Protein Profile of Fasciola gigantica Antigens

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منسج البحث

استخدمت بروتينات جسد المرونة المعالكة (Fasciola gigantica) ومنتجاتها الإخراجية والإقراضية (SO) ومنتجاتها الإخراجية والإقراضية (SO) بعد حصانتها في وسط غذائي (RPMI-1640). حللت هذه البروتينات بواسطة تقنية الرحلان الكهربائي لفصل مكوناتها المختلفة حسب أوزانها الجزيئية وذلك لإجراء مزيد من الدراسة لمعرفة البروتينات ذات التفاعل المناعي والتي يمكن أن تستخدم في التشخيص المبكر لحدث الإصابة بهذه الديدان وإيجاد لقاح ضدها.

أظهرت تقنية الرحلان الكهربائي أن جسد الطفيلي ومنتجاته الإخراجية والإقراضية تحتويان على مجموعة من البروتينات ذات الأوزان الجزيئية المختلفة، ووجد أن عدد البروتينات المستخدمة من منتجات جسد الطفيلي الإخراجية والإقراضية أقل من تلك المستخدمة من جسد. تجنبت الخصائص البروتينية الأكثر وجوداً والمستخدمة من منتجات الطفيلي في الأوزان الجزيئية 11، 27 و 30 كيلو دالتنون، وجهاز الخصائص البروتينية المهمة ذات الأوزان 44، 26، 11 و 7 كيلو دالتنون في منتجات الطفيلي.

فازيليا gigantica proteins were extracted from its somatic (SO) and excretory–secretary (E/S) products and analyzed for studying their protein structures that could further be used for detection of its immunoreactive proteins. These proteins can be used for early diagnosis of the disease and development of a protective vaccine. Separation of both parasite products by SDS-PAGE resulted in protein bands of different molecular weights. The excretory/secretory products (E/S) contained fewer bands compared to the somatic extracts (SO). Dominant bands for both SO and E/S products were clustered between 11, 27 and 30 Kda. Major bands of 44, 55 and 66 Kda were observed in E/S products.

The protein bands clustered between 11, 27 and 30 Kda contain the major enzymes that could play important biological and immune-modulatory functions in juvenile and adult parasites such as saponin-like
protein family (FSAP-2), cysteine proteinases, glutathione-S-transferase and haemoglobinase. These proteins can be considered as possible vaccine candidates for the disease control.

**Introduction**

Fasciolosis is cosmopolitan in distribution and occurs widely in cattle and sheep-raising areas. Two species of Fasciola: *Fasciola hepatica* and *F. gigantica* are generally recognized to cause the disease in domestic animals and man (WHO, 1995).

In the Sudan and other tropical countries, fasciolosis is caused by *F. gigantica*. The disease is enzootic in certain parts of the country, particularly The White Nile State. The highest risk of transmission rate occurs between May and December with a peak ova excretion between August and September; a period coincident with the rainy season (Goreish, 2000). The wet conditions are conducive for the development and hatchability of *Fasciola gigantica* ova and hence infection of the snail intermediate host, *Lymnaea natalensis*.

Diagnosis of fasciolosis is usually confirmed during the chronic phase of the disease when worms are established in the bile ducts and started to produce eggs. Fortunately, a number of serological tests such as ELISA (Burden and Hammet, 1978.) and Western blot (Duffus and Franks, 1981) are useful in detection of infection particularly at the prepatent period. However, these tests need selection of powerful antigens that recognize the infection during the early stage of its development. For this reason, careful analysis of the parasite antigenic structure is highly required for accurate early diagnosis.

Studies have already been carried out on the antigenic composition of *F. hepatica*. Excretory and secretory products that are produced by specialized excretory and secretory organs of the parasite and are commonly used to detect fasciolosis (Sinclair and Wassal, 1986; 1988; Bautista *et al.*, 1989). They are useful in the early diagnosis of fasciolosis, as they can detect infection before appearance of clinical signs and excretion of ova in faeces (Cupperlovic and Movsasijan, 1972; Hillyer *et al.*, 1985). Furthermore, enzymes present in the E/S products of *Fasciola* spp. have been shown to play a vital role in their survival (Chapman and Mitchell, 1982; Smith *et al.*, 1993). However, meagre information is currently available on *F. gigantica* antigens composition.

