IDENTIFICATION AND CHARACTERIZATION OF GUT ANTIGENIC PROTEIN OF ASCARIS SUUM

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Abstract: Ascaris suum is the most prevalent parasite in domestic swine, severely limiting the animal productivity throughout the world. However, anthelmintic resistance in nematodes population has focused attention on the prospect of developing effective anti-nematode vaccine. The present study was therefore, envisaged to focus on identifying and characterizing some specific antigenic proteins of A. suum responsible for protection. In perspectives, adult worms were procured from naturally infected pigs slaughtered at local abattoirs. After washed with 0.15M physiological saline, gut of the worms was dissected out, homogenized and the extract was prepared. Protein in all the processed extracts was fractionated separately by gel permeation chromatography on Sephadex G-200 and observed spectrophotometrically at 280nm. The presence of antigenic protein in elutes was identified by DID assay upon testing against hyper-immune sera, raised in rabbits. Upon SDS-PAGE, the dialyzed antigenic proteins of gut were resolved into 66 and 120 kDa. While immunoblotting analysis indicated that gut antigenic protein of 66kDa was identified to be the most immuno-dominant and therefore, can be explored for immuno-prophylactic purpose.

Key words: Ascaris suum, Gut antigenic protein

INTRODUCTION

Gastrointestinal helminthic infection is extremely widespread and contributes significantly both morbidity and mortality among livestock in developing countries [1]. One of these helminthes, Ascaris suum, was originally identified as ubiquitous, pathogenic parasite [2] that lives in micro-aerobic environment of pig intestine [3]. This is responsible for significant morbidity among domestic swine [4] because of their longevity and complex developmental stages within their hosts, afflicting thereby, heavy economic losses to the pig industry. At present the control of such parasitic diseases of livestock continues to rely on the traditional concept of clinico-helminthological diagnosis that involves direct visualization of eggs in feces. Moreover, widespread use of anthelmintic drugs concerns with the drug resistant parasites as well as the effect of drug residues on animal health and environment that have also focused attention on the prospect of developing effective anti-nematode vaccines [5-7]. Therefore, studies need to be focused on identifying, characterizing and exploring the nature of the antigenic proteins responsible for protection upon targeting the various developmental stages of the parasite during the course of its life cycle [5]. In perspectives, the present study was undertaken to characterize the antigenic protein in the gut of adult Ascaris suum, since these are known to be
Parasites: Adult Ascaris suum were collected from naturally infected pigs, slaughtered at local abattoirs at Jabalpur, Madhya Pradesh.

Preparation of crude extract of gut protein: The worms were brought alive to the laboratory within a short span of time that were collected from naturally infected pigs, slaughtered at local abattoirs at Jabalpur, Madhya Pradesh. After thorough washing in 0.15M physiological saline, dissected with the help of blunt forceps in a sterile glass petridish kept in a large trey containing ice cubes and recovered gut. Gut, thus dissected out, was washed 3-4 times and homogenized in 0.15M PBS (pH 7.2, 20%w/v) containing 1mm PMSF and 1mm EDTA in a Potter-Elvehjem homogenizer at 4°C. The homogenate was sonicated using Ultrasoniceter at 15 kHz for 10 cycles of 60 sec each with break of 60 sec after each cycle. The sonicated material was centrifuged at 14000 rpm for 20 min at 4°C, as for the method adopted earlier [8]. The supernatant was filtered through 0.22µm filter membrane and stored at -20°C till further use after estimation of protein using diagnostic kit.

Hyperimmune sera: New Zealand white strain rabbits of age 12-18 months old each weighing about 0.5-1.0 kg were allowed to be fed on diet prepared under hygienic conditions and water ad-libitum. The animals were immunized against gut products of the parasite by inoculating extract mixed with Freund's complete/incomplete adjuvant separately at multiple sites and at varying intervals by intramuscular route [9]. On 34th day, the rabbits were bled, serum separated and stored in aliquots at -20°C till further use after estimation of protein using diagnostic kit.

Identification of antigenic protein: Elutes, depicting the presence of protein, were tested for their antigenicity against hyper-immune sera, as obtained from the immunized rabbits. All the fractions depicting the positive results were pooled, subjected to ammonium sulfate precipitation and dialyzed against sucrose and later against PBS (pH 7.2). Finally, the dialyzed fractions were concentrated using polyethylene glycol (PEG) 6000 (Merek, India).

 Immunoblot: The dialyzed antigenic protein was subjected to sodium dodecyl sulphatepolyacrylamide gel electrophoresis [11]. The gel consisted of 4.5% stacking and 12.5% separating gel. Sample was dissolved in a sample buffer (50mM Tris-HCl buffer pH 6.8, containing 1% SDS, 20% glycerol and 0.1% bromophenol). Electrophoresis was carried out at a constant current of 20 mA, until the tracking dye reached the bottom of the gel in Tris-glycine electrode buffer (25 mm Tris, 192 mm glycine, pH 8.3). Molecular weight markers were incorporated to determine the molecular weight of the protein. Gel was stained with Coomassie brilliant blue dye (0.1%). Following the SDS-PAGE, the protein bands in unstained gel were transferred onto nitrocellulose membrane (Hybond C, Amersham, Sweden) in semi dry blotting apparatus (ATTO, Japan). The transblot was carried out at a constant current of 0.8 to 2.0 mA/cm² for 90 min. The membrane after transfer was incubated overnight at 4°C in blocking buffer. After blocking, the membrane was washed thrice with PBS-T and incubated with 1:400 diluted rabbit anti protein serum at 37°C for 1hr. Thereafter, the membrane was washed thrice with PBS-T as described above and incubated for 1 hr at 37°C with 1:2500 goat anti rabbit HRPO conjugate (Sigma, USA). After washing thrice with PBS-T, the antigen-antibody reaction was detected by incubating the membrane with substrate. The
color of the reaction was terminated by exhaustive washing with distilled water.

RESULTS AND DISCUSSION

In the present investigation, attention was also focused on exploring the possibility of gut somatic antigens for prophylactic purpose, since many hidden antigens are believed to reside in it [12]. Protein content of gut products was maximal and ranged from 3.0-4.0mg/ml as have also been reported earlier by many workers [13]. Chromatographic separation of proteins in crude gut extract, as resolved on sephadex G-200 indicated the presence of two clear and distinct peaks (Fig 3). The recovery rate of protein was more than 75% and was distributed in the ratio of 1:9 between both the peaks that revealed the presence of high molecular weight antigenic protein in gut which was correlated well with the observation on ES protein of Tricheneilla spiralis [14]. A clear precipitin line was observed upon DID assay in elute number from 16 to 21 (Fig 2). Upon SDS-polyamidamide gel electrophoresis, the dialyzed antigenic protein of gut of A. suum was resolved into two bands of 66 and 120kDa (Fig 4). The band width of 66kDa was thicker than that of 120kDa (Fig 5). Amongst all the bands, the band width of the gut antigenic protein at 66kDa was most thick (Fig 5).

CONCLUSION

The present investigation generates a precise knowledge on the antigenic proteins associated with gut of adult Ascaris suum with an aim to explore them as potential vaccine candidates against ascariasis in swine. SDS- electrophoratogram identified two antigenic proteins from gut of molecular weight of 120 & 66 kDa upon purification by gel permeation chromatography on Sephadex G-200. While immunoblotting analysis indicated gut antigenic protein of 66 kDa to be immunodominant that can be explored as vaccine candidates upon assessing their chemical and functional nature.

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REFERENCES


