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Comparative analysis of immunoglobulin molecules from catfish, *Clarias batrachus*, *Clarias gariepinus* and *C. batrachus*♀ x *C. gariepinus* ♂ hybrid

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ABSTRACT

Knowledge on the immunoglobulin molecules of teleosts is important in devising appropriate strategies for diagnosis and control of infectious diseases. In the present investigation, a comparative study of immunoglobulin molecules from *Clarias batrachus*, *Clarias gariepinus* and their F_1 hybrid (*C. batrachus* ♀ x *C. gariepinus* ♂) was undertaken to determine the size variations among these molecules and to assess their antigenic relatedness. The fishes were immunised with bovine serum albumin (BSA) and immunoglobulin molecules were purified from the immune serum of each species by affinity chromatography using BSA-Sepharose 4B. Native PAGE analysis of the purified immunoglobulins exhibited single band corresponding to a molecular weight of 863 kDa for *C. batrachus*, 864 kDa for the hybrid and 860 kDa for *C. gariepinus*, respectively. Two heavy (H) chains of ~66 and ~59 kDa, ~76 and ~66 kDa, ~86 and ~72 kDa, respectively for these species were identified by SDS-PAGE. However, there were two light (L) chain bands for *C. batrachus* and hybrid with molecular weights of ~27 and ~26 kDa, and one for *C. gariepinus* with a molecular weight of ~27 kDa. Western blot analysis using rabbit anti-*C.batrachus* Ig serum was shown to react with immunoglobulins of *C. batrachus* as well as the hybrid indicating their relatedness but not with the immunoglobulin of *C. gariepinus*. The study further indicated that the polyclonal antibody developed for *C. batrachus* can effectively be used to study the immune response in the hybrid catfish but not in *C. gariepinus*.

Keywords: Catfish, *Clarias batrachus*, *Clarias gariepinus*, Hybrid, Immunoglobulin

Introduction

Fish, like higher vertebrates can synthesise immuno globulins in response to antigenic stimulation. Five different types of immunoglobulins namely IgM, IgG, IgD, IgA and IgE are present in mammals with IgG being the major type. Major functional immunoglobulins reported in teleosts are the high molecular weight IgM (Magnadóttir, 2010). IgM is tetrameric in fish unlike mammals where it is pentameric. Other Ig isotypes like IgD, IgT/IgZ have also been described in some fish species (Ye *et al*., 2011). Studies on fish immunoglobulins are important in assessing health status as well as for developing immunological reagents. Immunoglobulins from several fish species have been characterised biochemically (Pilstrom and Bengten, 1996; Magnadóttir, 1998; Dacanay *et al*., 2006; Lim *et al*., 2009) including a number of indigenous species from India (Swain *et al*., 2004; Sood *et al*., 2008; Bag *et al*., 2009; Behera *et al*., 2009).

Intergeneric or interspecific fish hybrids are produced to gain characteristics from both parent species for better growth, production of sterile stock and higher environmental tolerance. Successful hybridisation has been achieved in laboratory conditions in several fish species (Müller *et al*., 2004; Glover *et al*. 2009; Sahin *et al*., 2011). Besides experimental hybridisation, hybrids have also been a success in commercial aquaculture, for *e.g.,* the catfish hybrid (*Clarias macro cepharus* x *C. gariepinus*) is one of the most important cultivable species in Thailand (FAO, 2006). Hybrids have been produced successfully in India between several carp species (Sahoo *et al*., 2012) and catfishes (Sahoo *et al*., 2003).

Catfishes are important cultivable species in India. The native Indian catfish, *Clarias batrachus* though a preferred species, possesses a slow growth rate. The African catfish, *C. gariepinus* grows fast, but has a low consumer preference due to massive appearance and body size. Hence, F_1 hybrids between *C. batrachus* ♀ x *C. gariepinus* ♂ have been produced in laboratory scale at the Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, India with an objective to increase the growth potential in hybrid progenies (Sahoo *et al*., 2003). In the present study, we did a comparative analysis of the immunoglobulin molecules from *C. batrachus*, *C. gariepinus* and their F_1 hybrid (*C. batrachus* ♀ x *C. gariepinus* ♂).

