Purification and Immunobiochemical Characterization of Sheep Hydatid Cyst Fluid Antigen

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ABSTRACT

In the present study, sheep crude hydatid cyst fluid antigen (SCHCFA) was prepared by ammonium sulfate precipitation, centrifugation and dialysis. SDS-PAGE of SCHCFA showed seven polypeptides of 72.8 kDa, 66.5 kDa, 60.2 kDa, 41 kDa, 25.1 kDa, 19 kDa and 8 kDa when stained with Coomassie brilliant Blue R-250. The initial part of the ascending loop of the first peak (PI) when resolved by gel filtration chromatography on Sephacryl S 200 depicted the well defined polypeptide of molecular weight of 66.5 kDa and 60.2 kDa showed on 12.5% SDS-PAGE. Double immunodiffusion test, indirect ELISA and western blot analysis demonstrated that the 66.5 kDa and 60.2 kDa polypeptides were immunoreactive when treated against hyperimmune sera and known positive sera. It was concluded that these polypeptides (purified sheep hydatid cyst fluid antigen) might prove to be a promising tool for the diagnosis of hydatid disease.

Keywords: SCHCFA, purification, immunobiochemical characterization, SDS-PAGE, ELISA, immunoreactive

INTRODUCTION

Hydatidosis is a silent, cyclozoontic and oldest infection of man and domestic animals and is caused by larvae of the cestode, Echinococcus [1]. The infected human beings and animals loose their normal functional capacities which lead to huge economic losses. The diagnosis of hydatid disease is based on clinical, radiological, microscopical and immunological methods of which the last one is most sensitive. The crude or fractionated sterile hydatid cyst fluid obtained from sheep may be used as antigen in a variety of immunoassays. For improving the efficacy of immunodiagnosis of hydatid disease in sheep the purification and characterization of hydatid cyst fluid is essential. Moreover researches so far were performed mostly on diagnosis of Echinococcus granulosus in human beings only; relatively less research work has been performed for the early diagnosis of hydatidosis in sheep. In this background the present study is carried out with the objectives of purification and Immunobiochemical characterization of hydatid cyst fluid antigen of sheep.

MATERIALS AND METHODS

Collection of sera and preparation of crude sheep hydatid cyst fluid antigen

During the study sera of five positive cases of sheep with presence of visible cysts in their visceral organs and five negative cases of sheep with the absence of visible cyst in their visceral organs were collected and preserved at -20°C. SCHCFA (Crude Sheep Hydatid Cyst Fluid Antigen) were
prepared from fertile hydatid cysts by centrifugation and dialysis. PMSF (0.03mM) was added. The globulinic antigen of the hydatid fluid was obtained through precipitation with ammonium sulfate at half saturation. The protein concentrations of SCHCFA were determined by the Lowry method [2]. The antigens thus prepared were stored at -20°C.

**Preparation of purified PSHCFA by gel filtration chromatography**

A sample of SCHCFA was purified by gel filtration chromatography in a column on Sephacryl S 200 (1.5 cm diameter and 60 cm in length) in a buffer containing PBS (pH 7.2), PMSF (0.03mM) and 0.02% sodium azide at a flow rate of 20 ml per hour. The elutes were collected in 65 fractions of 3 ml each. The distribution of protein was monitored by taking the absorbance at 280 nm in a UV/VIS spectrophotometer (Systronics - 119).

**Immunobiochemical characterization of PSHCFA**

**Analytical sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)**

The SCHCFA and PSHCFA were analyzed by SDS-PAGE (12.5%) according to Laemmli method [3], with some modification. Vertical mini slab gel electrophoresis system (Bangalore Genei) was used. The gel was then stained with monochromatic silver stain.

**Determination of molecular weight by SDS-PAGE**

Molecular weights were determined by standard protein markers (PMW-M, Bangalore Genei). Phosphorylase (97.4 kDa), BSA (66 kDa), Ova albumin (43 kDa), Carbonic anhydrase (29 kDa), Soyabean trypsin inhibitor (20.1 kDa) and Lysozyme (14.3 kDa) were electrophoresed with SCHCFA and PSHCFA.

