CARACTERIZACION BIOQUIMICA E INMUNOLOGICA DEL ANTIGENO B PURIFICADO DEL CISTICERCO DE LA *Taenia solium*

Tesis que para obtener el grado de
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Esta Tesis fué realizada bajo la Dirección de los Doctores Luis Cañedo D. y Juan Pedro Laclette, y la colaboración de las Doctoras Ana Flisser y Kaethe Willms del Departamento de Inmunología del Instituto de Investigaciones Biomédicas, UNAM.

A mi Madre, Padre y Hermanos,

A Juan Pablo y Pablito,

A mis compañeros de laboratorio,

A mis amigos.

INDICE

ANTECEDENTES*

Dentro de la familia Taenidae se encuentran algunas especies de helmintos que son parásitos importantes del hombre y animales domésticos. Como ejemplos pueden citarse las cisticercosis humana y porcina que representan un serio problema de salud pública y de economía agropecuaria, principalmente en regiones con malas condiciones higiénicas, como es el caso de algunos países de Asia, Africa y América Latina (1,2). En México se han reportado frecuencias para la cisticercosis cerebral humana que varían entre el 1 y 3% con base en estudios postmortem realizados en hospitales de la Cd. de México. Así mismo, estudios seroepidemiológicos en los que se muestrearon 51 localidades de todo el país, han mostrado que del 1 al 3.8% de los individuos presentan anticuerpos circulantes (3,4). Si bien el problema de las enfermedades parasitarias causadas por Céstodos se resolvería con un mejoramiento en las condiciones higiénicas de la población, también la investigación Biomédica puede aportar herramientas de uso inmediato que puedan ser adaptadas a las condiciones específicas de cada país. De este modo, las investigaciones sobre la inmunología de las cestodiasis han estado dirigidas principalmente al desarrollo de mejores métodos diagnósticos e inmunoprofilácticos.

Para estos propósitos se han utilizado como antígenos, extractos crudos de los gusanos en fase adulta o de metacéstodo, así como materiales de secreción obtenidos <u>in vitro</u> a través del cultivo de los parásitos. Sin embargo, existe poco conocimiento sobre las características fisicoquímicas de estos antígenos y solo en pocas ocasiones se ha logrado su

*Las citas corresponden a la bibliografía complementaria.

purificación. Asímismo, la búsqueda de antígenos útiles en el diagnóstico y vacunación contra las cestodiasis, seguramente contribuirá al conocimiento de la biología de estas relaciones huésped-parásito.

La información concerniente a la composición de antígenos que puedan tener utilidad diagnóstica, se inicia con los trabajos realizados por Biguet y cols. (5), y Capron y cols. (6), quienes analizaron comparativamente los componentes antigénicos de algunos miembros de la familia Taenidae. Utilizando sueros hiperinmunes contra Taenia saginata, encontraron que 16 de los 23 antígenos demostrados en este parásito, estaban presentes en otros seis géneros de Ciclofílidos, incluyendo a miembros de las familias de los Anoplocefálidos e Himenolepídidos. Además, entre 2 y 5 componentes antigénicos de T. saginata fueron detectados en varias especies de Nemátodos y Tremátodos. Similarmente, Madison y cols. (7) sostienen que Cisticercus cellulosae, Cisticercus tenuicolis y Cisticercus bovis, presentan al menos dos antígenos en común, mientras que no presentan ninguno en común con el flúido de Echinococcus granulosus. De igual modo Flisser y cols. (8) encontraron una correlación entre la cercanía filogenética y el número de antígenos que cruzaban entre las distintas especies de Céstodos, Tremátodos y Nemátodos.

A pesar de las reacciones cruzadas, Capron y cols. (9), identificaron por inmunoelectroforesis una proteína antigénica que parecía ser específica de E. granulosus a la que denominaron como antígeno 5 o Arco 5. El Arco 5 es el principal componente antigénico del flúido hidatídico de E. granulosus y tiene una gran capacidad inmunoreactiva frente a sueros de pacientes con hidatidosis confirmada (10). Recientemente, se ha demostra-

do que el Arco 5 está presente también en Taenia solium, Taenia hidatigena, Taenia ovis y Echinococcus multilocularis (11-15).

El Arco 5 que es sin duda el antígeno de Céstodos mas estudiado, fue inicialmente purificado por Bout y cols. (16), y sus propiedades fisicoquímicas han sido descritas detalladamente (17,18). El Arco 5 es una proteína con actividad enzimática de alfa y beta carboxilesterasa, de peso molecular cercano a 400 000, constituída por subunidades de 67 000 que migran como una sola banda en geles de poliacrilamida con dodecil sulfato de sodio (SDS). El uso de agentes reductores como el 2-mercaptoetanol permite disociar a las subunidades de 67 000 d, en dos proteínas de 47 000 y 20 000 (18). El Arco 5 que es relativamente estable a 56°C y en un rango de pH de 4 a 7, ha sido localizado en las células tegumentales del protoescólex, la membrana germinal y la pared externa de la cápsula (19). Rickard y cols y Davies y cols. han sugerido que el Arco 5 acumulado en el flúido hidatídico es el producto de secreción de las anteriores estructuras (20,21).

Desde hace tiempo, la terminología utilizada por algunos autores para describir a este antígeno y a otros componentes importantes, ha dado lugar a una cierta confusión. Por ejemplo, Chordi y Kagan asignaron el número 4 al arco de precipitación en inmunoelectroforesis mas prominente del flúido hidatídico (22). Pozzuoli y cols. utilizaron la misma designación (23). Oriol y cols. (24) describieron la purificación de dos lipoproteínas antigénicas del flúido hidatídico del borrego, denominadas antígenos A y B, siendo la lipoproteína A el antígeno mas predominante en inmunoelectroforesis. Los resultados descritos por estos autores, indican que

posiblemente estos antígenos corresponden al Arco 5 descrito inicialmente por Capron y cols. (9).

