Purification of the Major Concanavalin A-Binding Surface Glycoprotein from Adult *Schistosoma mansoni*

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**ABSTRACT:** Radiolabeled freeze--thaw and detergent-extracted antigens from adult *Schistosoma mansoni* were fractionated by concanavalin A--Sepharose 4B affinity chromatography to isolate specific surface membrane glycoproteins. SDS-PAGE and ampholine isoelectric focusing of the α-methylmannoside eluate reveal the major component to have a molecular weight of approximately 58,000 and pI value of approximately 4.8. Surface localization of the glycoprotein is inferred by its extraction by low concentrations of non-ionic detergent, and by the ability of fluorescein-labeled Con A to bind to the surface of adult worms but not internally. Surface binding of Con A is inhibited by the purified glycoprotein. The glycoprotein reacts with sera from infected humans, as shown by enzyme-linked immunoassay (ELISA). The protocol for large-scale purification of this antigen is described in detail. An ancillary result of this work is the isolation of a second glycoprotein, not necessarily a surface antigen, that has a molecular weight of approximately 300,000, pI value of approximately 4.2, and also reacts with sera from infected humans.

Numerous reports have documented the presence of parasite antigens that stimulate a wide spectrum of immune effector mechanisms involving antibodies, complement fixation, and host cells (see review by Ogilvie et al., 1980). Although the relevance of these reactions to protective immunity is not well established, there can be little doubt that the detection, extraction, and isolation of antigens is central to any understanding of their role in immunity.

The presence of surface antigens that bind concanavalin A has been demonstrated for both *Schistosoma mansoni* schistosomes (Murrell et al., 1978) and adult worms (Simpson and Smithers, 1980). By using Con A affinity chromatography, Bennett and Seed (1977) isolated a glycoprotein fraction from adult *S. mansoni* consisting of picogram quantities of several components. However, efforts to “scale up” production have been hampered by the heterogeneity of tegumental antigen preparations, nonspecific (and sometimes irreversible) binding of material to Con A--Sepharose columns, and low yields of the final product. By using a sequence of column separations, we have been able to isolate microgram amounts of a Con A-binding surface glycoprotein from adult *S. mansoni*. The purified antigen migrates as a single band on SDS gels.

**Materials and Methods**

**Collection of parasites**

Adult worms of a Puerto Rican strain of *S. mansoni* were obtained from anesthetized NIH/NMRI mice 7 wk after infection, by portal perfusion with citrated saline. Worms were collected on a 183-μm-mesh Nitex screen (Tetko Inc., New York) and washed several times with citrated saline; pieces of debris and visibly

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damaged worms were removed by pipette. In addition to live material, lyophilized *S. mansoni* adult worms were obtained in 100-mg (dry weight) aliquots from the WHO Special Programme for Research and Training in Tropical Diseases.

**Crude antigen preparation**

A freeze–thaw preparation of adult worms (AFT-1) was made as described previously (Murrell et al., 1974). Briefly, adult worms were frozen in Hanks’ balanced salt solution, pH 7.2, at −70°C. After thawing at room temperature, the mixture was filtered through a Nitex screen to remove worm carcasses and the filtrate centrifuged at 10,000 *g* for 30 min; the supernatant was then dialyzed against 0.15 M borate-buffered saline (BBS), pH 7.6, and concentrated by Amicon® PM-10 membrane filtration (10 µm) to a final concentration of 5–8 mg/ml.

A detergent extract was prepared by incubating approximately 1,000 worms in 5 ml borate-buffered saline (BBS) containing 0.5% aprotinin enzyme inhibitor (Trasylol®, FBA Pharmaceuticals, New York) and 0.05% Nonide P-40 for 15 min at 37°C. The mixture was centrifuged at 1,000 *g* for 5 min and the supernatant collected.

Lipids were extracted from the lyophilized worms with chloroform : methanol (following Hayunga et al., 1982). Approximately 50 mg (dry weight) of lyophilized *S. mansoni* was suspended in 5 ml chloroform : methanol (2:1) and disrupted in a ground-glass tissue homogenizer. After 2 hr extraction at room temperature, the mixture was centrifuged at 3,000 *g* for 30 min and the supernatant removed; a second extraction was done overnight at 4°C. The pellet was next resuspended in 1 ml of 0.5% Nonidet P-40 in BBS, incubated for 30 min at 37°C, and centrifuged at 3,000 *g* for 30 min. The supernatant was dialyzed against several changes of BBS.

