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## Purification and partial characterization of serum immunoglobulins of *Channa striatus* (Bloch)

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### ABSTRACT

*Channa striatus* immunoglobulin (Ig) was purified from serum by affinity chromatography using bovine serum albumin as capture ligand. The purified Ig had a molecular weight (MW) of 820 kDa as determined with gel filtration chromatography. Under reducing SDS-PAGE, the Ig molecule was shown to consist of two subunits, heavy and light chain having a MW of 70.4 and 25.3 kDa, respectively. A high MW band was observed in non-reducing SDS-PAGE, suggesting tetrameric structure.

### Introduction

Teleost fish possess the capacity to produce specific immunoglobulins in response to antigenic stimulation. The fish immune system contains five classes of immunoglobulins i.e., IgM (Warr, 1995); IgD (Hirono *et al.*, 2003); IgZ (Danilova *et al.*, 2005); IgT (Hansen *et al.*, 2005) and IgH (Savan *et al.*, 2005). The predominant immunoglobulin class in fish blood serum is tetrameric IgM-like molecule (Koumansvandiepen *et al.*, 1995) consisting of eight light (L) and eight heavy (H) chains. It has a molecular weight (MW) between 6,00,000 and 8,00,000 Da, depending on the species of the fish. Molecular weight for most of the teleost H and L chains are generally in the range of 70-81 and 23-28 kDa, respectively. In fish, immunoglobulins have been demonstrated in the plasma, gut, skin, bile and mucus (Ellis, 1982).

The structural characteristics of the fish immunoglobulins have been investigated in a few species including *Dicentrarchus labrax* (Bourmaud *et al.*, 1995), *Salmo salar* (Magnadottir *et al.*, 1996), *Oreochromis niloticus* (Al-Harbi *et al.*, 2000), *Thunnus maccoyii* (Watts *et al.*, 2001), *Lates calcarifer* (Crosbie and Nowak, 2002), *Cyprinus carpio* (Vesely *et al.*, 2006). In India, there are a few reports on purification and characterization of immunoglobulins of *Oreochromis mossambicus* (Rajavarthini *et al.*, 2000), *Clarias batrachus* (Swain *et al.*, 2004), *Clarias gariepinus* (Rathore *et al.*, 2006) and *Cyprinus carpio* (Sood *et al.*, 2007). The objective of the present study was to isolate, purify and partially characterize *Channa striatus* Ig in order to develop polyclonal and later monoclonal antibodies against snakehead Ig.

### Materials and methods

#### Fish

Fifteen healthy *C. striatus*, weighing 400-500 g, procured from fish farm, were divided in two groups (test and control). The test group comprised of 10 fish, whereas, five fish served as control.

#### Raising of hyperimmune serum

One milligram of bovine serum albumin (BSA) in 0.1 ml of phosphate buffered saline (PBS) was emulsified with equal volume of Freund's complete adjuvant and injected intra-peritoneally in each fish of the test group. This was followed by three injections of similar emulsion in Freund's incomplete adjuvant at 2, 4 and 6 weeks interval. The fish in control group were injected with PBS emulsified with adjuvant, in a similar manner. Pre- and post-immunization (7 days after 4<sup>th</sup> injection) blood samples were collected from the fish *via* caudal vein and serum was stored at 4°C.

#### Indirect haemagglutination (IHA) test

IHA test was used to assess the humoral immune response in immunized fishes following Cho *et al.* (1976). Briefly, nine volumes of 2.5% sheep RBCs were mixed with one volume of 0.6% glutaraldehyde and incubated for 10 minutes. The RBCs were then washed and sensitized with BSA (10mg/ml) for 30 minutes. The RBCs were then washed again and suspended in PBS to a final concentration of 2.5%. For the test, two-fold dilution of individual serum was done with PBS in a 96 well microtitre plate, except RBC control wells. Equal volume of sensitized RBCs (50 µl) was added to each well, incubated for half an hour and the highest dilution of serum showing agglutination was considered as the titre.

### Purification of Ig by affinity chromatography

BSA-CL agarose column was used to purify anti-BSA Ig from immunized fish following Sood *et al.* (2007). In brief, 5 ml BSA-CL agarose column was equilibrated with PBS. Two millilitre of serum from immunized fish was mixed with equal volume of PBS, filtered through 0.4 $\mu$  filter and loaded in the affinity column. After thorough washing with PBS, anti-BSA antibodies were eluted from the column with elution buffer (0.1M glycine NaOH buffer, pH 11.0) and collected as 2 ml fractions. The optical density (OD) of each fraction was measured by UV-spectrophotometer (280 nm) in order to determine the protein concentration. Fractions having an optical density of >0.1 were pooled and concentrated with Centrplus YM - 100 filter. The concentrated Ig was exchanged with PBS buffer and again concentrated to 1/10<sup>th</sup> of eluted volume and stored at 4°C.

