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RESEARCH ARTICLE

Isolation and partial characterization of serum immunoglobuline in two species of sturgeons, Siberian sturgeon (*Acipenser baerii*) and Stellate sturgeon (*Acipenser stellatus*).

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ABSTRACT

The immunoglobulins (Igs) were isolated and purified respectively from serum of Siberian sturgeon (*Acipenser baerii*) and Stellate sturgeon (*Acipenser stellatus*) by using 50% saturated ammonium sulfate precipitation. Analysis of the purified Ig of the two species of fishes on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions revealed two bands, heavy chains and light chains respectively. The molecular weight of the heavy chains of two fishes was about 70 kD and the molecular weight of the light chains was 27kD for Siberian sturgeon and 27.5 kD for Stellate sturgeon. The results suggested that the isolated and purified serum Immunoglobuline in two species of sturgeons, Siberian sturgeon (*A. baerii*) and Stellate sturgeon (*A. stellatus*) can be used to produce polyclonal and monoclonal antibodies against sturgeon Ig for developing diagnostic tests against a wide variety of pathogens.

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Introduction

Immunoglobulins are important components in immune response of fish. The major immunoglobulin class in fish blood serum is IgM molecule consisting of light (L) and heavy (H) chains (Koumans-van Diepen et al., 1995). It has a molecular weight (MW) between 6.00, and 8.00, kD, depending on the species of the fish (Ellis, 1982). The characteristics of the fish immunoglobulins have been investigated in a few species including *Labeo calbasu, Dicentrarchus labrax*, *Salmo salar*, *Oreochromis niloticus*, *Thunnus maccoyii*, *Lates calcarifer*, *Cyprinus carpio* (Behera et al., 2009; Vesely et al., 2006; Bourmaud et al., 1995; Magnadottir et al., 1996; Al-Harbi et al., 2000; Watts et al., 2001; Crosbie and Nowak, 2002). There are little studies on the serum immunoglobulins of chondroesti sturgeons. Study

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on immunoglobulin will be useful in developing immunodiagnostic methods against a wide variety of pathogens in aquaculture industry. The aim of this study was to isolate and partially characterize serum Igs from two important species of sturgeons, Siberian sturgeon (*A. baerii*) and Stellate sturgeon (*A. stellatus*) in order to develop polyclonal and monoclonal antibodies against sturgeon Ig.

Material and Methods

Fish

Eight healthy *A. stellatus* and *A. baerii* weighing about (5.47±0.34 kg, 5.27 kg±0.87 respectively) were collected from a fish farm in Kamfiruz city, Fars province, Iran. Blood samples were collected from the caudal vein and allowed to clot overnight at 4°C. The sera were collected and stored at -20°C until required for the assay.

Isolation and Purification of Fish Igs

Serum immunoglobulins (Igs) were precipitated with 50% saturated ammonium sulphate (SAS) for 6 hours at 4°C. The precipitate was centrifuged at 9000g for 15 min at 4°C. Then, the precipitate was suspended in 1 ml of PBS (1x) and dialyzed for 24 hours at 4ºC. Ammonium sulphate was removed by dialysis against phosphate buffer saline (PBS pH 7.2). The purified Ig was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Purified Ig was characterized by polyacrylamide gel electrophoresis under reducing conditions to determine purity and molecular weight of reduced Ig molecules. Electrophoresis was performed using a discontinuous buffer system at 3% stacking gel, pH 6.8 and 10% separating gel, pH 8.8 as described by Laemmli (1970). Samples were mixed with sample buffer (Tris HCL, pH 6.8; 10% SDS; Glycerol; 0.1% Bromophenol blue; 2-mercaptoethanol and distilled water) and allowed to boiled for 5 minutes at 100°C before application. After electrophoresis for about 2 hours at a constant voltage of 100v, the gel was stained with Coomassie Brilliant blue and destained with 10% acetic acid– 40% methanol solution until the gel became clear. Relative molecular weights of bands were calculated using Protein marker (Cinnagen [SL7012] prestained protein ladder).

Results

SDS-PAGE analysis from the purified serum Igs in two species, the Siberian sturgeon and Stellate sturgeon, resulted in two distinct bands at approximately 70 and 27-27.5kDa (Figure 1). These could be identified as the H and L chain of Ig, respectively. The presumed light chain of Stellate sturgeon Ig showed a slightly higher MW i.e. 27.5KDa, while Siberian sturgeon was similar i.e. 27KDa.

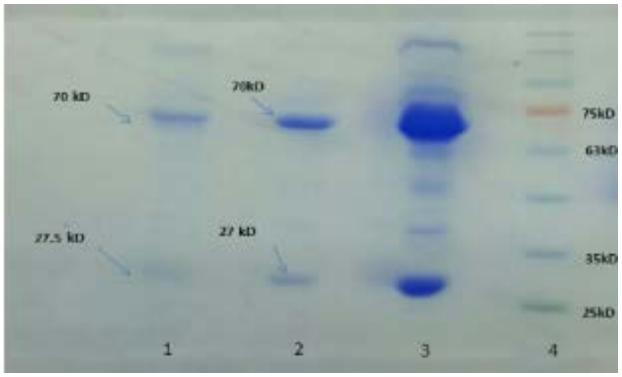

Figure 1. SDS-PAGE under reduceding conditions for estimation of molecular weight of Ig of the sturgeons. Lanes (1-2): Purified Igs from *Acipenser stellatus* and *Acipenser baerii* respectively, Lane (3): Unpurified Ig from *Acipenser baerii*, Lane (4): Marker (Cinnagen [SL7012] prestained protein ladder).

Discussion

Research on fish immunoglobulins is important for developing immunological reagents and in assessing health status. Immunoglobulin had been isolated from a variety of fish species such as European eel, Atlantic salmon, Asian sea bass, Japanese eel, Indian major carp, and Asiatic cat fish (Håvarstein et al., 1988; Buchmann et al., 1992; Uchida et al., 2000; Swain et al., 2004; Bag et al., 2009; Choudhury and Pani Prasad, 2011).

In the present study, immunoglobulin was purified from two important species of sturgeons, Siberian sturgeon (*A. baerii*) and Stellate sturgeon (*A. stellatus*) serum. The immunoglobulin was found with heavy and light chains of 70 and 27-27.5kDa, respectively., Pilström and Petersson (1991) found the heavy and light chains molecular weight of cod (*G. morhua*) Ig to be 81 kDa and 27.5 kDa, respectively. Kofod et al. (1994) reported one heavy chain of 79 kDa and two light chains of 27 and 29 kDa for turbot (*S. maximus*) Ig. Adkison et al. (1996) analyzed purified immunoglobulins from white sturgeon (*A. transmontanus*) serum. The MW of the heavy and light chains of the purified Igs determined by SDS-PAGE 73 and 27-30 kDa, respectively. Palenzuela et al. (1996) showed that European sea bass (*D. labrax*) Ig contains one heavy chain of 78 kDa and two light chains of 27.5 and 28.5 kDa.

Conclusion

The present results will be helpful for developing immunodiagnostic kits in aquaculture industry. The purified Ig of the Siberian sturgeon (*A .baerii*) and Stellate sturgeon (*A. stellatus)* can be used to raise polyclonal antisera and monoclonal antibodies which have applications in diagnosis of fish diseases.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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