Purification and partial characterization of serum immunoglobulins from Caspian Sea sturgeons

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Abstract
IgM-like immunoglobulins (Igs) were isolated from pooled anti-goat IgG sera raised in five species of sturgeons consisting of great sturgeon (Huso huso), Russian sturgeon (Acipenser guldenstedti), ship sturgeon (A. nudiventris), starred (stellate) sturgeon (A. stellatus) and Persian sturgeon (A. persicus). The Igs were purified by affinity chromatography. The molecular weight (MW) of the non-reduced IgMs from all species was identical with 870 kilo Daltons (kDa) estimated by dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The MW of the heavy and light chains was estimated at 77-84 and 28-30 kDa, respectively.

Introduction
The chondroesti sturgeons are valuable species of fish because of a strong demand for caviar. Industrial and agricultural pollutions and illegal fishing have significantly depleted many sturgeon stocks in the Caspian Sea, the spawning grounds of the most important sturgeon species such as Huso huso, Acipenser guldenstedti, A. nudiventris, A. stellatus and A. persicus. Also, the increased need for the artificial growing of these fish under intensive conditions has resulted in the identification of some economically important infectious diseases such as iridovirus (Irido-virus) disease, columnaris disease, vibriosis, yersiniosis and motile Aeromonas septicemia (Hedrick et al., 1992; Watson et al., 1995; 1998; LaPatra et al., 1994; Soltani and Kalbassi 2001). The analyses of fish immunoglobulins (Igs) are important for the development of tools to improve fish immunity against infectious diseases. Adkison, Basurco and Hedrick (1996) analyzed white sturgeon (A. transmontanus) immunoglobulin (Ig) that was purified from serum. The major portion of the Ig preparation consisted of two proteins with estimated molecular weights (MW) of 870 and 170 kDa. Also, the MW of the heavy and light chains of the purified Igs determined by SDS-PAGE was 73 and 27-30 kDa, respectively. The aim of this study was to purify and partially characterize IgMs raised against specific polyclonal antisera from five commercially important species from the Caspian Sea to

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enable further studies of the immune responses of these fish during occurrence of infectious diseases.

**Materials and methods**

**Immunization of fish**

Ten apparently healthy *Huso huso, A. guldendstelti, A. nudiventris, A. stellatus* and *A. persicus* weighing about 1 kg each from a fish farm in north Iran were acclimatized in 4000L fiberglass tanks at 20-22°C and were fed commercial fish food pellets. Fish were immunized by intraperitoneal injection with 2mg/fish of goat IgG (Sigma) in Freunds complete adjuvant (1:2 ratio). The immunization was repeated 2 and 4 weeks later and blood samples were collected from the caudal vein and allowed to clot overnight at 4°C. The sera was collected, pooled and stored at -20°C until required for the affinity chromatography procedure.

**Affinity chromatography**

The affinity chromatography described by Smith (1992) was used. Two ml of gravity-packed goat anti-mouse IgG agarose beads (Sigma) were added to a 10- x 0.5-mm glass column, washed with 150 ml phosphate-buffered saline (PBS, 0.01M, pH7.2) at a flow rate of 30ml/h, and then stripped with 10ml elution buffer (0.1M glycine, pH 11). The column was washed and equilibrated with 150ml PBS. Two ml of serum from the goat-IgG-immunized sturgeon were mixed with 2ml PBS and then filtered through a 0.45µm filter. The samples were applied to the columns and allowed to flow past the beads, and then the columns were washed with 100ml PBS. The fish anti-goat Igs were eluted from the columns with 5 ml elution buffer, collected as one fraction for each fish species, and neutralized with 0.6 ml of 1 M Tris buffer (pH 8.5). The fractions were extensively dialyzed against multiple changes of PBS for 18 hours at 4°C, concentrated by centrifugation (Centricon-30 micro concentrator) and stored at -20°C for further study by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

**SDS-PAGE**

The molecular weights of the fish sera Igs separated by affinity chromatography were determined by analytical SDS-PAGE under reducing and non-reducing conditions according to Sambrook *et al.* (2001) with a 8% resolving gel and a 5% stacking gel containing 29:1 acrylamide:bis acrylamide. Human IgM and a smaller marker (14.6-116 KD, SM0431, Fermentase, Germany) were used as approximate MW markers. The Ig samples were mixed 1:1 with electrophoresis sample buffer (2.3 ml 10% SDS; 1ml 0.625M Tris-HCL, pH6.8; 1ml glycerol; and 5.2 ml distilled water) containing 10mg Bromophenol Blue. The samples were boiled for 5 minutes, cooled at room temperature, and added to a single well of a vertical 5% gel over an 8% resolving gel. After electrophoresis for about 27 hours in an ice bath at a constant voltage of 70v, the gel was fixed and stained for 2 hours with a 10% acetic acid-40% methanol solution containing 0.125% Comassie Brilliant blue for 1 hour and de-stained with 10% acetic acid-40% methanol solution overnight and washed in distilled water until the gel became clear. The molecular weights of the proteins were estimated from the markers using software Uvitec, Gel Documentation, Model, Doc-008 XD, Version 10, UK.
Results and discussion

The study of sturgeon Igs is important for understanding the phylogeny and ontogeny of higher vertebrate Igs as well as improving the prevention and control of economically important infectious diseases. From the available literature, no studies of the Caspian Sea sturgeon Igs have been carried out to date. The aim of this work was to isolate and characterize these molecules, as a first step towards the production of anti-Ig monoclonal antibodies that could be used to study sturgeon immune responses.

After incubating the fish sera with the agarose beads, the fish anti-goat-IgG immunoglobulins were eluted in two peaks by the glycine elution buffer. The non-reduced affinity-purified serum Igs from all species of fish eluted from the columns in the second peaks and had identical estimated molecular weight of 870 kD by SDS-PAGE analysis (Figure 1). Also, SDS-PAGE analysis from the reduced affinity-purified serum Igs from all species resulted in two distinct bands at approximately 77-84 and 28-30 kDa (Figure 2). However, the presumed light chain of Persian sturgeon IgM showed a slightly higher MW i.e. 29.5 KDa, while those of great sturgeon, Russian sturgeon and ship sturgeon were similar i.e 28.68 KDa. The MW of starred (stellate) sturgeon IgM was estimated to be 29.23 KDa. The presumed heavy chain MW of Persian sturgeon was higher i.e. 84 KDa than other examined species, which were estimated to be 77.8-79.8 KDa.

In the study by Adkison et al. (1996) two types of Igs with MW of 870 and 170 kDa were observed using two methods of gel filtration and ion-exchange chromatography. In this study using the affinity chromatography procedure only one identical band with MW of 870 KDa were observed for all species of the Caspian Sea sturgeons. This difference may be in part due to the method of Ig
purification. However, similar to white sturgeon, the MW of presumably the heavy and light chains of the purified Igs in the Caspian Sea species were estimated at 77-84 and 28-30 kDa, respectively. These results indicate that these species of Caspian Sea sturgeon have a similar phylogeny to the American species in their Igs structures/patterns.

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References