This study was designed to analyze the E/S and somatic protein profiles of *F. gigantica* using SDS-PAGE electrophoresis. The resultants
would be used for further evaluation of their antigenicity for diagnostic purposes and attempts for developing a protective vaccine.

**Materials and Methods**

**Collection of parasites:**
Adult *F. gigantica* worms were collected from the bile ducts of infected bovine livers in the White Nile State, Sudan. They were washed 6 times in 0.01 M phosphate buffered saline solution (PBS; pH 7.2). The flukes were then used to prepare the parasite products.

**Preparations of parasite products:**

**Excretory/Secretory products (E/S):**
Collected parasites were washed six times with RPMI-1640 medium. The worms were then incubated with RPMI-1640 medium in addition to 2% glucose, 30 mM Hepes and 25 mg gentamycin per/ml of medium (one worm per 2.5 ml) at 37°C. Following the incubation period, the culture medium was removed, centrifuged at 14900 gpm for 30 min. and the supernatant was estimated for protein concentration as described by Warburg and Christian (1941). The antigen was then aliquoted and stored at -20°C until used.

**Somatic products (SO):**
After collection of E/S antigens, the worms were separated and ground in dry ice using a mortar and pestle. Approximately four volumes of PBS were added and the ground material was left with occasional shaking to stand for 30 min. on ice. The soluble somatic extract was collected by centrifuging the homogenate at 5000 g at 4°C. After dialysis, the homogenate was centrifuged again for 500 gpm at 4°C for 15 min. Protein concentration was determined by the method of Warburg and Christian (1941) and the antigen was aliquoted and stored at -20°C until used.

**F. hepatica antigens:**
Somatic extracts and purified cysteine L-proteinase from British *F. hepatica* isolates were kindly supplied by Dr. Diana Williams, Department of Veterinary Parasitology, Liverpool School of Tropical Medicine, University of Liverpool, UK.

**Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE):**
Fractionation of E/S and SO products was accomplished on 10% acrylamide gel slabs containing SDS (Laemmli, 1970). The two antigen preparations were diluted with equal volume of sample buffer (2% SDS
and 0.1M dithiotreitol, Sigma Chemical Co.) in trisbuffer (pH 6.8) and boiled for 3 min. Fifteen mgs of each E/S and SO protein products were each loaded into lane of separate gel. A sample from *F. hepatica* was loaded in a separate lane of each gel. Purified cysteine L-protease was also loaded in E/S gel. Molecular weight standard markers (BrioRad, Hemel Hempstead, and Hertfortshire) was run in one of the outside lanes of the gel. In about 60 mins, samples were subjected to electrophoresis at 25 mA per gel; 50 mA until the lowest molecular weight standard band had migrated to approximately 1 cm from the bottom of the gel; The gels were then stained with Coomasie Blue (Sigma, Chemical Co.,) to visualize the different bands, and destained several times with destaining solution to wash the excessive stain and to clarify the bands.

**Results**

The protein profiles of E/S and SO from *F. gigantica* Figures 1, 2, 3 and 4 under reducing conditions in 10% SDS-PAGE together with a purified cysteine protease. Several bands were identified in both extracts but the E/S extract had fewer bands compared with the SO extract. Dominant bands for both E/S and SO clustered between 27 and 30 Kda with the same position of the purified cysteine L-proteases (Fig 1). Major bands were also observed in E/S extracts at 40, 55 and 66 Kda; there were at least 8 polypeptides ranging between 27 and 66 Kda identified in E/S products. Somatic extract from British *F. hepatica* (Fig. 3, lane 1; Fig. 4, lane 8) which was run in the same gel with Sudanese *F. gigantica* gave almost the same banding pattern. Proteins of 17, 21, 37, 38, 43, 50, 62, 70, 72 and 110 Kda were only seen in E/S product. Also proteins between 17 and 21 Kda were identified in SO preparation of the parasites.