**Indirect ELISA**

PSHCFA was used as coating antigen and kept for overnight at 4°C. Blocking buffer (2% BSA in PBS) was added and kept for 2 hours followed by washing with PBS-T (0.05% Tween 20 in PBS). After that serial dilution of hyperimmune and normal rabbit sera (1:100, 1:200 and 1:300) were added accordingly and kept for 2 hours followed by washing. Conjugate solution containing anti-rabbit horse radish peroxidase conjugate (sigma) was added and kept for 2 hours. After washing with PBS-T, Substrate buffer (3 µl H₂O₂, 0.025 g of O-Phenylene diamine in 25 ml citrate buffer) was added and kept for 30 min in dark. After the development of colour 2N H₂SO₄ solution was added and the reading was taken in an ELISA reader at 492 nm (Multiskan, Labsystem, Model - 355).

**Western Blot**

The PSHCFA was characterized by western blotting technique according to method of Towbin [4] with some modifications. The antigen was separated by SDS-PAGE and then the resultant proteins were electroblotted to nitrocellulose filter paper from gel to a mini western blot apparatus (Bangalore Genei).

**Immonochemical analysis**

The antibody against PSHCFA was raised in healthy New Zealand white rabbits. The antigen was mixed with equal volume of Freund's complete adjuvant (FCA) and freund's incomplete adjuvant.
(FIA). Sera were collected from these rabbits 7 days after the last booster dose and were stored at -20°C. The Double immunodiffusion (DID) test was performed to detect the specific antibody raised against purified SHCFA.

RESULTS AND DISCUSSION

Protein fractions of SCHCFA showed mainly 2 peaks and were pooled together into 5 parts and named as PI (fractions of initial part of ascending loop of first peak), P2 (fractions of rest part of ascending loop first peak), P3 (fractions of descending loop of first peak), P4 (fractions between first and second peak) and P5 (fractions of second peak), as shown in figure 1. The 5-pooled fractions were concentrated and preserved at -20°C. 12.5% SDS-PAGE of the fraction PI showed two polypeptide of SCHCFA and this was considered as purified SHCFA.

![Figure 1. Purification of crude sheep hydatid cyst fluid antigen by gel filtration chromatography on Sephacryl S 200.](image)

The protein concentration of SCHCFA was 3.1 mg/ml. The protein concentration of the part PI was 2.9 mg/ml. The resolution of SCHCFA in 12.5% SDS-PAGE revealed polypeptides of 72.8 kDa, 66.5 kDa, 60.2 kDa, 41 kDa, 25.1 kDa, 19 kDa, and 8 kDa (Figure 2). The result was fairly similar to the findings of Jiang and his co-workers [5]. The bands of 66.5 kDa, 41 kDa and 19 kDa of the present study were almost similar to 66, 41 and 19 kDa bands as mentioned by Jiang and his co-workers [5] SCHCFA when resolved by gel filtration chromatography showed two polypeptide bands of 66.5 kDa and 60.2 kDa in 12.5% SDS-PAGE (Figure 2) and it was found to be immunoreactive when analyzed by western blot. The result was found to be similar to the findings of Felice and his co-workers [6]. Single precipitin line was observed in double immunodiffusion test when the PSHCFA was treated with the hyperimmune serum. No precipitin line or band was found against the normal control sera.
Table 1. Sero reactivity expressed as mean OD along with standard error values of PSHCFA against normal and Hyper immune Sera as assessed by indirect ELISA.

<table>
<thead>
<tr>
<th>Sera dilution (1:100)</th>
<th>Sera dilution (1:200)</th>
<th>Sera dilution (1:300)</th>
</tr>
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<tbody>
<tr>
<td>HS</td>
<td>0.66 ±0.01</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>NS</td>
<td>0.24±0.01</td>
<td>0.51±0.01</td>
</tr>
<tr>
<td>HS</td>
<td>0.24±0.01</td>
<td>0.43±0.01</td>
</tr>
<tr>
<td>NS</td>
<td>0.24±0.01</td>
<td>0.24±0.01</td>
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</table>

Values are expressed in mean of three observations ± standard error; HS: Hyper immune sera; NS: Normal sera.

Indirect ELISA using hyperimmune and normal rabbit sera at different dilutions assessed the seroreactivity, expressed as mean OD of SCHCFA. The mean OD along with their standard error values has been expressed in table 1. The mean OD value ranged from 0.66 ± 0.01 to 0.43 ± 0.008 for 1:100 to 1:300 hyperimmune sera dilution whereas the mean OD value ranged from 0.24 ± 0.01 for the same range of normal sera dilution. The present study of isolation, purification and characterization of hydatid cyst fluid antigen of sheep showed that the SCHCFA as 66.5 kDa and 60.2 kDa polypeptides are immunoreactive which may be used for immunodiagnosis of echinococcosis in sheep.

REFERENCES