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Biochemical properties of a purified protein in cystic fluid of *Taenia solium* metacestodes*

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Abstract: By affinity chromatography using a monoclonal antibody as ligand, Kim *et al.*(1986) purified a protein fraction in cystic fluid of *Taenia solium* metacestodes (CF). In this study, the biochemical properties of the purified protein were characterized. Discontinuous-polyacrylamide gel electrophoresis (disc-PAGE) of the protein at 4.5~10% separating gel concentration showed its molecular weight (MW) to be 150 kilodalton (kDa) in non-denatured state, while denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed that it was composed of 3 different subunits with respective MW of 15, 10 and 7 kDa. Subunit of 7 kDa was shown to be linked to other subunits by disulfide bonds. Isoelectric point of the protein was pH 6.8. The protein was relatively heat-stable for immunologic analysis. These properties indicated that the protein, comprising about 70% of total content in CF, had similar biochemical characters with antigen B of Oriol *et al.*(1971) in hydatid cyst fluid (HF).

Key words: *Taenia solium* metacestodes, protein in cystic fluid, protein antigen

INTRODUCTION

In antigenic preparations of *T. solium* metacestodes, a protein was purified by Guerra *et al.*(1982), which was a fibronectin of the metacestodes and composed of 95 and 105 kDa subunits; by immunoelectrophoresis with known patients sera of cysticercosis it made antigen B line of Flisser *et al.*(1980).

Kim *et al.*(1986) also purified a protein in CF of *T. solium* metacestodes and designated it as "A-antigen" meaning affinity-chromatography-purified protein. They showed that "A-antigen" was identical with "band C" of Choi *et al.*(1986) in disc-PAGE of CF. A-antigen was described to show higher specificity but lower sensitivity when tested with sera from patients of various parasitic diseases including cysticercosis.

In this paper, we described biochemical characters of the immunoaffinity purified protein (A-antigen) in CF of *T. solium* metacestodes.

MATERIALS AND METHODS

1. Antigens

Three antigenic preparations, CF of *T. solium* metacestodes, A- and U-antigen were used. CF was prepared as described by Choi *et al.* (1986). A-antigen was fractionated from CF by affinity chromatography through a monoclonal antibody (CFCc-298.18)-bound CNBr-activated sepharose 4B column. Affinity chromatography of A-antigen was repeated once

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again to remove contaminant. Unbound elute of CF was designated as U-antigen. The monoclonal antibody (CFCc-298.18) was known to bind 3 protein bands of 15, 10 and 7 kDa in CF by SDS-PAGE and enzyme-linked immunoelectrophoretic transfer blot(EITB) (Cho *et al.*, 1987).

2. Thermostability test

To observe thermostability of CF, A- and U-antigen, they were boiled at 100°C for 15 minutes. Antigenicity of 3 antigens were examined by enzyme-linked immunosorbent assay (ELISA) using 26 sera from confirmed neurocysticercosis patients. Antibody levels in the sera were all in positive range (0.23~1.22 in absorbance by ELISA; 0.18 or above are regarded as positive, Cho *et al.*, 1986).

Boiled and non-boiled antigens in protein concentration of 2.5μg/ml (as measured by Lowry *et al.*, 1951) were coated to polystyrene plates overnight at 4°C. Then 1 : 100 diluted sera and 1 : 10,000 diluted peroxidase conjugated goat IgG antihuman IgG (heavy- and light-chain specific, Cappel) were sequentially reacted. Differences in absorbance at 492 nm were compared between boiled and non-boiled antigens.

3. Disc-PAGE

To measure MW of component proteins in CF and A-antigen in nondenatured state, disc-PAGE method of Hedrick and Smith(1968) was applied. At separating gel concentration of 4.5%, 5%, 5.5%, 6%, 7%, 8%, 9% and 10%, standard proteins [α-lactalbumin (14.2 kDa), carbonic anhydrase (29 kDa), chicken egg albumin (45 kDa), bovine serum albumin (monomer 66 kDa; dimer 132 kDa), urease (trimer 272 kDa; hexamer 545 kDa), Sigma, USA] were electrophoresed with CF and A-antigen. Minus slopes of "100[log(Rf×100)]" of each standard protein against % gel concentrations were plotted against MW in log-log paper to make a standard calibration curve. Using minus slopes of the component proteins in CF and A-antigen, MW were estimated in the standard curve.

