

Anion Exchange Chromatography for Purification of Antigen B of Cystic Echinococcosis

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Abstract

Cystic echinococcosis or hydatidosis causes severe economic loss and public health problem to both human beings and livestock in many temperate and tropical areas of the world. Immuno diagnosis has been found to be useful not only in primary diagnosis but also for follow up of patients after surgical or pharmacological treatment in man. Various immune diagnostic tests for hydatidosis in man and animals have been attempted using hydatid cyst fluid antigens with varied sensitivity and specificity. However these assays using crude hydatid antigens have been non-specific due to cross reaction with *Cysticercus*, *Coenurus* and other helminthic infections. In order to overcome these difficulties various novel tests using purified antigens are essential for confirmative diagnosis of hydatidosis in man and animals. One of the subunit of antigen B, 8 kDa protein has been shown to be hydatid specific. Various types of chromatography have been used for purification of Hydatid antigens. However, Anion exchange chromatography had been used in this study to get more antigen B 8 kDa than other chromatographic methods.

Keywords: Hydatidosis; Antigen B; Anion exchange chromatography

Introduction

Cystic Echinococcosis

Cystic echinococcosis (CE) or hydatidosis is a chronic zoonotic parasitic infection in man and animals, with a cosmopolitan distribution, caused by the dog tapeworm *Echinococcus granulosus*. People become infected when they ingest eggs passed from tapeworms in dogs. This disease is of utmost public health problem in human beings and requires expensive and prolonged medical treatment with surgical intervention. The global annual monetary loss due to hydatidosis in man accounts for US\$ 193,529,740. The most tangible economic effects in animals of this are the loss of offal from food animals. The body weight of infected animal will be 1 per cent less than uninfected animals. The global annual livestock production loss due to CE is estimated to be US\$ 141,605,195 [1].

Diagnosis of CE can provide substantial improvements in the quality of the management and treatment of disease. In livestock, infection with hydatid cyst is asymptomatic and diagnosis is made usually at necropsy. Immunodiagnosis has been found to be useful not only in primary diagnosis but also for follow up of patients after surgical or pharmacological treatment in man. However assays using crude hydatid antigens have been non-specific due to cross reaction with *Cysticercus*, *Coenurus* and other helminthic infections. In order to overcome these difficulties various novel tests using purified antigens are essential for confirmative diagnosis of hydatidosis in man and animals [2].

Purification of hydatid antigens by chromatography

Immunodiagnostic tests using crude hydatid cyst antigen are far from satisfactory. Thus, purification of hydatid antigens is mandatory to remove host components and other cross reactive proteins. Various types of chromatography have been used for purification of Hydatid antigens.

Sephadex G 200 gel filtration chromatography had been commonly used to purify the antigens of sheep hydatid fluid. Sephadex G-200 chromatography (15 × 50 cm column) used for purification of hydatid

cyst fluid and membrane antigen. The elution profile of hydatid fluid revealed two peaks, while 3 distinct peaks were observed in case of hydatid membrane [3]. However Sephadex G-200 chromatography of sheep hydatid fluid gave three main peaks [4]. Six major peaks were got in elution profile of protoscolices aqueous soluble proteins on Sephadex G-200 column of 2 × 90 cm size [5].

A simple two step procedure involving Sephadex G 200, Sepharose 4B gel filtration chromatography was used to purify the lipoprotein antigens of sheep hydatid fluid [6]. Elution profiles showed two larger peaks designated as antigen A and B and two smaller peaks. Ovine liver *Echinococcus granulosus* hydatid cyst fluid was purified 24 and 30 fold by Sephadex G-200 and DEAE(Di- Ethyl Amino- Ethyl) - Sephadex chromatography in 25 and 31 cm column [7]. One major and 2 minor peaks were obtained in the sephadex G-200 column chromatography. Buffalo hydatid cyst fluid was fractionated in nine peaks by molecular exclusion chromatography using Sephadex G-150, 90 × 1.5 cm column. The third peak alone reacted with treated and untreated anti buffalo hydatid cyst fluid sera suggesting parasite derived antigen [8].