Materials and methods

Purification of immunoglobulins

Immunoglobulins from all three species were purified by affinity chromatography following the protocol of Swain *et al*. (2004) Ten numbers of each fish species (100-125 g) obtained from fish farm of the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, were maintained in wet laboratory in cement cisterns of 20 m^3 capacity with 15 cm soil base. The physico-chemical parameters were maintained at optimum levels and the fishes were fed once daily with a standard pellet feed during the period. The fishes were immunised intraperitoneally with bovine serum albumin (BSA, 200 µg per fish) emulsified with Freund's Complete adjuvant. (FCA). Two booster injections were subsequently administered on 14th and 28th day along with Freund's Incomplete Adjuvant (FIA). The fishes were bled on 14th day after administration of the second booster and the sera separated, pooled and stored at -20^{o}C until further analysis. Immunoglobulins were affinity purified from the respective serum samples using BSA-Sepharose 4B column (Genei, India). The eluted immunoglobulin fractions were concentrated by ultrafiltration (Amicon ultra, 100 kDa cut off, Millipore, USA) and stored at -20^{o}C until use.

Analysis of purified immunoglobulin samples in gel electrophoresis

The purified immunoglobulin samples from all three species were analysed by (i) native gradient PAGE to determine the type and molecular weight of native immunoglobulin molecules and, (ii) SDS-PAGE to determine molecular weights of constituent polypeptide chains.

Native gradient PAGE: All the three purified immunoglobulin samples were run in a native gradient PAGE in 2.8 to 22.5% acrylamide gradient along with molecular weight markers following the procedure of Behera *et al*. (2009). The gel was run in TBE buffer at 200V for approximately 10 h. The gel was stained with Coomassie blue R 250 and photographed after proper destaining. The molecular weight of the immunoglobulin molecules were analysed in comparison to the marker proteins using GS-800 gel scanner and Quantity One software (Bio-Rad, USA).

SDS-PAGE: Three purified immunoglobulin samples were also run in an SDS-PAGE with 12% separating gel following the method of Laemmli (1970). The samples were mixed with equal volume of 2X sample buffer (5% 2-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue and 2% SDS in 0.125 M trisHCl pH 6.8) and heated in boiling water bath for 2 min prior to loading on to the gel. The molecular weight markers (PMWM, Genei, India) were also prepared similarly and loaded onto the gel. The gel was run in Tris-Glycine-SDS buffer at 200V for 45 min approximately till the bromophenol blue dye reached the bottom of the gel. Staining and molecular weight determination of the Ig polypeptide chains were performed as mentioned for native gradient PAGE.

Western blot analysis

Western blot was carried out to check the reactivity of rabbit anti-*C. batrachus* Ig serum with the immunoglobulin molecules of all three species. Rabbit anti-*C.batrachus* Ig serum used in the present study was developed and pre-adsorbed with 10% liver tissue homogenate of *C. batrachus* to increase the specificity. This reacts with the H chains of *C. batrachus* immunoglobulin (Swain *et al*., 2004).

The purified immunoglobulin and serum samples from all three species were run in SDS-PAGE and were transferred to a nitrocellulose paper (NCP) (Towbin *et al.,* 1979) at 100V for 1 h. After transfer, the NCP was incubated overnight at 4^{o}C in 5% skim milk powder (Hi-media, India) for blocking. The NCP was washed three times in TBS-T (0.02M TrisHCl, pH 7.4 with 0.05% Tween 20) and then incubated with rabbit anti-*C. batrachus* Ig serum at 1:1000 dilution for 1 h. The NCP was washed again as before and incubated with 1:5000 dilution of anti-rabbit HRP conjugate (Genei, India). The NCP after washing was developed using substrate BCIP/NBT (Genei, India).

Results and discussion

During purification on chromatographic column, only one peak was observed for each species under study. The purified and concentrated samples were electrophoresed (Fig. 1 and 2). The types and molecular weights of native immunoglobulin molecule as well as those of the constituent polypeptide chains (H and L) were determined in native gradient PAGE and SDS-PAGE, respectively (Table. 1).

All samples showed a single band in native gradient PAGE (Fig. 1) indicating the existence of only one major immunoglobulin type in these species like in other teleosts (Magnadóttir, 2010). The molecular weight of native Ig molecules were similar and comparable to that from other fish species such as southern bluefin tuna, *Thunnus maccoyii* (845 kDa) (Watts *et al*., 2001), Gulf menhaden, *Brevoortia patronus* (850 kDa) (Shelby *et al*., 2002), Atlantic cod, *Gadus morhua* (840 kDa) (Dacanay *et al*., 2006) and kalbasu, *Labeo calbasu* (857 kDa) (Behera *et al*., 2009). Considering the higher molecular weight Ig in all three species, the Ig in these species can