La confusión aumenta con la designación dada por Pozzouli y cols. a un contaminante del antígeno 4 al que llaman antígeno 5 (23), que parece ser idéntico al antígeno B de Oriol y cols. (24). Ambos antígenos fueron identificados en sueros de pacientes con hidatidosis y producen una marcada reacción anafiláctica (23,25). Las características fisicoquímicas de estos antígenos han sido estudiadas detalladamente por Oriol y Oriol (26) y Piantelli y cols. (27). El antígeno B de Echinococcus granulosus es una proteína termoestable a 100°C con un peso molecular de 120 000 determinado por sedimentación al equilibrio. Presenta tres bandas con pesos moleculares que varían en un rango de 10 500 a 20 000 en geles de poliacrilamida con SDS, sin importar la presencia o ausencia de agentes reductores. Piantelli y cols. han sugerido que estas subunidades se asocian por uniones no covalentes para producir agregados de formas irregulares (18).

El antígeno B ha sido localizado en E. granulosus y E. multilocularis (20,27) y presenta una distribución mas difusa que el arco 5. Se localiza en la membrana germinal laminada, así como en la cápsula externa. En el protoescólex se encuentra en el tegumento de la región anterior a las ventosas. Utilizando métodos inmunocitoquímicos, las células subtegumentales se marcan de manera granular, lo que sugiere que junto con el parénquima de la cápsula externa, representan los sitios de mayor síntesis (27).

En relación a la purificación de antígenos de otras especies de la familia de los Taenidos, Campbell (29) ha reportado la obtención de fracciones proteicas, de nucleoproteínas y polisacáridos de Taenia taeniaeformis que confieren inmunidad a ratas al ser inyectadas subcutaneamente. También Kwa y Lew (29), reportaron la purificación de un antígeno de la forma larvaria de T. taeniaeformis que tiene un peso molecular de 140 000 con propiedades anafilácticas y capacidad inmunogénica en ratas. Con respecto al metacéstodo de Taenia pisiformis, Rickard y Katiyar (30) reportaron la purificación parcial de dos proteínas que las formas larvarias secretan al medio de cultivo, que producen marcada reacción anafiláctica en conejos. Sin embargo no se ha logrado su caracterización. Leid y Williams (31) han logrado la purificación de un alergeno obtenido de Cisticercus faciolaris, que muestra una carga neta negativa y un peso molecular aproximado de 50 000. Esta proteína no es secretada y se encuentra en mayor cantidad en la forma larvaria que en la adulta.

En cuanto a la purificación de antígenos de T. saginata, la información disponible es muy pobre. Sin embargo se sabe que en el gusano adulto hay al menos siete componentes antigénicos que presentan poca reacción cruzada con otros helmintos (32). Tres de estos antígenos tienen algún valor en el diagnóstico de la cisticercosis bovina. Recientemente se ha publicado la purificación parcial de dos fracciones antigénicas a partir de extractos crudos de proglótidos de T. saginata (33,34).

Finalmente, la información acerca de las características de antígenos de la T. solium adulta así como de su forma larvaria, era practicamente nula y solo se disponía como antecedente del trabajo de Flisser y cols. (35) en el que se reportaba que el antígeno denominado como "B" era

el mas frecuentemente reconocido por sueros de pacientes cisticercosos. - Las razones que justifican el intento de purificación del antígeno B, se - encuentran expuestas en la Introducción del sobretiro anexo.

BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF ANTIGEN B PURIFIED FROM CYSTICERCI OF *TAENIA SOLIUM*

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I. INTRODUCTION

The high incidence of cysticercosis among Mexicans due to the metacestode of *Taenia solium* is indicated by the fact that cerebral cysticercosis is found in approximately 2 % of the autopsies performed on hospitalized patients [1] and in 1.0–3.8 % of the population surveyed in seroepidemiological studies [2–4]. Although immunodiagnosis of human cysticercosis is currently carried out by complement fixation [5] or immunoelectrophoresis [6], these methods, as well as others that have been employed or tested [7], show a lack of sensitivity and/or specificity. It has been argued that the use of crude extracts in immunodiagnosis is the principal cause of these deficiencies [8].

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The humoral immune response of individuals with brain cysticercosis is very heterogeneous. When tested against a crude extract of cysticerci in immunoelectrophoresis carried out at pH 8.6, the sera of such patients display different numbers and types of antigens. A total of eight precipitation bands have been detected, with the isoelectric band (antigen B) being by far the most frequently recognized [6]. Protection by IgG antibodies has been demonstrated in several experimental models of cysticercosis [9–11]. As this is the most frequent class of human anti-B antibodies [6], the availability of purified antigen B would permit an evaluation of its immunizing properties, as well as of its use in the improvement of methods currently employed for the immunodiagnosis of this parasitosis. This communication presents the purification and the biochemical and immunological characterization of antigen B from cysticerci of *T. solium*.

II. MATERIALS AND METHODS

Source of metacestodes. Cysticerci were dissected from the skeletal muscles of highly infected pigs within 6 h after slaughter. The vesicular fluid was removed by puncture and the larvae were stored at –70 °C until use.

Antigen preparation. A crude extract was obtained as previously described [6]. Briefly, the larvae were homogenized in 3 M KCl, centrifuged at 1000 *g* for 30 min at 4 °C, and the supernatant was dialyzed against 0.15 M NaCl in 0.015 M phosphate buffer, pH 7.4 (PBS).

A purified protein fraction was obtained by a substantial modification (summarized in Fig. 1) of a previously published procedure [12]. The cysticerci were homogenized in 0.45 M NaCl (5 ml/g of humid larvae) containing 2.5 mM EDTA, 0.04 o/o (w/v) *p*-hydroxymercuribenzoate (PHMB; Sigma), and 0.006 o/o (w/v) phenylmethylsulfonylfluoride (PMSF; Sigma) in a Polytron homogenizer (Brinkmann Instruments) at maximal speed for 1–2 min at 4 °C. The homogenate was a whitish emulsion which was cleared by centrifugation at 27,000 *g* for 60 min at 4 °C. The precipitate was discarded and the supernatant (S1) was immediately dialyzed against 0.5 M acetic acid, pH 2.5, at 4 °C using standard cellulose dialysis tubing (molecular weight cut-off: 12,000–14,000). The turbid emulsion obtained from dialysis was centrifuged at 27,000 *g* for 60 min at 4 °C, and the clear supernatant (S2) was diluted to a protein concentration of 0.5 mg/ml using 0.5 M acetic acid.

