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PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF IgG FROM SERUM OF YAK (*BOS GRUNNIENS*)

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ABSTRACT: In the present study Immunoglobulin G was purified from serum of Yak by gel filtration chromatography on Sephacryl S-200. SDS- PAGE of purified yak IgG showed four major polypeptides of 62.94 kDa, 53.29 kDa 29.22 and 28.21 kDa. The purified Immunoglobulin has been found to be immuno-reactive by DID test and Western Blot analysis when treated against hyper-immune sera.

Key Words: Immunoglobulin G, Purification, Gel filtration Chromatography, Immunoreactive.

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 yak (*Poephagus grunniens* or *Bos grunniens*), which provides milk, meat, wool and much needed pack on the precipitous slopes, must be regarded as one of the world's most remarkable domestic animals as it thrives in conditions of extreme harshness and deprivation. According to the 17th livestock census the total yak population in India is about 65,000. The yak population in India has decreased over the years, and the yak is now considered a threatened species (Bandyopadhyay *et al.* 2007). 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. Nowadays, it is of interest to develop good method for purification of this IgGs from various animals except Yak. So, the present study has been taken to purify and characterize the immunoglobulin G from Serum of Yak.

MATERIALS AND METHODS:

The blood sample of yak was collected from animals maintained in National Research Centre on Yak, Dirang of Arunachal Pradesh and serum was separated in a sterile vial.

Fifty percent ammonium sulphate precipitation was carried out by addition of 3.14 gm of salt. 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

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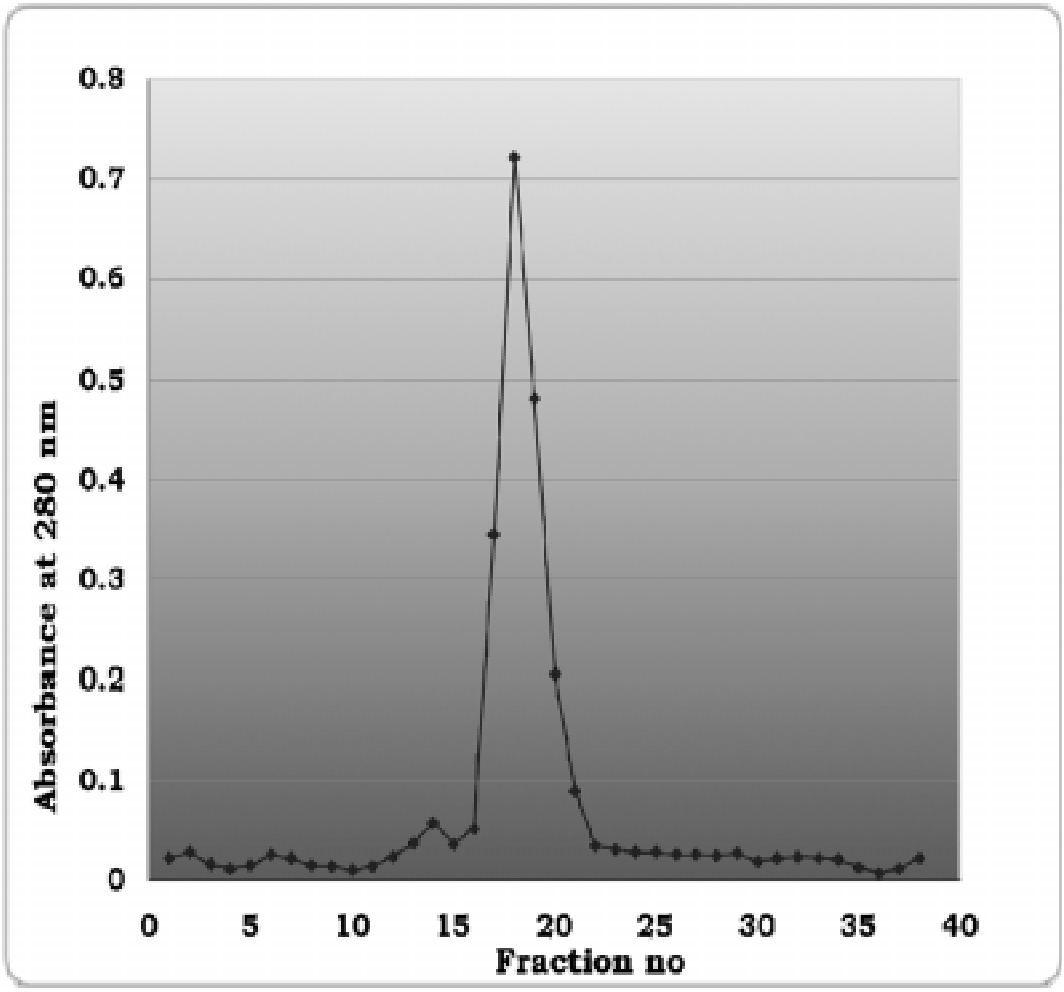

