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PARTIAL PURIFICATION AND IMMUNE-BIOCHEMICAL CHARACTERIZATION OF DOG SERUM IMMUNOGLOBULIN G

Manoj Kumar, Subhasis Batabyal*, Shyamsundar Kesh and Saibal Chattopadhyay

ABSTRACT: In the present study Immunoglobulin G was purified from serum of dog by gel filtration chromatography on Sephacryl S-200. SDS- PAGE analysis of purified dog IgG showed major polypeptides of 66 kDa, 52.40 kDa and 20.72 kDa. The purified Immunoglobulin has been found to be immune-reactive by DID test and Western Blot analysis when treated against hyperimmune sera which was raised in rabbit.

Key words: Immunoglobulin G, Purification, Gel filtration Chromatography, Immuno-reactive.

INTRODUCTION

Antibodies are members of a family of molecules, the immunoglobulins that constitute the humoral branch of the immune system. IgG is the predominant immunoglobulin in the blood and it is initiated in secondary immune response (Talwar and Gupta 1992) and it is the only antibody that can cross placenta.

The dog *(Canis familaris)*, generally considered to be the first domesticated mammal, learned to live with humans more than 14,000 years ago. In this modern era, with the changing scenario the dog has assumed a significant position especially in a nuclear family. It is considered as brother or sister in such family. There are lots of immunological diseases, like immune-mediated hemolytic anaemia, idiopathic thrombocytopenic purpura, chronic inflammatory demyelinating polyneuropathy etc. to affect the health of a dog. Immunoglobulins are required not only for the treatment of these diseases but also for strict disease monitoring system, for which purified IgG may be necessary. Purification of IgG is simple due to its charge properties, high molecular weight and abundance in normal serum. So, the present study has been taken to purify and characterize the immunoglobulin G from serum of dog.

MATERIALS AND METHODS

The blood sample was collected from adult mongrels of either sex. Blood was allowed to clot at 37 º C for 1 hour and then stored at 4 º C overnight to facilitate clot retraction. Following this the serum was drawn off , centrifuged at 3000 rpm for 5 minutes and stored.

Fifty percent ammonium sulphate precipitation was carried out by addition of 3.14 gm of salt (in 10.8 ml. of dog serum).

Department of Veterinary Biochemistry, Faculty of Veterinary and Animal Sciences,
West Bengal University of Animal and Fishery Sciences, Kolkata-700037, West Bengal, India.
**Corresponding Author*

Ammonium sulphate was used due to its high solubility. 50 % saturation was followed as this would give a relatively high yield of IgG (Hudson and Hay 1989). The precipitated sample was centrifuged at 10,000 rpm for 15 minutes. The supernatant was removed and again 50% salt precipitation was carried out by using the PBS (pH 7.2) as the original serum volume. Then the precipitate obtained by centrifugation after 2 hrs was dissolved in PBS, and dialysis was carried out against the several changes of PBS (pH 7.2) for 24 hours at 4ºC.

Protein concentration was estimated for the dialyzed crude IgG of dog using bovine serum albumin (BSA) as standard (Lowry *et al.* 1951). The samples were preserved at - 20 ºC for further use.

Crude IgG was purified by the gel filtration chromatography on Sephacryl S-200 (2.1cm diameter and 43 cm in length) in a buffer containing PBS (pH 7.2), phenyl methyl sulfonyl fluoride (PMSF) (0.03 mM) and 0.02% sodium azide and flow rate was maintained at 35 ml per hour. Fractions of 4 ml each were collected in 50 different sterile test tubes. The distribution of protein was monitored by taking the absorbance at 280 nm in a UV-VIS spectrophotometer (Systronics-119). A graph was plotted by taking fraction numbers in the X axis and absorbance in the Y axis, which revealed a curve with major peak. Then the fractions of the major peak were pooled and concentrated by dialysis against sucrose using dialysis membrane (cut of value 12,000).

The crude and purified samples were analyzed by One-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method descried by Laemmli (1970). After separation, the gel was then stained with Coomassie Brilliant blue R250 staining solution followed by destaining it with destaining solution after 6 hours.