A salting-out precipitation was carried out on S2 by slowly adding a concentrated solution of NaCl while stirring, until a final concentration of 0.83 M NaCl was reached. This mixture was stirred for 2 h at 4 °C and centrifuged at 27,000 *g* for 60 min at 4 °C. The supernatant was discarded and the pellet (P3) was solubilized by dialysis against 0.5 M acetic acid. The unsolubilized material was eliminated by centrifugation as above, and the clear supernatant (S4) was chromatographed on Sephadex G-200 (40 x 2.6-cm column) and eluted with 0.5 M

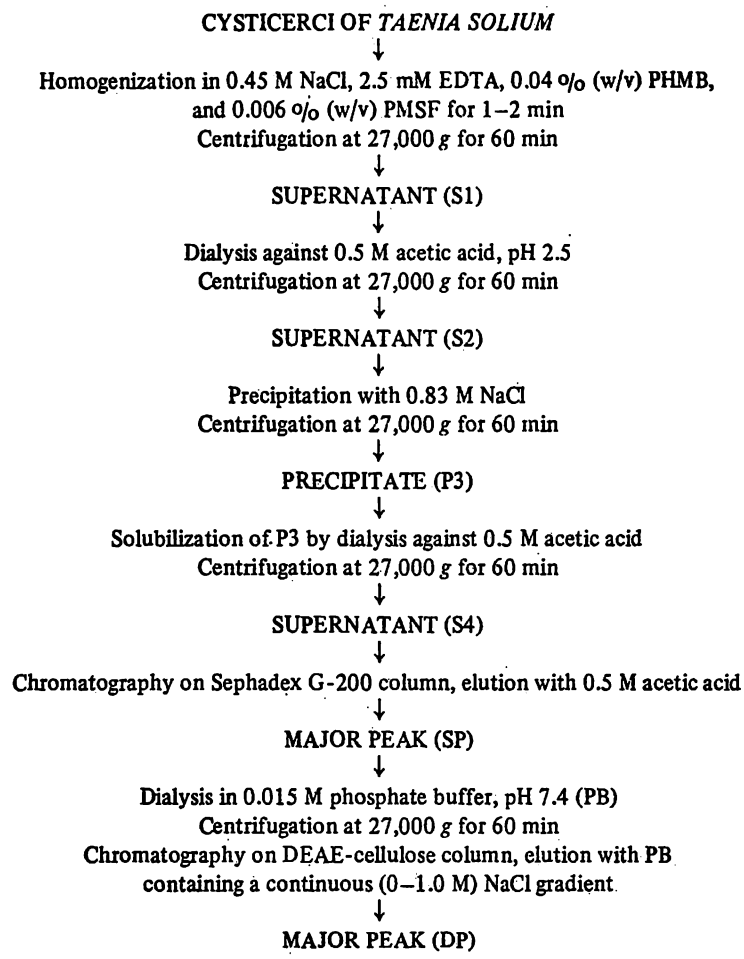

FIG. 1. Summary of the purification protocol for the isolation of antigen B from cysticerci of Taenia solium. *All procedures were carried out at 4 °C. For a detailed explanation, see Section II.*

acetic acid (flow rate: 5 ml/cm^2/h). Fractions of 2.5 ml were collected and their absorbance at 230 nm was determined using a PMQII Zeiss spectrophotometer. Absorbance at 230 nm instead of at 280 nm was selected in order to enhance the sensitivity of the detection. In a typical chromatographic run, 10 ml of S4 with a protein concentration of 0.5 mg/ml were applied to the column. The proteins recovered in the major peak (Fig. 2) accounted for 45–50 % of the applied sample, and the proteins recovered in the other peaks accounted for 15–25 %. Therefore, the quantitative recovery from this column was approximately 70 %.

The fractions corresponding to the major peak were pooled and concentrated 4–5 times using immersible CX-10 ultrafiltration units (Millipore Corp.). This

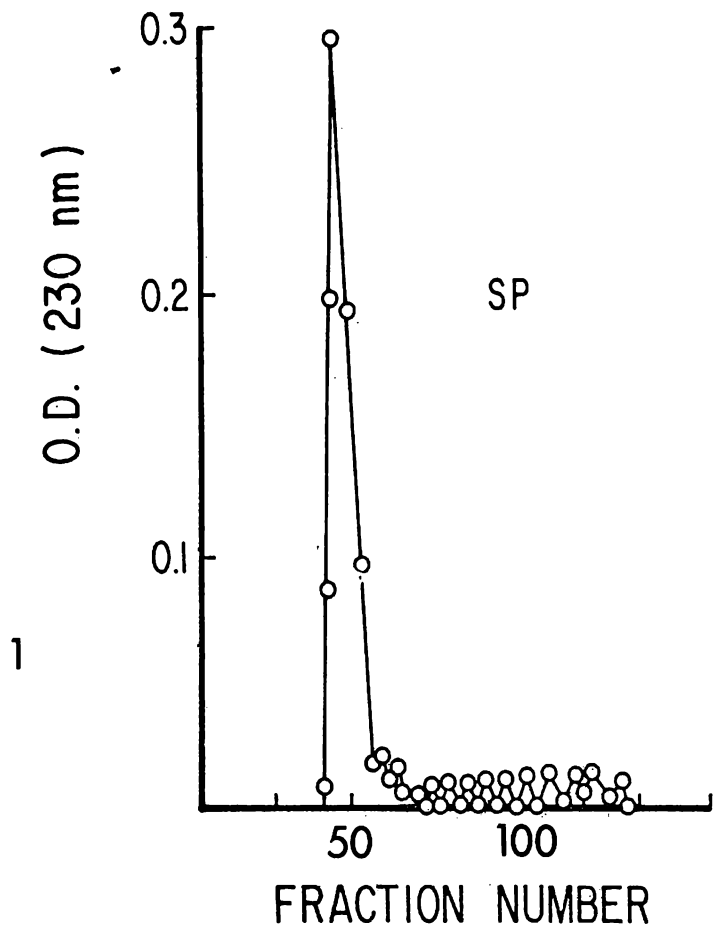

FIG. 2. Profile of S4 eluted from a Sephadex G-200 column with 0.5 M acetic acid. The protein present in the major peak (SP) was used in all experiments with the exception of amino acid analysis and molecular weight determination.