**Protein radioiodination**

Protein labeling with ¹²⁵I-iodinated *p*-hydroxyphenylpropionic acid, N-hydroxysuccinimide ester, was carried out following the method originally described by Bolton and Hunter (1973). Approximately 100 µg protein in a 300-µl volume was radioiodinated with 0.5 mCi Bolton–Hunter reagent (specific activity 4,000 Ci/mmol, molecular weight 511.2) (New England Nuclear) in each experiment. The detergent extract was dialyzed against 0.1 M borate buffer, pH 8.5, prior to labeling; the more concentrated freeze–thaw antigen was simply diluted 1:10 in the buffer. After labeling for 30 min at 4°C, the reaction was terminated by the addition of 500 µl 1% glycine in borate buffer. Samples were then dialyzed against 1-liter volumes of 0.1 M sodium acetate buffer (pH 6.8, containing 0.5 mM Ca++ and Mn++) to remove unbound label and to facilitate the next step in the fractionation procedure. Dialysis was considered complete when the buffer contained less than 2,000 cpm/ml and when at least 75% of the labeled sample could be precipitated by 20% w/v trichloroacetic acid (TCA); four or five changes of buffer were usually sufficient.

**Affinity chromatography**

Analytical separation using concanavalin A or *Lens culinaris* (lentil) lectin was performed as described previously (Hayunga et al., 1982). After exhaustive dialysis
against the 0.1 M sodium acetate buffer, an aliquot (=approximately 1,000,000 cpm) of labeled antigen was applied to a 0.5 × 10-cm column of concanavalin A–Sepharose 4B (Pharmacia) or lentil lectin–Sepharose 4B (Pharmacia) at a flow rate of 0.2 ml/min. After collection of the initial peak, the column was thoroughly washed with 300 ml acetate buffer, then eluted with acetate buffer containing 0.1 M α-methylmannoside (methyl-α-D-mannopyranoside). The eluate was dialyzed against distilled water, then fractionated by ampholine isoelectric focusing column, or lyophilized and reconstituted in sample buffer for SDS-PAGE, as described below.

Preparative-scale affinity chromatography was performed using a 0.9 × 20-cm concanavalin A–Sepharose 4B column equilibrated with acetate buffer, at a flow rate of 0.2 ml/min. Initially, samples consisted of 1–2 ml (approximately 5–10 mg) AFT-1 antigen that had been dialyzed against acetate buffer. Bound material was eluted from the column using acetate buffer containing 0.1 M α-methylmannoside, followed by 0.15 M BBS (pH 7.6), then 0.45 M borate buffer (pH 6.0). Later separations were done by application of 15–20 ml (approximately 3 mg) of eluate from a Sephacryl S-200 column (described below), thorough washing with approximately 500 ml acetate buffer, then elution using 0.45 M borate. In all cases, the eluted fractions were pooled and dialyzed against distilled water for subsequent analysis.

Gel filtration

Aliquots of 5 mg of lipid-extracted antigen (1 mg/ml protein) in 0.1 M acetate buffer, pH 6.8, were applied to a 1.5 × 25-cm column of Sephacryl S-200 Superfine (Pharmacia). The column was eluted with acetate buffer at a flow rate of 3 ml/hr and the eluate was collected in 2-ml fractions. Absorbance at 280 nm was determined using a Gilford Model 250 spectrophotometer.

SDS-PAGE

Lyophilized samples were resuspended in 150–200 μl sample buffer consisting of 0.04 M Tris-HCl (pH 7.0) containing 2% SDS, 20% glycerol, 50 mM dithiothreitol (DTT), and 20 μg/ml bromophenol blue. Electrophoresis was performed using 5-mm-i.d. tubes in a continuous buffer system at 2.5 mA per tube for 2.5 hr. The electrophoresis buffer consisted of 14.4 g/liter glycine, 3 g/liter Tris, and 1 g/liter SDS, pH 8.0. The 7½% running gel consisted of 76.4 g/liter acrylamide, 2.01 g/liter N,N'-methylenebisacrylamide, 0.52 g/liter SDS, 0.3 ml/liter TEMED, and 0.37 g/liter ammonium persulfate in 0.4 M Tris-HCl, pH 7.0; total volume was 2.5 ml per tube. The stacking gel consisted of 30 g/liter acrylamide, 0.79 g/liter N,N'-methylenebisacrylamide, 1.43 g/liter SDS, 0.36 ml/liter TEMED, and 0.8 g/liter ammonium persulfate in 0.14 M Tris-HCl, pH 7.0; total volume was 200 μl per tube. Gels were removed from the glass tubes, frozen, cut into 2-mm slices using a Bio-Rad gel slicer, and counted using an LKB 1270 RackGamma II.