4. SDS-PAGE

Vertical electrophoresis system of 17×12cm was used for SDS-PAGE (Laemmli, 1970). Separating slab gel of 9 cm long and 1.5 mm thick was linear gradient gel of 10~15% polyacrylamide in 1.5M Tris buffer (pH 8.8) containing 0.4% SDS. Sample buffer containing 0.125% Tris (pH 6.8), 20% glycerol, 4.5% SDS and 10% 2-mercaptoethanol (2-ME) and 0.01% bromphenol blue, was heated with same amount of CF, A- and U-antigen at 95°C for 5 minutes. Thirty μl of each antigen(containing 30μg of protein) were applied. Samples were electrophoresed at 30 mA for 3~4 hours. Protein bands were stained with 0.125% Coomassie brilliant blue R-250.

To observe the presence of disulfide bonds within molecule of A-antigen, SDS-PAGE were done after: (1) heating A-antigen with sample buffer containing 10% 2-ME at 95°C for 5 minutes, (2) mixing A-antigen with sample buffer containing 10% 2-ME without heating (3) heating A-antigen at 95°C for 5 minutes with sample buffer without 2-ME and (4) mixing A-antigen with sample buffer without 2-ME and without heating

5. Enzyme-linked immunoelectrophoretic transfer blot (EITB)

To confirm the purity of A-antigen, SDS-PAGE/EITB of CF, A- and U-antigen were done by method of Tsang *et al.*(1983). After protein bands were transferred to nitrocellulose paper by electrophoresis in Towbin buffer, a patient serum of neurocysticercosis (our serum No. HSC-1090), which was previous confirmed to react with major bands in CF including 15, 10 and 7 kDa, was treated.

6. Isoelectric focusing (IEF)

CF and A-antigen were focused isoelectrically in 5% polyacrylamide gel containing pharmalyte (pH 3-10) in Pharmacia Phast system. Fifty μl of antigens(protein concentration 1 mg/ml) were applied.

RESULTS

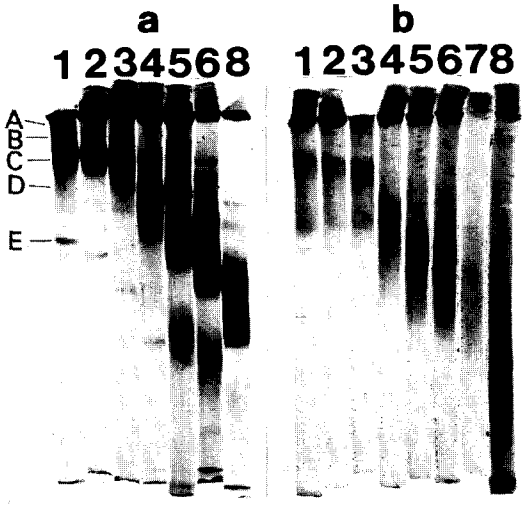
1. Molecular weight and subunits

(1) Non-denatured state

As shown in Fig. 1a, CF showed 5 protein bands in disc-PAGE. The results of disc-PAGE in purified A-antigen were shown in Fig. 1b at different gel concentrations. Calculating slopes of "100×[log (Rf×100)]" against % gel concentrations in standard proteins, standard curve of molecular weights was prepared (Figs. 2 and 3). Molecular weights of component proteins in CF were estimated to be over 600 kDa in band A, 220 kDa in band B, 150 kDa in band C (=A-antigen), 120 kDa in band D and 30 kDa in band E respectively in order of mobility.