fraction, designated as SP, was dialyzed against 0.015 M phosphate buffer, pH 7.4. After centrifugation at 27,000 *g* for 60 min at 4 °C, this fraction was added to a DEAE-cellulose (Sigma) column (10–15 ml bed volume) and was eluted with 0.015 M phosphate buffer, pH 7.4. Usually, 5–15 ml of SP (0.5 mg protein/ml) were applied to the column with a flow rate of 25 ml/h. After loading the column, the unbound proteins were washed out from the resin with 10–15 ml of phosphate buffer. The bound proteins were eluted from the column with phosphate buffer containing a continuous (0–1.0 M) NaCl gradient. Fractions of 2.5 ml were collected and their absorbance at 230 nm was determined. Fractions included in the only peak obtained with the NaCl gradient (Fig. 3) were

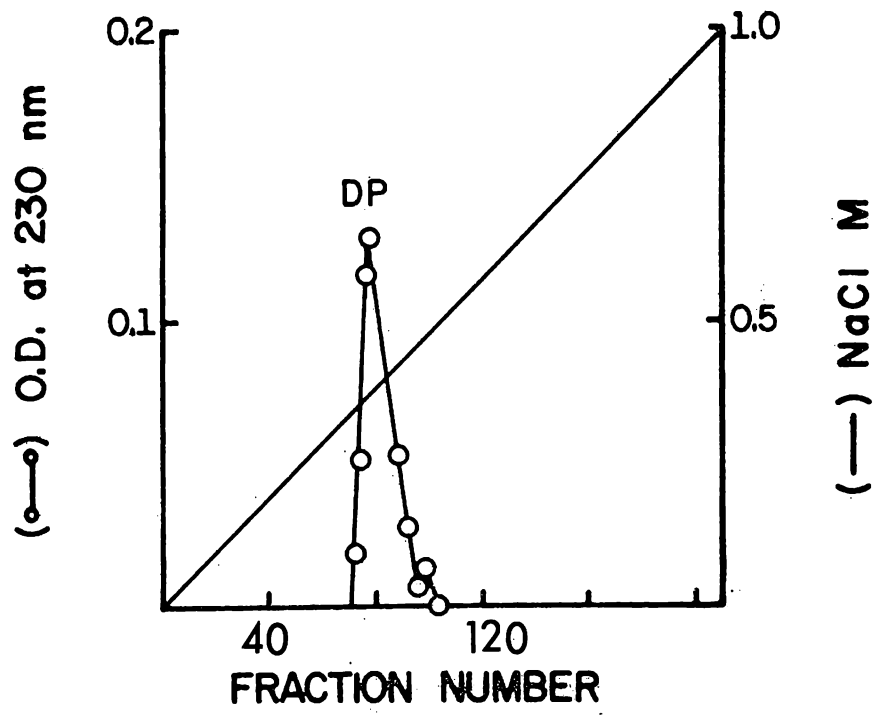

FIG. 3. Profile of SP eluted from a DEAE-cellulose column. The bound protein was eluted with a phosphate buffer containing a continuous (0–1.0 M) NaCl gradient. The major peak (DP) was used for amino acid analysis and molecular weight determination.

pooled and concentrated 2–3 times. The resulting material was called the DP fraction. The protein and carbohydrate content of samples from each step of the purification procedure was determined using the Lowry method as modified by Hartree [13] and the phenol-sulfuric method [14], respectively.

Polyacrylamide gel electrophoresis (PAGE). Sodium dodecyl sulfate (SDS)-PAGE was carried out in 7.5 o/o (w/v) cylindrical polyacrylamide gels (70 x 6 mm) with 1 o/o SDS according to Payne [15]. For the molecular weight determinations, globular proteins were used as standards. The protein bands were stained with 0.06 o/o (w/v) Coomassie blue in 10 o/o (v/v) acetic acid, and destained in 10 o/o acetic acid. The relative amount of protein in each band was determined by densitometry of the gels.

Isoelectric-focusing. Gel isoelectric-focusing was carried out in 5 o/o (w/v) cylindrical polyacrylamide gels (70 x 6 mm) containing 2 o/o (w/v) carrier Bio-Lyte ampholytes (Bio-Rad) with a pH range of 3–10 [16]. The pH gradient was determined at the end of the run by cutting 5-mm discs along the gels; each disc

was suspended in 1 ml of distilled water for 2 h and the pH was measured with a microelectrode. The gels were stained as described above.

Amino acid analysis. A sample of the major peak obtained from DEAE-cellulose chromatography (Fig. 1, DP fraction) was hydrolyzed in duplicate in 6 M HCl at 105 °C for 24 h. The amino acids were resolved in a Beckman Spinco model 120C analyzer. Tryptophan and cysteine were not determined, and threonine and serine values were not corrected.

Hyperimmune sera. After PAGE of the Sephadex G-200 peak (SP), the gel was frozen. The sections corresponding to the upper and lower bands (UB and LB, respectively), which appeared as dense white discs, were carefully cut out, minced, diluted with 1 ml of PBS, and mixed with adjuvant. New Zealand white rabbits were immunized subcutaneously according to the following schedule: an initial sensitizing dose in complete Freund's adjuvant was administered at day 0, followed by three booster doses in incomplete Freund's adjuvant at days 15, 30, and 45. One rabbit was injected each time with 330 μg of the proteins present in SP, while two others received doses of approximately 120 μg protein from either the UB or LB. All animals were bled 7 days after each booster and the sera (anti-SP, anti-UB, and anti-LB) were separated by centrifugation and stored at –20 °C until use.

Human sera. Sera from patients with confirmed brain cysticercosis were obtained from various hospitals in Mexico City and kept at –20 °C until use.