Fig. 1: Purification of Yak (Bos grunniens) crude IgG by Gel filtration chromatography on Sephacryl S- 200

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 IgG of yak by Lowry's method (Lowry *et al.* 1951).

Crude IgG was purified by the gel filtration chromatography on Sephacryl S-200 (2.1cm diameter and 43 cm in length) and flow rate was maintained at 35 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). Then the fractions 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) in vertical

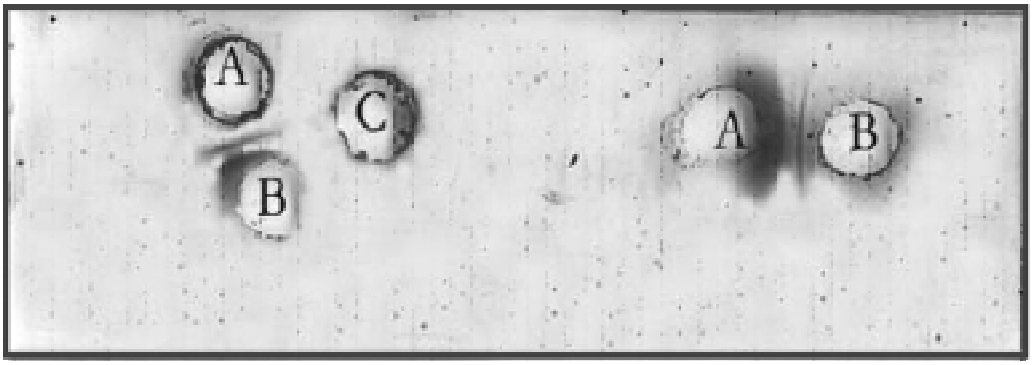

Fig. 2

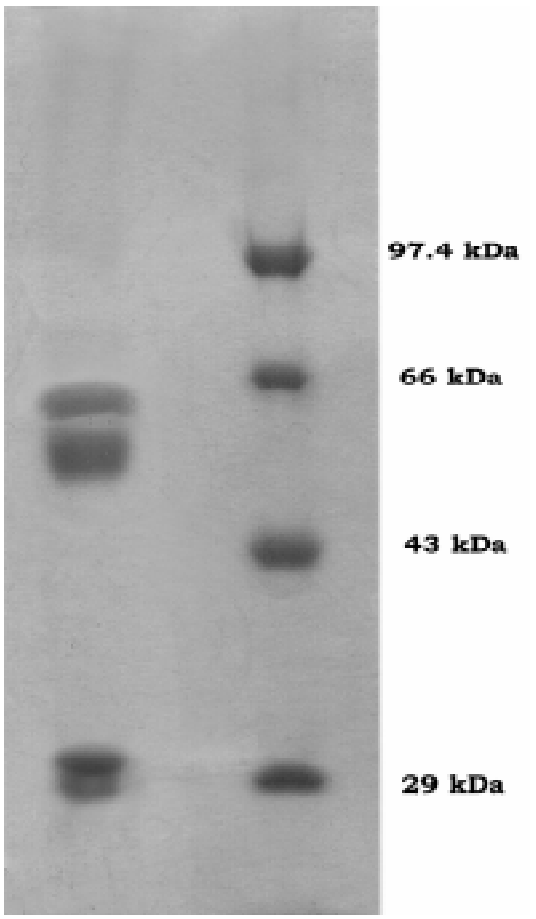

Fig. 1

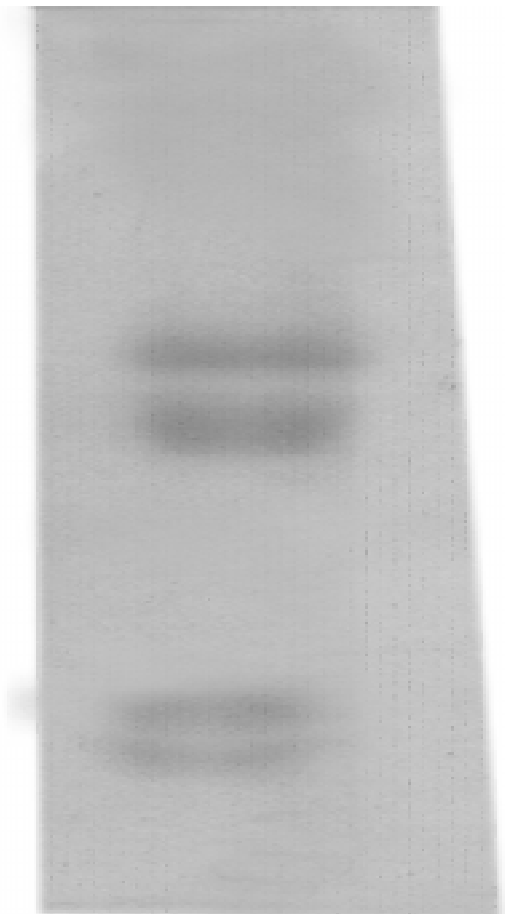

Fig. 3

slab gel electrophoresis instrument method descried by Laemmli (1970). 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 yak serum was raised in healthy New Zealand white rabbit. The DID test was performed according to Hudson and Hay (1989). Immunochemical analysis of purified protein was done by Western blot technique according to Towbin *et al.* (1979).

RESULTS AND DISCUSSION:

Crude IgG obtained after 50% ammonium sulphate precipitation was dialyzed against PBS. The protein concentration of the crude IgG from yak serum, determined by the method of Lowry *et al.* (1951) was 13.65 mg/ml. Purified yak 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. (Fig.1). The protein concentration of the pooled fractions P1 and P2 determined by the method of Lowry *et al.* (1951) were 0.757 mg/ml and 0.426 mg/ml respectively. The fractions were concentrated by using sucrose over the dialysis bag (cut of value 12,000).

The P1 fraction of yak was checked for purity by SDS PAGE analysis (10% gel), which showed bands at 62.94 kDa, 53.29 kDa and 29.22, 28.21 kDa suggestive of heavy chains and light chains respectively (Fig. 1). The molecular weight of purified IgG determined by SDS PAGE was 173.66 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 affigel 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 IgG1 and IgG2 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 IgG1 and IgG2 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 Yak. 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 IgG2 infraclass purification that showed single precipitin line (Krishnamohan & 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 yak showed imunoreactivity in the NCP when Immunoblotting was done against the hyperimmune serum which was raised in rabbit against the crude IgG of Yak (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 Ig. This observation by Antalikova *et al.*

(2006) corresponds some similarity to our study.

CONCLOSION :

Immunoglobulin G of Yak could be purified from serum by gel filtration chromatography and is immuno-reactive.

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