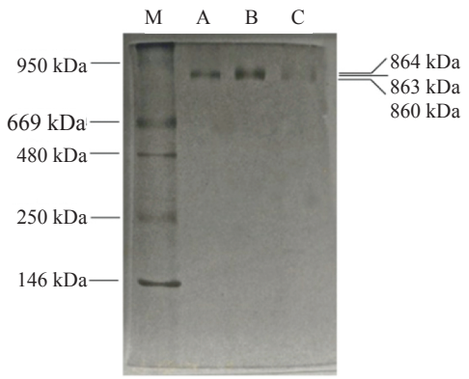

Fig. 1. Native gradient PAGE of purified immunoglobulins on a 2.8 to 22.5% polyacrylamide gradient gel stained with coomassie blue
Lane M : Molecular weight markers, Lane A: *C. batrachus* Ig
Lane B: Hybrid Ig, Lane C: *C. gariepinus* Ig

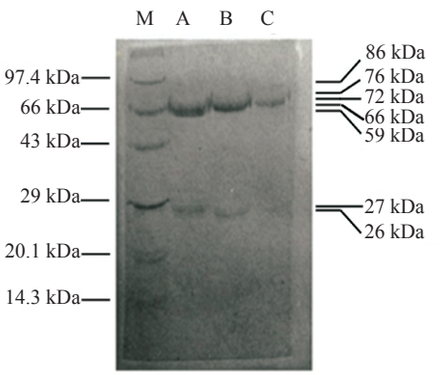

Fig. 2. SDS-PAGE of purified immunoglobulins on a 12% polyacrylamide separating gel stained with coomassie blue
Lane M : Molecular weight markers, Lane A: *C. batrachus* Ig
Lane B: Hybrid Ig, Lane C: *C. gariepinus* Ig

Table 1. The types and molecular weights of native immunoglobulin and constituent H and L polypeptide chains in *C. batrachus*, *C. gariepinus* and its hybrid

Species	Ig types (Mol. wt)	H chain types (Mol. wt)	L chain types (Mol. wt)
C. batrachus	One (~863 kDa)	Two (~66 and ~59 kDa)	Two (~27 and ~26 kDa)
Hybrid	One (~864 kDa)	Two (~76 and ~66 kDa)	Two (~27 and ~26 kDa)
C. gariepinus	One (~860 kDa)	Two (~86 and ~72 kDa)	One (~27 kDa)

be considered as IgM type. The present findings from *C. batrachus* were similar to our previous results (Swain *et al*., 2004). Rathore *et al*. (2006) reported the molecular weight of native Ig from *C. gariepinus* to be 840 kDa. The minor difference in molecular weight of Ig (860 kDa) observed in the present study might be due to the different techniques used for determination of molecular weights; *i.e*., gel filtration chromatography in later. The molecular weights of one H and L chains (72 kDa and 27 kDa, respectively) observed in the present study for *C. gariepinus* Ig were in similar range of 74.8 kDa and 27.2 kDa, respectively as reported by Rathore *et al*. (2006). However, an additional low intense band of an H chain with a molecular weight of 86 kDa was found in this study. At present no information is available pertaining to the Ig molecule from the hybrid catfish (*C. batrachus* ♀ x *C. gariepinus* ♂).

In western blot, the rabbit anti-*C.batrachus* Ig serum reacted with the H chains of *C. batrachus* and the hybrid Ig in both the purified fractions and serum samples (Fig. 3). But it did not react with the Ig from *C. gariepinus*. This indicated that immunoglobulin molecule in hybrid was similar to that of *C. batrachus* (maternal species). A similar maternal effect has been reported by Nynca *et al*. (2010) in electrophoretic profiles of semen proteinase inhibitors in hybrids of pike perch, *Sander lucioperca*♀ and Volga pike perch, *Sander lucioperca*♂. Predomination of maternal effects in fish hybrids have

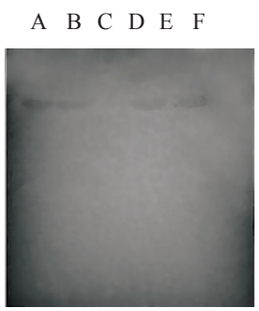

Fig. 3. Reactivity of rabbit anti-*C.batrachus* Ig serum with the immunoglobulins from three species in Western blotting. Samples were run in SDS-PAGE, electroblotted to nitrocellulose paper and the Ig detected with the rabbit antiserum
Lane A : *C. batrachus* Ig, Lane B : Hybrid Ig
Lane C : *C. gariepinus* Ig, Lane D : *C. batrachus* serum
Lane E : Hybrid serum, Lane F : *C. gariepinus* serum

also been identified in morphometric studies (Specziár *et al*., 2009) and biochemical parameters in blood (Di Marco *et al*., 2011).

In the present study, only one IgM type of immunoglobulin molecule with similar molecular weight was found in all the three species. The type of polypeptide subunits and their molecular weights were found to be varying among the three fish species and the Ig molecule in hybrid was similar to Ig from C. *batrachus* (maternal species).

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