Double immunodiffusion and immunoelectrophoresis. Double immunodiffusion (DID) was carried out in 1 % (w/v) agarose in 0.05 M barbital buffer, pH 8.6, according to the method of Ouchterlony [17]. For immunoelectrophoresis (IEP) [17], microscope slides were layered with 1 % agarose in barbital buffer and 25 μl of the crude extract (CE) or SP were applied to wells cut in the gel. After electrophoresis at 1.5–2.0 mA per slide for 90 min, the sera were added to the troughs and allowed to diffuse in a humid chamber for 48 h. The slides were then washed thoroughly with 0.5 M NaCl and dried. Finally, 0.1 % (w/v) amido black in 10 % acetic acid was employed for staining the gels, and 10 % acetic acid for destaining. The crude extract (CE) and purified fraction (SP) were used at protein concentrations of 15 and 4 mg/ml, respectively.

Electron microscopy. One drop (50 μl) of SP (0.22 mg protein/ml) was applied to a double layered (Formvar-carbon) grid, followed by a second drop of 60 μM ATP in 0.25 M acetic acid (final concentration, 30 μM ATP) and left for 15–30 min at room temperature. After exhaustively washing the grid, 1 % uranyl acetate was added to the sample and left for 2–3 min. The grid was again washed with distilled water, dried, and examined in a JEOL 100B electron microscope. Control samples were treated similarly except that no ATP was added.

III. RESULTS

Figure 4 illustrates the band patterns obtained in SDS-PAGE of fractions from each step of the purification procedure. The material extracted in the first supernatant (S1) obtained after homogenization of the cysticerci was very heterogeneous, as evidenced by the number of protein bands found in SDS-PAGE. Dialysis against 0.5 M acetic acid eliminated several of the medium molecular weight proteins from the S2 fraction. The low molecular weight proteins were selectively excluded from the S4 supernatant obtained by the salting-out of S2 and dialysis of P3, producing a sizeable enrichment in two high molecular weight proteins and a less marked enrichment in two proteins of medium molecular weight. One major peak (SP) obtained following Sephadex G-200 chromatography, which eluted in the void volume of the column, included the two proteins

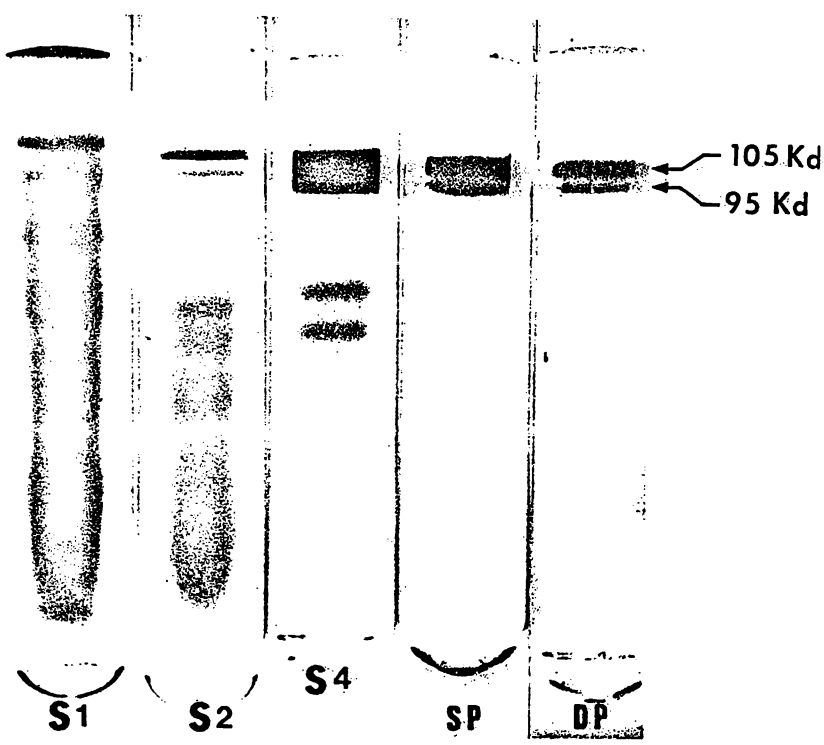

FIG. 4. SDS-PAGE of the fractions obtained during purification of T. solium *cysticerci (see Figure 1). The arrows indicate the position of the upper (105 kd) and lower (95 kd) bands (UB and LB, respectively). By densitometry of the gels, the combined UB and LB proteins from the SP and DP fractions were determined to represent 85 o/o and 95 o/o of the total protein, respectively.*

TABLE I. Protein Recovered During Purification of Antigen B

Fraction	*mg Protein/10 g Dry Tissue Weight*[a]
S1	*1100*
S2	*630*
S4	*30*
SP	*21*
DP	*7.2*

[a] *The dry weight of tissues from cysticerci was determined by desiccation of the larvae to a constant weight. Ten grams of dry tissue were obtained from approximately 37 g of fresh larvae.*

of high molecular weight as shown in the SDS-PAGE of this fraction. These two protein bands comprised 85 % of the proteins in the SP fraction, as determined by densitometry of the gel. The amount of protein recovered in each step of the purification procedure is shown in Table I.

Both the CE and SP, when reacted in DID against anti-SP serum, gave one precipitation arc (Fig. 5), indicating that no antibodies against other antigens were formed when the rabbit was immunized using the SP fraction. In IEP, both antigens gave one isoelectric band when reacted against either anti-SP (Fig. 6a) or anti-CE (Fig. 6b) serum. This result indicated that none of the antigens rec-

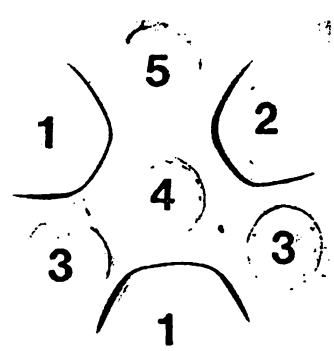

FIG. 5. Double immunodiffusion patterns of SP (1) and the crude extract (CE, 2) in their reaction against anti-SP (3), anti-upper band (UB, 4) and anti-lower band (LB, 5) sera. The anti-SP serum recognized only one protein in both antigens, indicating its monospecificity. The precipitation arc formed between SP and both the anti-UB and anti-LB sera showed complete identity.

