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PARTIAL PURIFICATION AND IMMUNE-BIOCHEMICAL CHARACTERIZATION OF ROYAL BENGAL TIGER (*PANTHERA TIGRIS TIGRIS*) SERUM IMMUNOGLOBULIN G

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ABSTRACT: In the present study Immunoglobulin G was purified from serum of Royal Bengal Tiger by gel filtration chromatography on Sephacryl S-200. SDS-PAGE analysis showed the molecular weight of purified tiger IgG was 170.52 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. Immunoglobulin G (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 tiger is the largest member of the cat family. Indian tigers, the Royal Bengal Tigers are the most graceful animal found in the Sunderbans in West Bengal. In India we had 40 thousand tigers at the beginning of the 20th century. Today it is very sad to note that the statistics is much lower. To save the animal from being extinct, we have to pay proper attention. Besides different policies and projects proper conservation of this species is only possible under strict disease monitoring system for which purified IgG may be necessary for enzyme immunoassays. Purification of IgG is simple due to its charge properties, high molecular weight and abundance in normal serum. Now a days, it is of interest to develop good method for purification of Ig Gs from various animals except tiger. So, the present study has been taken to purify and characterize the immunoglobulin G from Serum of tiger.

MATERIALS AND METHODS

The blood sample of Tiger was collected from animals maintained in Alipore Zoological Garden, Kolkata and serum was separated in a sterile vial and stored at -20 ºC.

Fifty percent ammonium sulphate

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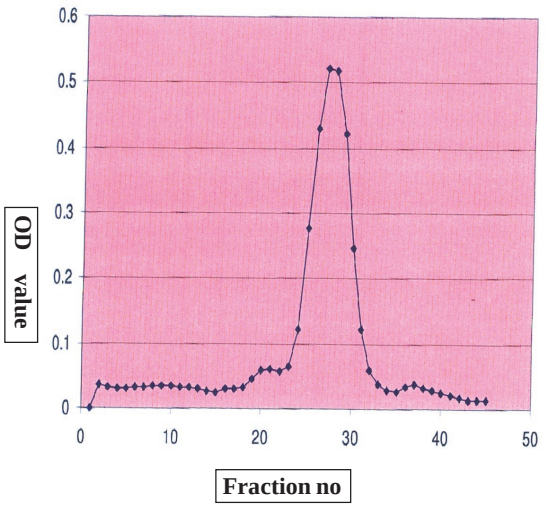
precipitation was carried out by addition of 3.14 gm of ammonium sulphate. Ammonium sulphate was used due to its high solubility, 50% saturation was followed, as this would give a relatively high yield of IgG (Hudson and Hay 1989). The precipitated sample was centrifuged at 10,000 rpm at 4°C (Sigma 3K30) for 15 minutes. The supernatant was removed and 50% salt precipitation was repeated 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 4 ºC.

Protein concentration was estimated for the dialyzed crude Ig G of tiger using bovine serum albumin 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), PMSF (0.03 mM) and 0.02% sodium azide at a flow rate of 20 ml per hour. Fractions of 4 ml each were collected in 38 different sterile test tubes. The distribution of protein was monitored by taking the absorbance at 280 nm in a UV-VIS spectrophotometer (Systronics-119) peak. 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 kDa).

The crude and purified samples were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in vertical slab gel electrophoresis instrument (AE-8450) with Power pack (ATTO



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

Corporation, Japan), method described by Laemmli (1970). After separation, the gel was then stained with Coomassie Brilliant blue R250 staining solution followed by de-staining it with de-staining solution after 6 hours.

Molecular weights were determined by SDS-PAGE by using protein markers (PMW–M, Bangalore Genei) and analyzed by Gel Documentation System (Bio-Rad).

The antibody against crude Tiger serum was raised in healthy New Zealand white rabbit. The crude antigen (500 µl) 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 (100 µl in each dose) 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

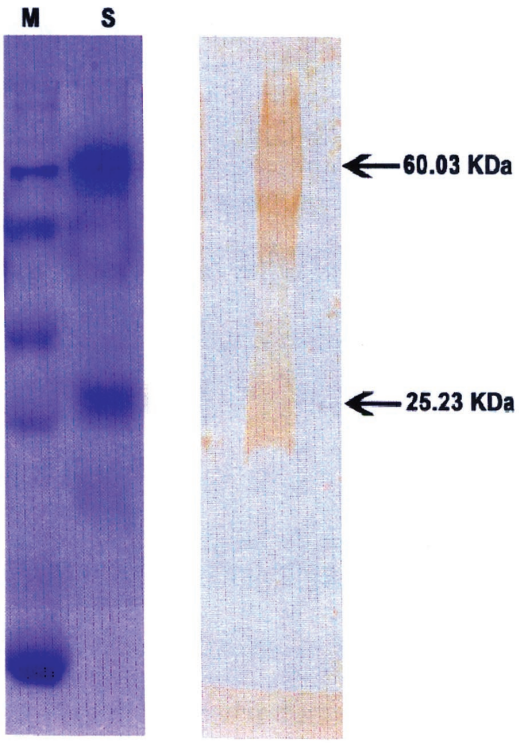


Fig. 1

Fig. 2

centrifugation at 5000 rpm for 10 min at 4°C. The antiserum was stored at –20^{0}C for further use.

The DID test was performed according to the methodology of Hudson and Hay (1989) with some modifications. The slides were then placed in humid chamber (37^{0}C) and incubated overnight. After washing the slide in PBS (pH 7.2), it was stained with Coomassie Brilliant blue R250 staining solution and destained with destaining solution.

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. The proteins were separated by 10% SDS-PAGE and then electroblotted to nitrocellulose membrane (Immobilon-NC).

RESULTS AND DISCUSSION

Crude IgG obtained after 50% ammonium sulphate precipitation was dialyzed against PBS (pH 7.2). The protein concentration of the crude IgG from tiger serum, determined by the method of Lowry *et al*. (1951) was 11.89 mg/ml.

Purified tiger 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 0.44 mg/ml and 0.41 mg/ml respectively. The fractions were concentrated by using sucrose over the dialysis bag (cut of value 12,000 dalton).

The P_1 fraction of tiger was checked for purity by SDS PAGE analysis (10% gel), which showed bands at 60.03 kDa and 25.23 kDa suggestive of heavy chains and light chains respectively (Fig. 1). The molecular weight of purified IgG determined by SDS PAGE was 170.52 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 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.

Hyperimmune serum was raised in rabbit against crude IgG of tiger. 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 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 tiger showed imunoreactivity in the NCP when Immunoblotting was done against the hyperimmune serum which was raised in rabbit against the crude IgG of Tiger (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 24 kDa 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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