### Molecular weight determination by gel filtration chromatography

The purity and MW of eluted Ig was determined by gel filtration chromatography on a 1.6 x 57 cm column bed of Sephacryl S-300 (Pharmacia, Sweden) having a total volume (Vt) of 114.6 ml. The column was run at a flow rate of 5.4 ml per hour using 0.1M PBS (pH 7.2) and fractions of 1.8 ml were collected using automatic fraction collector (Pharmacia). The optical density (OD) of elutes was measured at 280 nm and void volume (Vo) of the column bed was 54 ml as determined with blue dextran. The column was calibrated with protein MW markers (Sigma) viz., thyroglobulin (669 kDa), apoferritin (443 kDa), amylase (200 kDa), alcohol dehydrogenase (150 kDa) and bovine serum albumin (66 kDa). The elution volume (Ve) for each marker was determined from the peak OD at 280 nm. Thereafter, 1 ml of concentrated snakehead Ig containing 1.5 mg protein was applied to the column. The MW of purified Ig molecule was estimated from a standard curve of the markers against their 'Ve' divided by 'Vo' of blue dextran.

### Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Purified Ig was characterized by polyacrylamide gel electrophoresis under both reducing and non-reducing conditions to determine purity and MW of reduced Ig molecules. The MW of the Ig in reduced form was determined by SDS-PAGE using Labworks Version 4.5 software. Similarly, Ig was analyzed under non-reducing conditions in a gradient gel (3-12%), with samples diluted in loading buffer that did not contain  $\beta$ -mercaptoethanol, so as to determine the different forms of Ig.

## Results and discussion

The post-immunization IHA titre in the test group ranged from 1:32 to 1:256, whereas, the titre was <1:2 in pre-immunization serum and control group. In affinity chromatography, a single peak was observed on elution with glycine buffer. The concentrated Ig fraction showed an IHA titre of 1:128. Affinity chromatography using BSA-CL agarose column was quite successful in purification of serum Ig and the yield was dependent on IHA titre.

By gel filtration chromatography, a single peak was observed at an elution volume of 72 ml corresponding to MW of approximately 820 kDa (Fig. 1). Previously, reported MW of approximately 670 kDa for *C. striatus* Ig

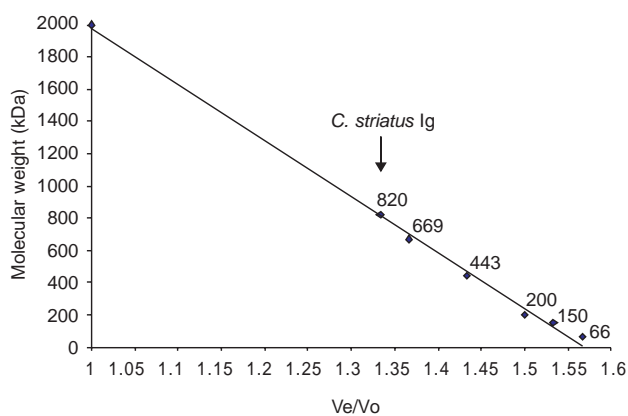


Fig.1. Molecular weight determination of purified *Channa striatus* immunoglobulins by gel filtration chromatography. (V<sub>e</sub> - Elution volume, V<sub>o</sub> - Void volume)

(Chanphong and Adams, 1994). Variability in MW of snakehead Ig may be due to the use of different techniques for estimation of MW. Variable MW of Ig has also been reported in *Lates calcarifer* and *Cyprinus carpio*. Crosbie and Nowak (2002) indicated that native *L. calcarifer* Ig molecule had a MW of 929 kDa while Bryant *et al.* (1999) reported a MW of 768 kDa. Similarly, MW of *C. carpio* Ig has been reported to be 740 kDa, 608 kDa, 800 kDa and 760-768 kDa (Shelton and Smith, 1970; Richter *et al.*, 1973; Kusuda *et al.*, 1987; Zhong *et al.*, 1999).