**Discussion**

The protein composition of E/S and SO products of *F. gigantica* isolates were investigated by SDS-PAGE. Separation by SDS-PAGE allowed the determination of the relative molecular weight and demonstrated the abundance of the protein components present in these parasite preparations. The E/S products of the adult parasite contains proteins bands fewer than those of SO products. Electrophoresis patterns showed some similarities and some differences between the two-parasite products. A complex range of bands was identified in SO products between 40 and 90 Kda. A group of proteins clustered between 27 and 30 Kda, was common to E/S and SO preparations. Proteins within these
molecular weight ranges were also identified in both parasite preparations by Ajanusi et al (1993).

Fig. 1: Protein profile of S/E extract of *F. gigantica* from different bovine livers from The White Nile State, Sudan (lane 2-8) together with Purified Cysteine L-Proteinase (lane 1); M= Molecular Marker.

Fig. 2: Protein Profile S/E extract of *F. gigantica* from different bovine livers from The White Nile State, Sudan (lane 9-17), M= Molecular marker.
Fig. 3: Protein Profile of somatic extract of *F. gigantica* from different bovine livers from The White Nile State, Sudan (lane 2-7) and *F. hepatica* (lane 1); Molecular marker (lane M).

Fig. 4: Protein Profile of somatic extract (SO) of British *F. hepatica* isolate (Lane 8) and 8 isolates of *F. gigantica* (lane 9-16); Molecular marker (lane M).

A 28 Kda protein present in E/S products of adult parasites was also identified by Ruiz-Navarrete *et al* (1993). A protein of 27.5-29 Kda is identified in the E/S products of *F. gigantica* in the present study. This protein corresponds to cysteine-L-proteinase which was identified and purified by Dalton *et al* (1996) and separated from the E/S products of *F.*
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Furthermore, important enzymatic components, such as glutathione-S-transferase (Hillyer et al, 1992), haemoglobinase (Coles and Rubano, 1988) and cysteine-L-proteinases of *F. hepatica* (Simth et al, 1993; Dowd et al, 1994) have been identified within the 27.5 to 29 Kda range in E/S products of the parasite. Cysteine proteinases of the E/S products of Fasciola have become the focus of research since they play important biological and immuno-modulatory functions in the juvenile and adult parasites (Chapman and Mitchell, 1982; Smith et al, 1993). Moreover, these proteinases have also been shown to induce a high protective immunity in experimentally infected cattle (Dalton et al, 1996). A protein of 12.5 Kda is also separated; it corresponds to SAP2 protein which is proved to have protective potentiality against *F. hepatica* infection (Espino and Hillyer, 2003; Hillyer, 2005).

Proteins of 40, 55 and 66 Kda were identified in both parasite preparations in the present investigation. This is not in accordance with the finding of Espino et al (1993) who identified common components between 13 and 37 Kda in these parasite products. These differences may be due to the parasite strain used or to the technique used in the preparations of the antigens. Tegumental proteins may be found in E/S products; therefore, the 66 Kda protein identified in E/S products of the parasite may be a tegumental protein shed into the medium during incubation. Surface proteins are shed from the external tegumental surface of the parasite when cultivated *in vitro* (Lammas and Duffus, 1983; Lightowlers and Richard, 1988). The 45 Kda protein identified in E/S products of the parasite may correspond to the 48 Kda proteins in E/S products of adult parasites identified by Ajanusi et al (1993). Several proteins between 21 and 110 Kda were found in SO products of *F. gigantica* in the present study. These proteins may correspond to T1 and T2 tegumental glyocalyx proteins present in the adult parasite. It is known that production of T1 tegumental antigen is reduced as the fluke enters the bile ducts (Hughes, 1987) and T2 tegumental antigen becomes the predominant type (Hanna, 1980). The identified 50 Kda SO protein may correspond to T1 tegumental antigen which has a molecular weight of 50 Kda with sub-units of 40 and 25 Kda (Hanna and Trudget, 1983).

The present results partially agree with previous findings (Santiago and Hillyer, 1986; 1988; Ruiz-Navarrete et al, 1993).
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