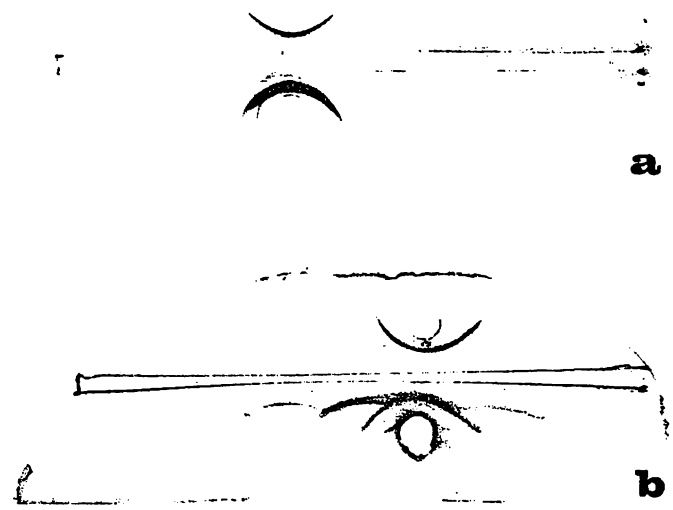

FIG. 6. Immunoelectrophoretic patterns of SP (upper wells) and the crude extract (CE, lower wells) against anti-SP serum (a) *and anti-CE serum* (b). *Both the anti-SP and anti-CE sera recognized one isoelectric antigen in SP.*

ognized by the anti-CE serum, except antigen B, was present in sufficient amounts in the SP fraction to be detected. Antigen B (and other precipitation bands) was also present in S1, S2, and S4, but it could not be detected in the precipitates obtained from S1 and S2 (data not shown).

Since the isoelectric pattern of SP suggested that this fraction contained antigen B, sera from seven cysticercotic patients which had precipitating anticysticercus antibodies [6] were assayed by IEP against both CE and SP. All sera formed only one isoelectric precipitation band with each antigen as exemplified in Figure 7.

FIG. 7. Immunoelectrophoresis of the crude extract (upper well) and SP (lower well) against the serum of a patient with neurocysticercosis. The precipitation arc characteristic of antigen B can be seen in both systems.

These results demonstrated that SP contained antigen B in a semipurified form. However, as shown before, the SP fraction was composed principally of two proteins (85 %), designated as the upper (UB) and lower (LB) bands in SDS-PAGE (Fig. 4). In order to determine which band corresponded to antigen B, rabbits were immunized with either UB or LB. The resulting hyperimmune anti-UB and anti-LB sera were reacted in DID with SP. As shown in Figure 5, both antisera formed a precipitation band against SP which showed identity, indicating that the two principal proteins were antigenically identical. Therefore, B is an antigen composed of two proteins or subunits.

To eliminate contaminants still present in SP, ion exchange chromatography through DEAE-cellulose was carried out. As illustrated in Figure 3, a major peak (DP) was eluted from this column. SDS-PAGE of DP (Fig. 4) revealed molecular weights of 105,000 and 95,000 for the upper and lower bands, respectively. Additionally, densitometry of the gel indicated that the combined bands (UB plus LB) represented over 95 % of the protein content in DP. When DP was reacted in DID against anti-SP, anti-UB, or anti-LB, the isoelectric band characteristic of antigen B was obtained (data not shown). The preliminary

TABLE II. Amino Acid Composition of Antigen B Obtained from Cysticerci of Taenia solium[a]

Amino Acid Residue	*Mole Percent*
Alanine	*11.85*
Valine	*3.50*
Leucine	*12.74*
Isoleucine	*2.70*
Proline	*0*
Hydroxyproline	*0*
Phenylalanine	*0.71*
Methionine	*2.25*
Glycine	*2.69*
Serine	*5.20*
Threonine	*5.15*
Tyrosine	*0.65*
Aspartic acid	*12.58*
Glutamic acid	*22.00*
Lysine	*7.20*
Arginine	*9.65*
Histidine	*0.72*
Total	*99.59*

[a] *Tryptophan and cysteine were not determined.*

amino acid analysis of DP showed a very high content of charged amino acid residues (Table II); approximately 31 % were acidic and 21 % were basic, and there was a low content of aromatic and sulfur residues.

Other biochemical features of antigen B were determined using the 85 %-pure SP fraction. The isoelectric point of the upper and lower bands, obtained upon isoelectric-focusing in gel containing ampholyte carriers, was between 5.0 and 5.3. The carbohydrate content was found to be approximately 12 %. As previously shown [12], a semi-crude fraction of *T. solium* cysticerci precipitates in the form of fiberlike structures when incubated in the presence of ATP. In order to determine whether antigen B was responsible for this precipitation, SP was incubated in the presence of ATP. As shown in Figure 8, the fiberlike precipitation was obtained with as little as 30 μM ATP; because no ATPase activity could be determined in SP, following the method of Sumner [18], this process did not appear to be dependent on ATP hydrolysis.