Molecular weights were determined by SDS-PAGE by using protein molunlar weighy marker (Bangalore Genei) containing Phosphorylase (97.4 kDa), bovine serum albumin (66 kDa), Ova albumin (43 kDa), Carbonic anhydrase (29 kDa), Soabean trypsin inhibitor (20.1 kDa) and Lysozyme (14.3 kDa) and analyzed by Gel Documentation System (Bio-Rad).

The antibody against crude dog serum was raised in healthy New Zealand white rabbit. The crude antigen was thoroughly mixed with equal volume of Freund's complete adjuvant (FCA) (1:1) and a total of 1 ml was injected intramuscularly at the thigh muscles. Four booster doses of the same antigen emulsified with Freund's incomplete adjuvant (FIA) (1:1) were given with subsequent increase in amount after 14 days interval following the first injection. Blood was collected from the rabbit 7 days after the last booster dose and serum was separated by centrifugation at 5000 rpm for 10 min. The antiserum was stored at -20^0C for further use.

The double immuno diffusion (DID) test was performed according to the methodology of Hudson and Hay (1989) with some modifications.

Immunochemical analysis of purified protein was done by Western blot technique according to Towbin *et al.* (1979) and Svoboda *et al.* (1985) with some modifications to the labeled antibody and its substrate.

RESULTS AND DISCUSSION

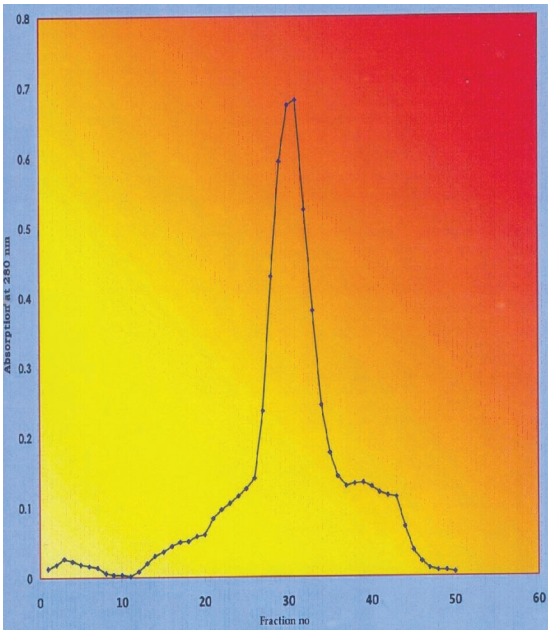
Crude IgG obtained after 50% ammonium sulphate precipitation was dialyzed against PBS. The protein concentration of the crude IgG from dog serum, determined by the method of

Lowry *et al.* (1951) was 38.342 mg/ml.

Purified dog IgG was prepared by gel filtration chromatography on Sephacryl S-200 with the flow rate of 35ml/hr in which the proteins were resolved into one major peak. (Graph No. 1)

The protein concentration of the pooled fractions P_1 and P_2 determined were 2.152 mg/ml and 0.957 mg/ml respectively. The fractions were concentrated by using sucrose over the dialysis bag (cut of value 12,000).

The P_1 fraction of dog was checked for purity by SDS PAGE analysis (10% gel), which showed bands at 66.00 kDa, 52.40 kDa suggestive of heavy chains. Overlapping band at 20.72 kDa suggests the light chains (Fig. 1). The molecular weight of purified IgG determined by SDS PAGE was 159.84 kDa. The present study corresponds almost to the results of Ninfali *et al.* (1994) who obtained two heavy chains of 50 kDa and two light chains of 25 kDa of goat Immunoglobulin purification on phosphocellulose and Dicthyl amino ethyl (DEAE) affi-gel blue chromatography followed by 7.5 % SDS-PAGE. Boden *et al.* (1995) obtained bands at 55 kDa and 25 kDa for heavy and light chains for purification of goat immunoglobulin by immobilized metal ion affinity chromatography. Purified buffalo IgG_1 and IgG_2 were found to be of heavier 58 kDa chains, identified as H chains and lighter 24 kDa chains identified as L chains, observed by Kakker and Goel (1993) which corresponds to some similarity to our study. Ngah *et al.* (1982) found that there were only minor amounts of proteins with molecular weights of 54,000 or 59,000, which is the weight of heavy chains from IgG_1 and IgG_2 using SDS-PAGE. They also stated that the purified preparation did not cross react with monospecific goat IgG antisera.