Isoelectric focusing

After dialysis against distilled water (to remove salts), samples were applied to a 110-ml LKB Multiphor® ampholine isoelectric focusing column and run for 18 hr at 15 W, maximum voltage 1,600 V. A discontinuous sucrose gradient was
made by manual layering of sucrose solutions containing ampholine. Sample was added to the middle of the gradient. Separations were performed using pH range of 3.5–10.0 or 2.5–6.0. Detailed description of ampholine mixtures and sample application may be found in literature supplied by the manufacturer.

Reactivity with antisera

The ability of the purified antigen to react with antisera was evaluated by enzyme-linked immunoassay (EIA) as described by Stek and Kassim (1983). Briefly, 0.2–1.0 μg of antigen was used to coat the plastic wells of microtiter plates, then was incubated with antisera followed by alkaline phosphatase-labeled goat anti-human IgG and p-nitrophenyl phosphate substrate in a “sandwich” fashion. Sera from patients infected with either *S. mansoni* or *Schistosoma haematobium* were obtained from Dr. E. Higashi, NAMRU-3, Cairo; *Schistosoma japonicum*, from Dr. J. Cross, NAMRU-2, Manila.

Inhibition of antibody binding

Approximately 0.4 μg of antigen was used to coat the plastic wells of microtiter plates, as in a typical EIA. The next incubation, however, consisted of specific antibody (human *S. mansoni*-infection serum) diluted 1:100 in solutions of various monosaccharides. Thus, if the monosaccharide were to compete with structurally similar binding sites of the antigen, antibody binding and subsequent colorimetric reaction would be reduced. Inhibition was measured using nine sugars and amino sugars (D-galactose [gal], D-galactosamine [galNH], N-acetyl-D-galactosamine [galNAc], D-glucose [glc], D-glucosamine [glcNH], N-acetyl-D-glucosamine [glcNAC], D-mannose [man], D-fucose [fuc], and methyl-α-D-mannopyranoside [Me-α-man]) prepared in concentrations of 1, 10, 100, and 1,000 μg/ml, and 1% and 10% w/v. Incubations using conjugate (goat anti-human IgG bound to alkaline phosphatase) and substrate (p-nitrophenyl phosphate) were conducted as before.

Inhibition of concanavalin A surface binding by purified antigen

Aliquots of approximately 10–15 *S. mansoni* adult worms were incubated at 37°C for 30 min in Earle’s balanced salt solution (without phenol red indicator) containing fluorescein isothiocyanate-conjugated (FITC) Con A (E.Y. Laboratories, San Mateo, California) both with and without the addition of the purified 58,000-molecular-weight glycoprotein. Incubation was carried out using 25 μg/ml and 2.5 μg/ml FITC–Con A. Antigen concentrations were 180 μg/ml and 0 μg/ml (control). After incubation, worms were washed three times in 10 ml Earle’s salts (without phenol red indicator) at room temperature. Worms were placed on a glass slide containing one drop of Earle’s salts, and were examined using a Nikon “Optiphoto” fluorescent microscope at 530 nm.Slides were scored subjectively using a scale of — to ++++. 

Reproducibility

Antigen fractionation by affinity chromatography, ampholine isoelectric focusing, and SDS-PAGE was repeated over a dozen times with similar, if not identical, results. Enzyme-linked immunoassay (EIA) was also found to be routinely repro-
ducible; assays described in this paper were performed at least three times. Data presented in the figures and tables are, in each case, the results of a single representative experimental run. Inhibition of FITC–Con A surface binding by antigen was done only once to conserve limited amounts of the purified antigen.

Buffers

Acetate buffer: 0.1 M sodium acetate to which was added 0.5 mM Ca\(^{2+}\) and Mn\(^{2+}\) (99 mg/liter MnCl\(_2\)-4H\(_2\)O and 56 mg/liter anhydrous CaCl\(_2\) ); pH was adjusted to 6.8 by the addition of 1 N acetic acid.

Borate buffer for radiolabeling: 0.1 M borate buffer prepared using 6.18 g/liter boric acid and 0.50 g/liter NaOH; pH was adjusted to 8.5 by the addition of approximately 5 ml 1 N NaOH.

Borate buffer for elution: 0.45 M borate buffer with molarity calculated as boron equivalents (following Svensson et al., 1970) prepared using 42.86 g/liter sodium tetraborate (Na\(_2\)B\(_4\)O\(_7\)-10H\(_2\)O) and adjusted to pH 6.0 by the addition of approximately 56 g/liter solid KH\(_2\)PO\(_4\).

