Indian Journal of Experimental Biology
Vol. 56, June 2018, pp. 402-410

Isolation and characterization of immunoglobulin from African catfish, *Clarias gariepinus* (Burchell, 1822)

Vipin Kumar Verma[1], Neeta Sehgal[2] & Om Prakash[1]*

[1]Department of Zoology, Sri Venkateswara College, University of Delhi, Dhaula Kuan, New Delhi-110 021, India

[2]Department of Zoology, University of Delhi, Delhi-110 007, India

Received 10 September 2015; revised 09 April 2017

Immunoglobulins are an important aspect of adaptive immune system and tetrameric IgM are the most prevalent immunoglobulins in Pisces.. In this study, we made an attempt to isolate and characterize immunoglobulins from the Afrcian cat fish, *Clarias gariepinus* (Burchell, 1822). The immunoglobulins were induced by immunization with BSA. Various methods such as ammonium sulphate precipitation, ion exchange chromatography (DEAE cellulose), gel permeation chromatography and affinity chromatography (Protein A & CNBr-activated agarose conjugated with BSA) were employed for purification of immunoglobulins. But for affinity chromatography involving BSA conjugated agarose, all other methods could purify immunoglobulins only partially, i.e., there was contamination of other proteins. Whereas with affinity chromatography, immunoglobulins could be isolated in purified form. Electrophoresis under denaturing condition resulted in one heavy and two light chain bands of molecular weights of 74.5 and 29.7 & 30.5 kDa, respectively. It resolved into single band on electrophoresis under native conditions. The molecular weight of immunoglobulin was estimated to be 890 kDa by gel filtration chromatography on Ultrogel AcA34. The immunoglobulin was further characterized by western blotting and MALDI-MS and N-terminal analysis. Rat anti-fish Ig generated against heavy chain showed cross reactivity with fish antibody raised against BSA or *Aeromonas hydrophila*.

Keywords: Affinity chromatography, Gel permeation chromatography, IgM, Ion-exchange chromatography, MALDI, N-terminal sequencing