(2) Subunits

SDS-PAGE of CF, A- and U-antigen were shown in Fig. 4. In CF, at least 23 bands were recognized; bands of 94, 64, 48, 39, 24, 15, 10 and 7 kDa were major bands. Densitometry revealed that concentration of 15, 10 and 7 kDa



Figs. 1a & 1b. Disc-PAGE of CF of *Taenia solium* metacestodes(Fig. 1a) and purified A-antigen (Fig. 1b) at different separating gel concentrations. No. on the top of gels are; 1: 10% gel, 2: 9% gel, 3: 8% gel, 4: 7% gel, 5: 6% gel, 6: 5.5% gel, 7: 5% gel, and 8: 4.5% gel. A-E on left of Fig. 1a are component protein bands in CF.

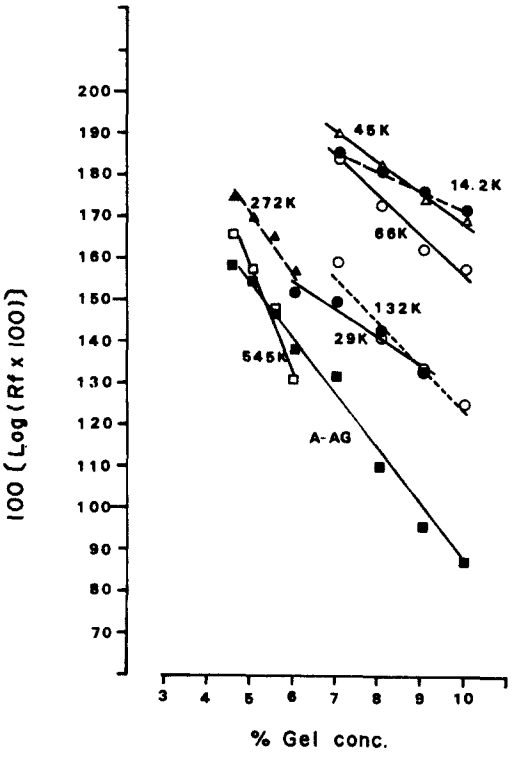


Fig. 2. Slope of "100×[log(Rf×100)]" against % gel concentrations in standard proteins and purified A-antigen.

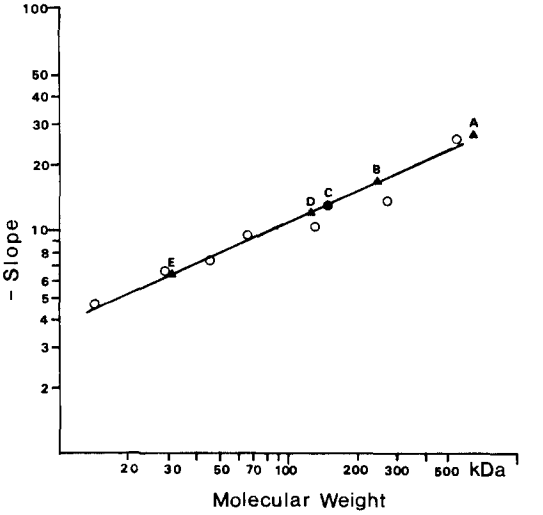


Fig. 3. Standard curve of molecular weight by minus slope in Fig. 2. Open circle (○): standard proteins, closed circle (●): A-antigen(=band C in disc-PAGE), closed triangle (▲): component proteins in CF.

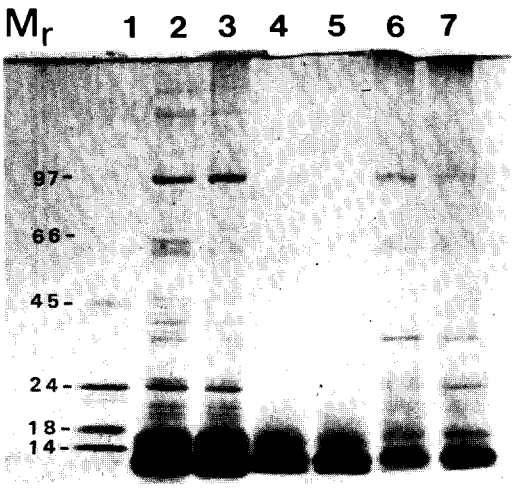


Fig. 4. SDS-PAGE of CF, A- and U-antigen in 10~15% linear gradient gel. M_r: Molecular weight in kDa. Lane 1: standard proteins, lane 2: CF, lane 3: CF after boiling at 100°C for 15 minutes, lane 4: A-antigen, lane 5: A-antigen after the boiling, lane 6: U-antigen, lane 7: U-antigen after boiling.

