-PAGE Analysis of Partially Purified STC from the Fish *Notopterus notopterus* (Figure 1)

Lane-1 is molecular weight morker; phosphorylase (97 kDa); bovine serum albumin(66kDa); ovalbumin (43kDa); carbonic anhydrase (29kDa); lane-2 crude, lane-3 ammonium sulphate, lane-4 after dialysis, lane-5 G-100.

DISCUSSIONS

The study on the SDS-PAGE application of CS tissue homogenate indicates that the product of CS of *N. notopterus* is a protein having a molecular weight of 41 kDa which might be a hypocalcemic hormone reported in other fishes (Lafeber *et al*., 1988). Because of its high molecular weight, this product from CS of the fish *N. notopterus* can be called as hypocalcin. As this name (hypocalcin) was proposed for a CS hypocalcemic principle with a molecular weight about 10 kDa by (Pang *et al*., 1974,), whereas the name teleocalcin was first given to a hypocalcimic principle with a molecular weight of 3 kDa isolated from CS of Salmon (Ma and Copp, 1978).

Hypocalcin is present in relatively large amount in the CS of the six freshwater species such as European eel, Tilapia, gold fish and carp after SDS-PAGE under reducing conditions found that the product with an apparent molecular weight of approximately 54 kDa and concluded that after SDS-PAGE under reducing conditions the product from CS of the fishes appears as a dimer identified as a 41 kDa band. It has also been isolated and purified a glycoprotein from the Corpuscles of Stannius of trout, which is considered hypocalcin, the major hypocalcemic hormone of fish. This product is present in relatively large amounts in the Corpuscles of Stannius of several species (i.e., European eel, tilapia goldfish, and carp). Hypocalcin is typically released from the Corpuscles of Stannius in response to an experimentally induced increase of the blood calcium concentration. Ultrastructural observations show that after this treatment the hypocalcin-producing cell type of the corpuscles of stannius are almost completely degranulated. The isolated glycoprotein has an apparent molecular weight of 54 Kda)). Lafeber *et al*., 1988).

The present observation on the molecular weight of 41 kDa for the product (hypocalcemic principle) of *N. notopterus* were in agreement with the reports of Lafeber *et al*., (1988) in six species of fish studied showing that isolation

of hypocalcin yields a band of 41 kDa after SDS-PAGE under non-reducing conditions. There are similar reports on other fishes (Wendelaar Bonga *et al*., 1985; Wagner *et al*., 1986). Hence, it is indicated that the CS of the freshwater fish *N. notopterus* secretes a product having molecular weight of 41 kDa as that of other fishes and this product of CS can be called as hypocalcin hormone.

CONCLUSIONS

SDS-PAGE application of corpuscles of Stannius tissue of *Notopterus notopterus* is a protein having molecular weight 41 kDa which might be a hypocalcemic hormone reported in other fishes and can be called as hypocalcin. Stanniocalcin (STC).The cells of CS conten three types of cells and type-I cell is responsible for synthesis of Stanniocalcin

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APPENDICES

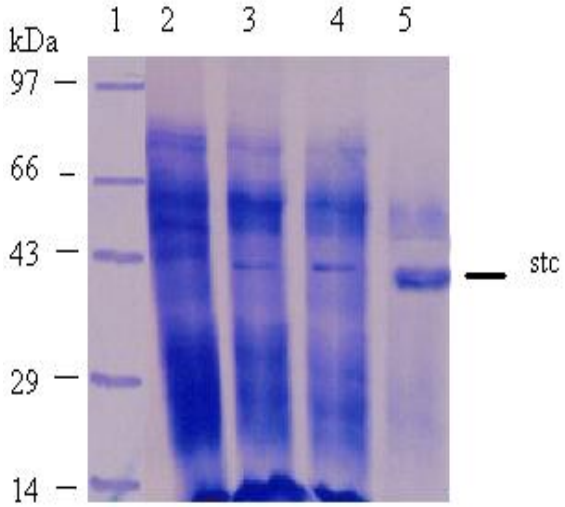

Figure 1: Showing SDS-PAGE Analyses of Partly Purified Steniocalcin in the Corpuscles of Stannius Extract of the Fish, *Notopterus notopterus*

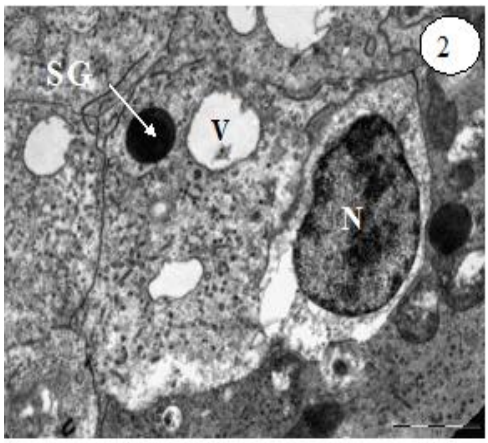

Figure 2: Section of the Corpuscles of Stannius under Electron Microscope Showing a Cell Type -I Cell with Secretary Granules and Vacuoles, the Nucleus Contain Chromatin Patches × 9300