Borate buffered saline (BBS): 0.15 M BBS prepared using 11 g/liter boric acid and 6.3 g/liter NaCl; pH was adjusted to 7.6 by the addition of 1 N NaOH.

Detergent solution: 0.05% v/v Nonidet P-40 (non-ionic) detergent prepared in BBS containing 5 ml/liter aprotinin enzyme inhibitor (Trasylol\(^{®}\)).

Electrophoresis buffer: 14.4 g/liter glycine, 3 g/liter Tris, and 1 g/liter sodium dodecyl sulfate (SDS), pH 8.0.

Glycine solution: 1% glycine prepared in 0.1 M borate buffer, pH 8.5.

Hanks' balanced salt solution (Hanks' BSS): prepared commercially by Gibco, Grand Island, New York.

Sample buffer (for SDS-PAGE): 0.04 M Tris-HCl (pH 7.0), containing 2% w/v SDS, 20% v/v glycerol, 50 mM dithiothreitol (DTT), and 20 \(\mu\)g/ml bromophenol blue (BB).

Sugar solution for elution: 0.1 M \(\alpha\)-methylmannoside prepared in 0.1 M acetate (pH 6.8), containing 0.5 mM Ca\(^{2+}\) and Mn\(^{2+}\) (as above).

Results

Analytical separation using radiolabeled material

Bolton–Hunter radioiodinated freeze–thaw antigen in acetate buffer was applied to a 0.5 \(\times\) 10-cm Con A–Sepharose 4B column; the column was thoroughly washed, then eluted with 0.1 M \(\alpha\)-methylmannoside to yield a single peak. Analysis of the labeled eluate by SDS-PAGE gave variable results: between one and four peaks might be observed with material eluted in a given experimental run. A peak of approximately 58,000 molecular weight was found in all instances; in some experiments, peaks of approximately 30,000, 94,000, and 150,000 molecular weight were also detected (Fig. 1A). In order to rule out nonspecific adsorption by Con A–Sepharose, the eluate was dialyzed (to remove the \(\alpha\)-methylmannoside) and then run on a second Con A column. The \(\alpha\)-methylmannoside eluate from the second column was comprised primarily of the 58,000-molecular-weight peak (Fig. 1C), although minor peaks persisted. This was true for both unreduced samples and samples reduced using 50 mM dithiothreitol (DTT). Fractionation of the eluate by ampholine isoelectric focusing revealed a single peak with a pI
Figure 1. SDS-PAGE profile of radiolabeled *Schistosoma mansoni* proteins after separation by lectin affinity chromatography, 7½% gels, 2-mm slices. A. Alpha-methylmannoside eluate of Bolton-Hunter labeled freeze-thaw antigen separated by Con A-Sepharose 4B. B. Eluate of freeze-thaw antigen separated by lentil lectin-Sepharose 4B. C. Eluate of freeze-thaw antigen separated by two successive Con A columns. D. Eluate of detergent extracted tegumental antigen separated by Con A-Sepharose 4B.

Figure 2. Flow diagram depicting the fractionation protocol for purifying the Con A-binding antigen from *Schistosoma mansoni* adult worms.
Figure 3. Fractionation of antigen from lyophilized adult worms by gel filtration using Sephacryl S-200 Superfine.

The value of approximately 4.8. The ampholine peak, in turn, yielded a single peak when run on SDS gels. Thus, we concluded that the 58,000-molecular-weight component was the major Con A-binding glycoprotein extracted from *S. mansoni* adult worms. The minor peaks may have represented (a) glycoproteins that react only weakly with Con A, (b) labile Con A-binding glycoproteins that are unable to withstand excessive handling, or (c) material nonspecifically adsorbed by the column.

Tegumental material extracted by incubation of worms in 0.05% Nonidet P-40 detergent was also labeled and analyzed. SDS gels of the α-methylmannoside eluate of this material showed only a single peak of approximately 58,000 molecular weight (Fig. 1D). This would suggest that the 58,000-molecular-weight component is the major Con A-binding site associated with the parasite’s surface.

For comparison, radiolabeled freeze–thaw antigen was applied to a lentil lectin–Sepharose 4B column, and the α-methylmannoside eluate was analyzed by SDS-PAGE. As shown in Figure 1B, this procedure yielded a single peak of approximately 94,000 molecular weight; the 58,000-molecular-weight peak that bound to concanavalin A did not react with lentil lectin. Data from affinity chromatography using other lectins will be described in detail elsewhere.