Graph No. 1 : Purification of Dog crude IgG by Gel filtration chromatography on Sephacryl S- 200.

Hyperimmune serum was raised in rabbit against crude IgG of dog. Single precipitin line was observed in DID test when the partially purified IgG was reacted with the hyperimmune serum. No precipitin line or band was found against the normal control serum (Fig. 2). This corresponds to the bovine IgG_2 infraclass purification that showed single precipitin line (Krishnamohan and Giridhar 1991). Ramesh and Krishnamohan (1992) observed a single precipitin line with DEAE cellulose purified ovine IgG. Rantamaki and Muller (1995) showed precipitin lines in purified IgG desorbed from Streptococcus dysgalactiae against anti-goat whole serum. Cortihier *et al.* (1984) demonstrated a precipitin line for goat and sheep IgG purification on counter immunoelectrophoresis and a single band in

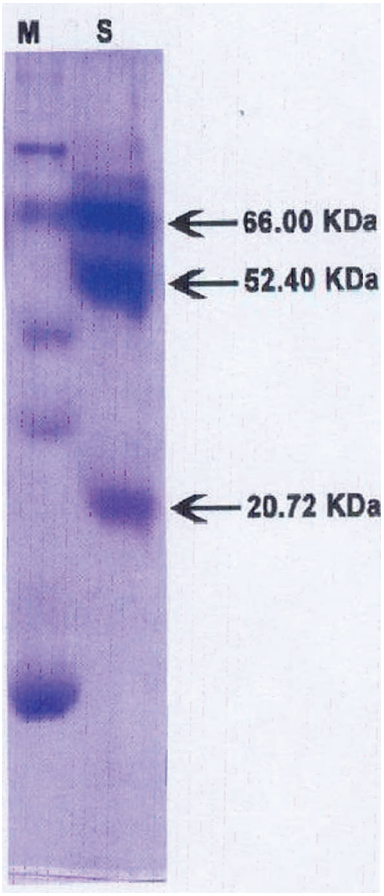


Fig. 1

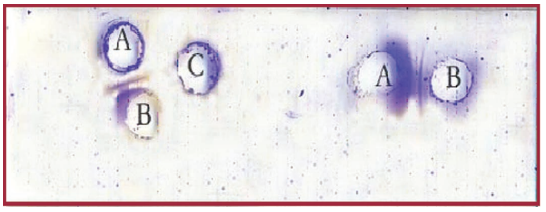


Fig. 2

acetate gel electrophoresis.

The partially purified IgG was found to be immunoreactive against hyperimmune serum by Western Blot technique. Both the heavy and light chain of purified Immunoglobulin G of

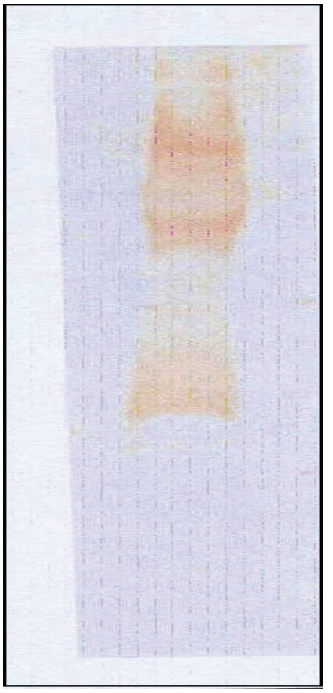


Fig. 3

dog showed imunoreactivity in the nitro cellutose paper, when Immunoblotting was done against the hyperimmune serum which was raised in rabbit against the crude IgG of Dog (Fig. 3). Azwai *et al.* (1993) reported the camel IgG was immunoreactive when it was immunoblotted against the rabbit antiserum and development of this solid phase reactants was visualized with 3,3, diamino-benzene-tetra-hydrochloride. Western blot analysis of bovine IgG and IgM purified from bovine blood serum as well as whole immunoglobulin fractions of bovine and ovine serum with IVA-285 showed a molecular weight in range from 24kDa to 27 kDa, corresponding to the immunoglobulin light chain of bovine Immunoglobulin. This observation by Antalikova *et al.* (2006) corresponds some similarity to our study.

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