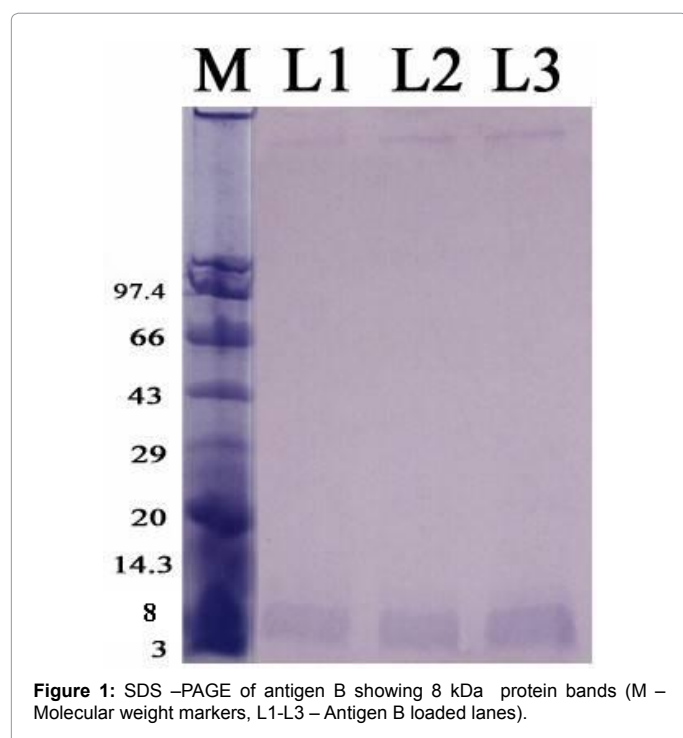
Affinity chromatographic purification may be a useful first step in production of species specific immuno diagnostic antigens for hydatid infections. Affinity chromatography of crude hydatid cyst fluid using CNBr (Cyanogen Bromide) activated sepharose 4B column was carried out by various workers [9]. Con (Concavalin) -A sepharose column was also used for preparation of fraction rich in glycoprotein from crude hydatid cyst fluid [10].

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Anion exchange chromatography

Anion exchange chromatography had been used to get more antigen B than other chromatographic methods [11].

Experimental

Collection of hydatid cysts

The hydatid cysts were collected from sheep slaughtered at Corporation Slaughter House in Perambur and Department of Meat Science and Technology, Madras Veterinary College, Chennai, India.

Anion exchange Chromatography

The immunodominant antigen B was prepared from hydatid cyst fluid by Anion exchange chromatography using DEAE- sepharose fast flow (Sigma, USA) by following the standard protocols [12,13].

Reagents

Application buffer pH 7.4.

20 mM sodium phosphate 1.2 g
200 mM sodium chloride 5.84 gm
TGDW (Triple glass distilled water) – 500 ml

Elution buffer pH 7.4.

20 mM sodium phosphate 1.2 g
500 mM sodium chloride 14.6 g
TGDW 500 ml

Conductivity buffer pH 7.4.

20 mM sodium phosphate 1.2 g
2 M sodium chloride 58.0 g
TGDW 500 ml

Preparation of hydatid fluid antigen

The hydatid cysts were thoroughly washed in distilled water to remove the adhering dirt and clotted blood. The fluid was aspirated slowly using 20 ml syringe. The aspirated fluid was pooled together and kept in a glass beaker for settling of brood capsules, protoscolices and dead tissues. The supernatant was collected and clarified by centrifugation at 10,000 rpm for 30 minutes to remove other sediments. The conductivity of hydatid fluid was adjusted with conductivity buffer as equal to that of application buffer. The hydatid cyst fluid was loaded bit by bit into the column.

Preparation of Anion exchange column

DEAE sepharose fast flow (Sigma, USA) was slowly packed to a 5 × 2.5 cm size column (Bio-rad, USA). The column was equilibrated with application buffer. Typically 1.5 liters of the hydatid cyst fluid supernatant were loaded in the column. The flow rate was adjusted to 3 ml / minute and the chromatography was undertaken at 4°C. The column was washed with 5 column volumes of application buffer. The bound antigen fractions were eluted with elution buffer. The antigen B was eluted in 7, 8, 9 and 10th fractions. These fractions were pooled together and concentrated using PEG 6000. The protein concentration of antigen B was estimated by BCA method [14]. The protein content was 0.987 mg / ml. SDS-PAGE analysis of DEAE Sepharose fast flow anion exchange chromatography fractions revealed the antigen B protein bands at 8 kDa (Figure 1).

Conclusions

Hydatid cyst fluid (HCF) is a complex mixture of glycol lipoproteins, carbohydrates and salts. Crude HCF has a high sensitivity, ranging typically from 75 per cent to 95 percent. However, its specificity is often unsatisfactory and cross-reactivity with sera from patients infected with other cestode (89 per cent), nematode (39 percent) and trematode (30 per cent) species is commonly observed. Hence, the crude HCF is specifically recommended for mass serological screening and it has now become more frequent to purify components such as the lipoproteins antigen B and antigen 5, the most relevant components of HCF for diagnostic purposes. Antigen B 8 kDa may have the opportunity to accumulate in the cyst fluid after being secreted by the parasite in such a way that the protein has the chance to aggregate into a form that is more immunogenic before the antigen gains contact with the host immune system [15]. Various authors have used different chromatographic protocols to isolate antigen B from hydatid cyst fluid, [16], however, the quantity of antigen available from the above methods was scanty. Therefore the Anion exchange chromatography using DEAE Sepharose fast flow was followed and it resulted in production of a large quantity of antigen B. The 8 kDa fraction can be separated from antigen B by SDS-PAGE method.

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