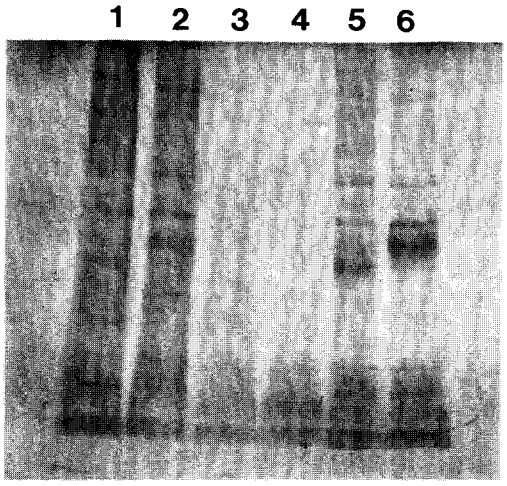


Fig. 5. SDS-PAGE/EITB of CF, A- and U-antigen using a polyclonal antibody (a patient serum). SDS-PAGE was done as in Fig. 4. Lanes 1 & 2: CF before (1) and after (2) boiling at 100°C for 15 minutes, lanes 3 & 4: A-antigen before (3) and after (4) the boiling, lanes 5 & 6: U-antigen before (5) and after (6) boiling. Peroxidase conjugated antihuman IgG and 3, 3'-diaminobenzidine/H_2O_2 as substrate were used.

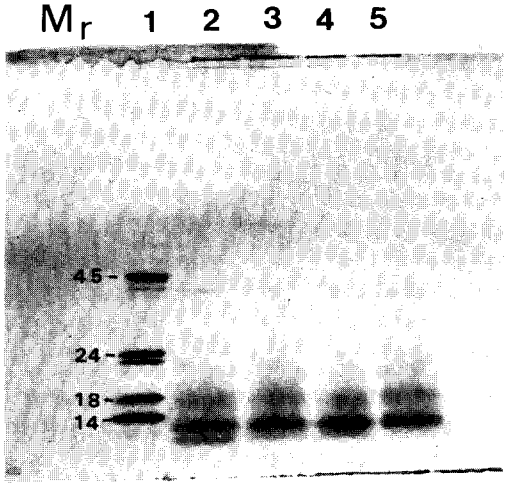


Fig. 6. SDS-PAGE of A-antigen at different conditions of sample treatment. M_r: molecular weight in kDa, lane 1: standard proteins, lane 2: A-antigen heated at 95°C for 5 minutes with same amount of sample buffer containing 10% 2-mercaptoethanol, lane 3: A-antigen mixed with sample buffer containing 2-mercaptoethanol without heating, lane 4: A-antigen heated at 95°C for 5 minutes with sample buffer without 2-mercaptoethanol, lane 5: A-antigen mixed with sample buffer without 2-mercaptoethanol and without heating.

bands were 25.7%, 30.5% and 20.0% (total 76.2%) of total protein in CF (densitogram not shown). In A-antigen, 3 bands of 15, 10 and 7 kDa were recognized. In U-antigen, 18 bands including the 3 bands of 15, 10 7kDa were found.

Fig. 5 showed the result of SDS-PAGE/EITB with a patient serum against CF, A-and U-antigen. Bands of 15, 10 and 7 kDa in 3 antigens reacted with antibody in common. Eleven bands in CF and 8 bands in U-antigen reacted with the patient serum in addition to components in A-antigen.