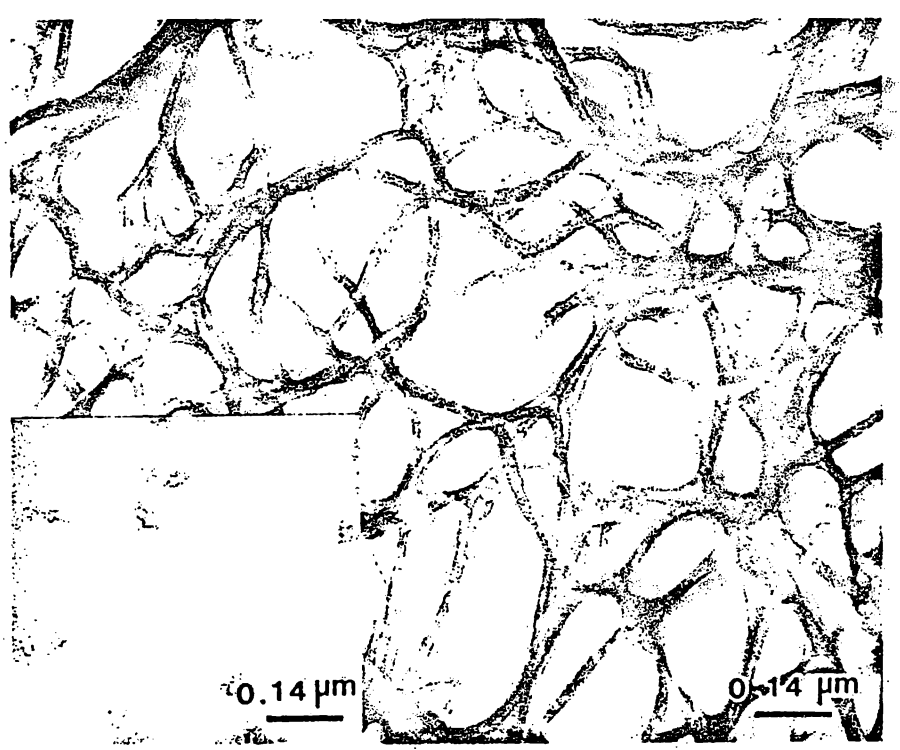

FIG. 8. Electron micrographs of negatively stained SP samples in the absence (inset) or presence of 30 μM ATP. This precipitation in the form of fibers is not dependent on ATP hydrolysis since no ATPase activity could be detected.

IV. DISCUSSION

Antigen B from *T. solium* metacestodes, the antigen in the crude extract most frequently recognized by sera from patients with cysticercosis, was isolated to 95 % purity. The conclusion that antigen B is the main component in the SP and DP fractions, obtained from the last two steps of purification, was based on their immunoelectrophoretic precipitation patterns with sera from cysticercotic patients. Antigen B in the SP fraction showed identity with only one component of the crude extract, indicating that its antigenic features were retained throughout the purification procedure.

As mentioned above, 85 % of the protein content in SP was constituted by two proteins, UB and LB, having molecular weights of 105,000 and 95,000, respectively. Complete identity was obtained when rabbit hyperimmune sera raised against each protein (anti-UB and anti-LB) were reacted in DID with SP, indicating that the purified antigen B was formed by two proteins or subunits. At least two possibilities arise: namely, either the larvae synthesize two antigenically identical proteins differing in molecular weight by 10,000, or the 95,000-dalton protein is a product of hydrolysis of the 105,000-protein, formed either as a normal post-transcriptional product of the parasite, or as an artifact during the purification procedure. However, since the homogenization of the cysticerci was carried out rapidly (1–2 min) and in the presence of highly effective inhibitors of proteolytic activity, it is more probable that these proteins were extracted from two preexisting pools in the parasite. Experiments are in progress to examine these alternatives.

Since the two proteins of antigen B showed antigenic identity and since we have not obtained them in a separate soluble form, amino acid analysis was carried out on the DP fraction. Consequently, the figures obtained represent the average content of residues in the two proteins. This analysis indicated that antigen B is composed of proteins that are uncommon with respect to their amino acid composition [19]. The mole fractions for Asp, Glu, Lys, and Arg represented approximately 52 % of the total, implying that about half of the residues may be charged. This high charge density could explain the following odd behavior of antigen B: antigen B, composed of proteins of 95,000 and 105,000 daltons, was excluded from a Sephadex G-200 column (Fig. 2); antigen B did not migrate in immunoelectrophoresis at pH 8.6 (Figs. 6 and 7), although its isoelectric point was between 5.0 and 5.3 as determined by isoelectric-focusing; and in the presence of as little as 30 μM ATP, SP precipitated as fiberlike structures having no ATPase activity (Fig. 8).

These properties may result from the abundance of charged residues in the protein that could lead to a great variety of ionic interactions with other charged molecules, as well as with itself. If antigen B at pH 2.5 interacts with itself, it could form aggregates that would be excluded from Sephadex G-200. It is possible that at pH 8.6, different types of aggregates are formed that by their size or by having an average neutral surface charge would be isoelectric in immunoelec-

trophoresis [20]. With respect to the ATP-induced precipitation, since it was not dependent on hydrolysis of the nucleotide, it is likely that ATP was simply acting as a polyanion to produce this precipitation.

Purification of antigen B is of interest because of its possible use in immunodiagnosis and vaccination. As cysticercosis is a common disease in several countries [21–25] and produces very heterogeneous clinical manifestations, the development of a specific and sensitive method for the identification of this disease is desirable. Throughout this century immunological methods have been used in many countries for the diagnosis of cysticercosis [5, 6, 26, 27]. However, all methods that have been tested lack sensitivity and/or specificity [28]. Several reasons for false negative results have been postulated, among others, the inherent lack of sensitivity of the method used [7]; the existence of immunologically nonresponsive patients [6]; and the use of nonrepresentative antigen preparations [29]. The use of a crude extract as a source of antigen may explain the lack of sensitivity and/or specificity of the immunodiagnostic methods [8]; also, this could be the reason for inadequate concentrations of prominent antigens in the immunological test. The purification of parasite antigens and their use in serological methods could improve the immunodiagnosis of cysticercosis. Since antigen B is the one most frequently recognized by sera of patients, it is a good choice for use in the development of improved immunological techniques for the diagnosis of cysticercosis [see chapter by B. Espinoza *et al.* in this volume].

Vaccination in experimental cysticercosis has demonstrated that protection can be obtained against the disease, although with variable results and efficiencies [7]. IgG has been recognized as a protective class of antibodies in cysticercosis [9–11]. Semipurified antigens from other cyclophyllidean cestodes have been reported to produce very high levels of protection. Of these, the fractions isolated from the larvae of *T. taeniaeformis* [30] and from *Echinococcus granulosus* [31, 32] are particularly interesting because of the similarity of their molecular weight with that of antigen B.

Now that antigen B – the antigen most frequently recognized by the sera of cysticercotic patients and which induces IgG [6] – has been successfully purified, it is a likely prospect for pig and human vaccination, although the immunizing and toxic properties of this antigen have yet to be properly studied.

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COMENTARIOS FINALES

En el presente trabajo se describe un procedimiento para la purificación del antígeno B del cisticerco de la T. solium. Las características bioquímicas e inmunológicas de este antígeno están descritas y discutidas en el sobretiro anexo.

La disponibilidad de antígeno B en forma pura, ha permitido el estudio de algunos aspectos de la biología de la cisticercosis y ha abierto nuevas posibilidades de estudio en el inmunodiagnóstico e inmunoprofilaxis de esta parasitosis. A continuación solo se mencionarán algunas de las nuevas líneas de investigación que están siendo exploradas en parte, con base en los resultados de esta Tesis.

