SDS-PAGE analysis of corpuscles of Stanniuss 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.

Keywords: SDS-PAGE, *Notopterus notopterus*, hypocalcin.

I. Introduction

The corpuscles of Stannius (CS) have been most consistently implicated in the control of plasma calcium metabolism (Wendelaar Bonga and Pang, 1991). Stanniocalcin (STC), the hormone 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*.

II. 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.

III. 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.

SDS-PAGE: (SDS-PAGE analysis of partially purified STC from the fish *Notopterus notopterus* (Fig-1)

Lane-1 is molecular weight marker: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.
Fig. 1: Showing SDS-PAGE analyses of partly purified steniocalcin in the corpuscles of Stannius extract of the fish, Notopterus notopterus.

IV. Discussion

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 (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.

V. Conclusion

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

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References