As shown in Fig. 6, A-antigen, which was treated with sample buffer containing 10% 2-ME and heated at 95°C for 5 minutes, showed dissociation of 7 kDa band (Lane 2). In the remaining conditions of sample treatment without 2-ME and/or without heating, the band

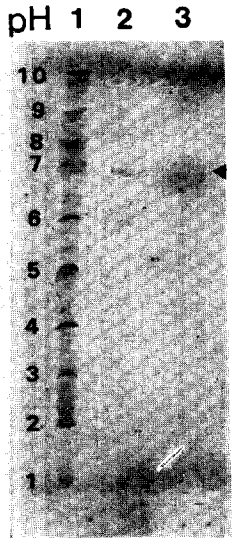


Fig. 7. IEF findings of CF and A-antigen. Lane 1: standard proteins with pH, lane 2: CF of *T. solium* metacestodes, lane 3: purified A-antigen(arrow head).

of 7 kDa was not dissociated.

2. Result of IEF

By IEF, CF was separated into a discrete band at pH 6.8 and a number of bands at pH 5.5~6.5. A-antigen showed only one band at pH 6.8 (Fig. 7).

3. Thermostability test

Antigenicity of 3 antigenic preparations was compared before and after boiling at 100°C for 15 minutes (Table 1). In 26 patients sera, mean absorbances by ELISA against non-boiled CF and U-antigen were comparable each other (0.63/0.68). However, mean absorbance against non-boiled A-antigen was lower (0.46) and was about 73% of CF.

After boiling, however, mean absorbances were similar each other between 3 antigens. Therefore, absorbance was changed less in A-antigen by ratio than CF or U-antigen. In addition, as shown in Figs. 4 and 5, band of 64 kDa was most affected by heating both in CF and U-antigen.

Table 1. Change of antigenicity in 3 antigenic preparations(CF, A- and U-antigen) before and after boiling at 100°C for 15 minutes. Absorbance of specific IgG antibody in 26 patients' sera were measured by ELISA (Cho *et al.*, 1986)

Antigen	Mean absorbance±S.D.		%(mean±S.D.) of absorbance after boiling
	before boiling	after boiling	
CF	0.63±0.31	0.35±0.23	55.9±16.1
A-antigen	0.46±0.28	0.29±0.22	62.8±15.5
U-antigen	0.68±0.33	0.29±0.20	41.0±15.6

DISCUSSION

In this study, the A-antigen purified by Kim *et al.* (1986) was characterized. This was relatively thermostable, 150 kDa protein with pI 6.8, composed of 3 subunits such as 15, 10 and 7 kDa. Polypeptide of 7 kDa subunit was illustrated to dissociate in reducing conditions.

As reviewed by Williams and Sandeman (1982), Harrison and Parkhouse (1985) and Schantz and Gottstein (1986), two proteins in HF were well characterized. One of them, "antigen 4" of Chordi and Kagan(1965), "Arc 5 antigen" of Capron *et al.* (1968) and "Antigen A" of Oriol *et al.* (1971), which are now agreed to be identical, was a heatlabile lipoprotein with MW of about 400 kDa and was composed of non-covalently linked subunits of MW 67 kDa. This subunit dissociated into two smaller subunits of MW 47 and 20 kDa on reducing gels. Another major protein in HF, that is "Antigen 5" of Chordi and Kagan (1965) or "Antigen B" of Criol *et al.* (1971), was heat-stable protein of MW 120~150 kDa (Oriol *et al.*, 1971). It gives 3 component subunits in 10.5~20 kDa on SDS-PAGE with or without reducing conditions; these subunits may, however, become associated through non-covalent linkages to produce aggregates in a range of sizes (Piantelli *et al.*, 1977).

Out of the two proteins in HF, A-antigen in CF had similarity with "Antigen B" of Oriol *et al.* (1971), in aspects of its MW in non-denatured state, composing subunits and their MW, and its thermostability. A-antigen was different from "Antigen E" of Flisser *et al.* (1980) in *T. solium*, which was a fibronectin

of MW 95 and 105 kDa (Guerra *et al.*, 1982). Considering the taxonomic kinship between *T. solium* and *Echinococcus granulosus* in family Taeniidae, structural similarity in a component protein in cystic fluid of the metacestodes may be quite possible. "Antigen A" of Oriol *et al.* (1971) was known to occur also in *T. hydatigena*, *T. ovis*, *T. solium* and *E. multilocularis* and cause cross reactions between them (Capron *et al.*, 1968).