La información existente en la literatura sobre las características bioquímicas e inmunológicas de antígenos purificados de otros Céstodos, indica que se trata de un antígeno no descrito anteriormente. Sin embargo, esto no significa que el antígeno B está ausente en otros parásitos. Al respecto, Olivo, D.A. (36) ha buscado el antígeno B en Platelmintos, Nemátodos y Acantocéfalos por inmunoelectroforesis, utilizando sueros anti-antígeno B en contra de extractos crudos de los organismos probados. Los resultados indicaron que el antígeno B está presente en las formas adultas de T. solium, T. saginata, T. taeniaeformis, en Cisticercus bovis, Cisticercus tenuicolis, Cisticercus pisiformis, Cisticercus crassiceps, Dipylidium caninum y Fasciola hepatica. Estos resultados sugieren que el antígeno B es una proteína muy conservada en organismos parásitos

del filum de los Platelmintos.

Recientemente, Plancarte y cols. (37,38) han descrito que el antígeno B puro fluoresceinado cuando se aplica en cortes de diferentes -- tejidos, se une específicamente al tejido conectivo. Adicionalmente, el - antígeno B posee la capacidad de aglutinar eritrocitos de varias especies, de orientar fibroblastos en cultivo in vitro y de unirse a colágena con alta afinidad. Estas propiedades son consistentes con una proteína del tipo de las fibronectinas (38). Asimismo, las propiedades de fibronectina del antígeno B sugieren una participación activa de esta proteína en los mecanismos involucrados en la relación huésped-parásito: los cisticercos vivos en el huésped intermediario están rodeados por una respuesta inflamatoria granulomatosa rica en tejido conectivo (39). Una actividad de fibronectina en las cercanías del parásito podría protegerlo de la respuesta -- del huésped al inducir la formación de una pared de componentes del huésped que sean inofensivos para el parásito y que impidan el contacto con -- moléculas y células del sistema inmune del huésped. Esta sugerencia del - papel defensivo del antígeno B con propiedades de fibronectina, representan una hipótesis novedosa dentro de los mecanismos propuestos para la evasión de la respuesta inmune del huésped por parásitos.

Los siguientes hallazgos sugieren que el antígeno B unido al tejido conectivo de la cápsula inflamatoria podría distraer el ataque por anticuerpos del huésped: 1) Las células plasmáticas presentes en la cápsula inflamatoria alrededor del cisticerco sintetizan anticuerpos anti-antígeno B (40), 2) El antígeno B se encuentra en la cápsula inflamatoria -- (Laclette, J.P., comunicación personal) y 3) El factor C-3 del complemen-

to se encuentra asociado al tejido, conectivo de la cápsula inflamatoria - (40,41). Sin embargo, no sería necesario que el antígeno B se uniera al - tejido conectivo del huésped para impedir el ataque por anticuerpos. Una liberación contínua de este antígeno podría tener el efecto de una "pantalla de humo" (smoke screen effect) que bloquearía a los anticuerpos antes de que estos llegaran a la superficie del parásito (42). Aunque la utilidad de el antígeno B puro en pruebas para el inmunodiagnóstico de la cisticercosis se ha visto limitada por el hecho de que se encuentra en otros -- parásitos (36), una preparación semi pura del antígeno B ha sido utilizada para establecer un método de ELISA (Enzyme-linked-Immunosorbent-Assay) que permite la búsqueda de anticuerpos en sueros y líquido cefalorraquídeo de pacientes cisticercosos. De este modo ha sido posible detectar anticuerpos anti antígeno B en el 73% de los sueros y en el 85% de los líquidos cefalorraquídeos de pacientes (43). Estos resultados indican que se producen anticuerpos anti B en la mayor parte de los pacientes, la cual apoya nuevamente la importancia de este antígeno en la relación huésped-cisticerco. - Sin embargo, el uso de un extracto crudo del parásito en lugar de antígeno B en la prueba de ELISA, aumenta la sensibilidad de la misma: varios autores (44,45) han reportado la detección de anticuerpos en el 79% de los sueros de pacientes cisticercosos. Por lo tanto, aunque la utilización de -- antígeno B en el ensayo de ELISA mejoró la sensibilidad que se conseguía - por los métodos tradicionales, parece claro que el uso de mezclas de varios antígenos relevantes podrían incrementar aún mas la sensibilidad, incluso por arriba de la que se logra usando un extracto crudo.

Con respecto a las líneas de investigación que sobre antígeno B se llevan a cabo en el laboratorio, cabe mencionar los esfuerzos para lo

calizar a este antígeno en el tejido del parásito. Resultados de experi-- mentos de inmunofluorescencia muestran que éste antígeno se encuentra primordialmente en las células subtegumentales (Laclette, J.P., comunicación personal). Adicionalmente, experimentos de incorporación de isótopos ra-- diactivos a componentes de superficie del cisticerco indican su exposición en la superficie (Laclette, J.P., comunicación personal). Puesto que el - papel secretor de las células subtegumentales ha sido ampliamente documentado en Taenidos (46), parece válido pensar que el antígeno B es sintetizado en estas células para luego ser secretado a la interfase huésped-cisticerco. Finalmente, se encuentran en proceso de desarrollo la producción - de anticuerpos monoclonales anti-antígeno B (Díaz Camacho, S., comunicación personal). El uso de anticuerpos monoclonales en técnicas de separación - por afinidad se espera posibilite la purificación de mayores cantidades de antígeno B de las que actualmente se obtienen. La disponibilidad de anticuerpos monoclonales y cantidades considerables de antígeno B puro, permitirá el inicio de algunas líneas de investigación que a la fecha no han sido abordadas. Una de ellas es el estudio de la reactividad de fragmentos polipeptídicos de las dos subunidades del antígeno B con anticuerpos provenientes de varias clonas para determinar si existen regiones de la molécula con alta actividad epitópica, así como para dilucidar si además de los epitopos comunes con antígeno B de otras especies, existen determinantes - antigénicos específicos del cisticerco de la T. solium.

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