In our study, the affinity purified Ig revealed two bands of 70.4 kDa and 25.3 kDa by SDS-PAGE under reducing conditions, corresponding to the heavy and light chain, respectively (Fig. 2A). In earlier study, molecular weight of heavy chains and light chains was estimated at about 67 and 29 kDa, respectively (Chanphong and Adams, 1994). Zhong *et al.* (1999) observed H and L chain of 71 and 24-26 kDa for common carp Ig. However, Sood *et al.* (2007) reported MW of H and L chain of *C. carpio* Ig as 73.7 and 25.3 kDa. Rathore *et al.* (2006) reported that MW of H chain in *Clarias gariepinus* was 74.8 kDa while that of L

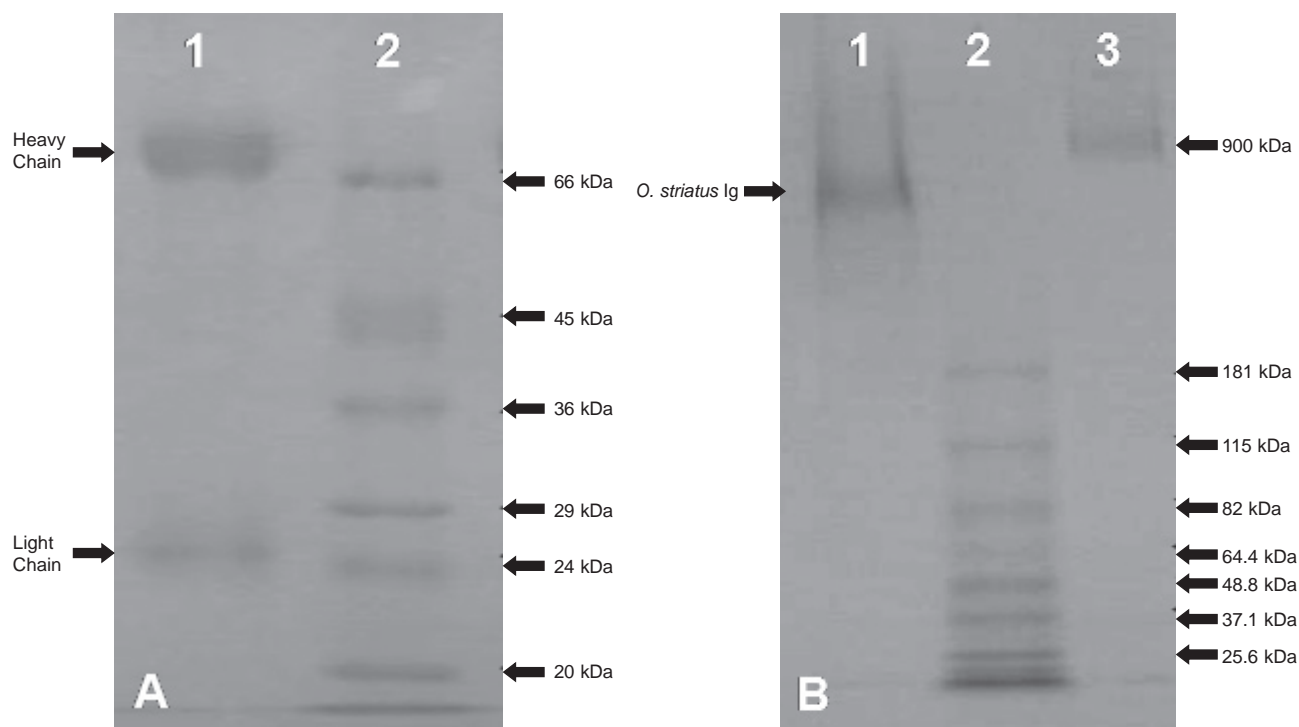


Fig 2. Analysis of *Channa striatus* immunoglobulin by polyacrylamide gel electrophoresis

A. Denaturing SDS-PAGE (12%), Lane 1- Affinity purified Ig, Lane 2 - Molecular weight marker (Sigma)

B. Non-reducing SDS-PAGE (3-12% gradient gel), Lane 1- *C. striatus* Ig; Lane 2 - Molecular weight marker (Invitrogen); Lane 3 - Bovine IgM

chain was 27.2 kDa. As an Ig molecule in its monomeric form is composed of two heavy and two light chains, the calculated MW of this molecule would be 191 kDa. Therefore, a molecule of 820 kDa would have a tetrameric structure in accordance with observations from other teleost fish.

Under non-reducing conditions, the affinity purified Ig was shown to have one high MW band (Fig. 2B) which is presumed to be of tetrameric Ig, in accordance with earlier reports (Morrison and Nowak, 2001). Further, the purified Ig of the snakehead, *C. striatus* can be used to raise polyclonal antisera and monoclonal antibodies which have applications in monitoring humoral immune response and sero-surveillance.

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