It is still uncertain whether A-antigen in *T. solium* metacestodes is species specific. Kim *et al.* (1986) described the higher value of A-antigen than CF especially in aspect of specificity. But they did not observe cross reactivity of A-antigen with sera from hydatid disease. Therefore, its value as a diagnostic antigen needs further studies.

In serodiagnosis of human cysticercosis by ELISA, CF is now increasingly recognized as more sensitive and specific than parenchymal extracts (Choi *et al.*, 1986; Larralde *et al.*, 1986; Bailey *et al.*, 1988). In the mean time, sensitive and specific fractions for diagnostic purpose have been searched for by SDS-PAGE/EITB. In parenchymal extracts or in CF, protein bands of 64 kDa were agreed to be most sensitive (Grogl *et al.*, 1985; Joo *et al.*, 1987; Cho *et al.*, 1987; Hur *et al.*, 1988). A-antigen bands were also sensitive (Cho *et al.*, 1987). Gottstein *et al.* (1986 & 1987) reported that bands of 26 and 8 kDa are specific for diagnosis of cysticercosis.

Just like the unsettled reports on the sensitive and specific protein bands, hitherto reported results on fractionated proteins for diagnosis are inconsistent by author. Since approaches to fractionate crude antigens were different, it is difficult to compare their results each other. For example, gel filtration and ion exchange chromatography (Guerra *et al.*, 1982), chromatofocusing (Coker-Vann *et al.*, 1984), affinity chromatography using monoclonal antibody (Kim *et al.*, 1986), preparative isoelectric focusing (Pammenter and Rossouw, 1987), a mmonium sulfate precipitation (Chen *et al.*, 1988) *etc.* have been used. Therefore, the biochemical and the immunologic characterization of individual protein is necessary for its identification in *T. solium* metacestodes.

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＝國文抄錄＝

有鉤囊尾蟲 囊液에서 親和性 크로마토그래피로 分離한 抗原 蛋白質의 生化學的 性狀

中央大學校 醫科大學 寄生蟲學敎室
趙昇烈・金錫一・姜信榮・孔 允

Kim *et al.*(1986)은 有鉤囊尾蟲 囊液을 感作시켜 만든 單細胞群 抗體를 CNBr活性化 Sepharose 4B에 連結시켜 親和性 크로마토그래피를 實施하고 囊液에서 A-抗原을 分離한 바 있다. 이 硏究는 그 抗原 蛋白質의 生化學的 性狀을 觀察한 것이다.

Disc-PAGE에 依해 4.5～10% 폴리아크릴아마이드 젤에서 나타내는 Rf값을 基礎로 分子量을 測定한 結果 A 抗原의 分子量은 150,000 dalton이었다. A-抗原은 SDS-PAGE에서 15,000, 10,000, 7,000 dalton에 해당하는 polypeptide로 分劃이 區別되었다. 10% 2-mercaptoethanol로 處理하지 않거나 95°C에서 5분간 處理하지 않은 A-抗原의 SDS-PAGE에서는 7,000 dalton의 polypeptide가 分離되지 않았다. A-抗原의 等電點(PI)은 pH 6.8이었다. 囊液抗原의 各 分劃에 反應하는 抗體를 갖는 患者血淸으로 A-抗原을 SDS-PAGE/EITB한 바 患者血淸은 15,000, 10,000, 7,000 dalton에 該當하는 部位에서만 反應하였다.

囊液 蛋白質의 約 70%를 차지하는 A-抗原은 免疫學的으로 耐熱性을 가졌으며 包蟲(hydatid cyst) 囊液 抗原中 Oriol *et al.*(1971)의 "Antigen B"와 生化學的 性狀이 비슷한 蛋白質이었다.