**Isolation and purification of the antigen**

Starting with lyophilized *S. mansoni* adult worms, the isolation protocol involves chloroform: methanol extraction, Sephacryl S-200 gel filtration, Con A–Sepharose 4B affinity chromatography, and ampholine isoelectric focusing (Fig.
Table 1. Amount of purified glycoprotein isolated from *Schistosoma mansoni* adult worms.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Con A-binding glycoprotein</th>
<th>High-molecular-weight glycoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,000 adult <em>S. mansoni</em></td>
<td>5 ml</td>
<td>5 ml</td>
</tr>
<tr>
<td>Lyophilized worms</td>
<td>275 mg</td>
<td>275 mg</td>
</tr>
<tr>
<td>Pellet after chloroform : methanol extraction</td>
<td>215 mg</td>
<td>215 mg</td>
</tr>
<tr>
<td>Detergent extract</td>
<td>5 mg</td>
<td>5 mg</td>
</tr>
<tr>
<td>Sephacryl “Fraction I”</td>
<td></td>
<td>1.9 mg</td>
</tr>
<tr>
<td>Ampholine peak, pi = 4.27</td>
<td></td>
<td>~350 μg</td>
</tr>
<tr>
<td>Sephacryl “Fraction II”</td>
<td>2.7 mg</td>
<td></td>
</tr>
<tr>
<td>Concanavalin A eluate</td>
<td>300 μg</td>
<td></td>
</tr>
<tr>
<td>Ampholine peak, pi = 4.85</td>
<td>~40 μg</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Enzyme-linked immunosassay (EIA) to detect reactivity of crude and purified antigen preparations. Data expressed as absorbance at 405 nm after 1-hr incubation with *p*-nitrophenyl phosphate substrate.

<table>
<thead>
<tr>
<th>Antigen preparations*</th>
<th>S. mansoni AFT-1 (4 μg/ml)</th>
<th>S. mansoni AFT-1 (8 μg/ml)</th>
<th>S. mansoni AFT-1 (10 μg/ml)</th>
<th>S. mansoni Con A (4 μg/ml)</th>
<th>S. mansoni Con A (6 μg/ml)</th>
<th>S. japonicum Con A (4 μg/ml)</th>
<th>Serum blank (no antigen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mansoni infection serum</td>
<td>0.482</td>
<td>0.673</td>
<td>0.643</td>
<td>0.344</td>
<td>0.408</td>
<td>0.628</td>
<td>0.107</td>
</tr>
<tr>
<td>S. haematobium infection serum</td>
<td>0.304</td>
<td>0.502</td>
<td>0.571</td>
<td>0.360</td>
<td>0.431</td>
<td>0.576</td>
<td>0.086</td>
</tr>
<tr>
<td>S. japonicum infection serum</td>
<td>0.277</td>
<td>0.293</td>
<td>0.324</td>
<td>0.499</td>
<td>0.522</td>
<td>0.656</td>
<td>0.083</td>
</tr>
<tr>
<td>Normal human serum (no serum)</td>
<td>0.067</td>
<td>0.063</td>
<td>0.080</td>
<td>0.090</td>
<td>0.098</td>
<td>0.113</td>
<td>0.037</td>
</tr>
</tbody>
</table>

* *Schistosoma mansoni* freeze-thaw antigen (AFT-1) prepared following Murrell et al. (1974); *S. mansoni* Con A-binding antigen described in the present paper; *S. japonicum* Con A-binding antigen prepared following Hayunga et al. (1982).

Table 3. Enzyme-linked immunosassay (EIA) to detect reactivity of crude and purified antigen preparations. Data expressed as absorbance at 405 nm after 90-min incubation with *p*-nitrophenyl phosphate substrate.

<table>
<thead>
<tr>
<th>Antigen preparations*</th>
<th>S. mansoni AFT-1 (4 μg/ml)</th>
<th>S. mansoni AFT-1 (8 μg/ml)</th>
<th>S. mansoni Fx I (2 μg/ml)</th>
<th>S. mansoni Fx I (4 μg/ml)</th>
<th>S. mansoni Fx I (8 μg/ml)</th>
<th>Serum blank (no antigen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mansoni infection serum</td>
<td>0.563</td>
<td>0.615</td>
<td>0.483</td>
<td>0.598</td>
<td>0.785</td>
<td>0.107</td>
</tr>
<tr>
<td>S. haematobium infection serum</td>
<td>0.502</td>
<td>0.548</td>
<td>0.411</td>
<td>0.469</td>
<td>0.624</td>
<td>0.095</td>
</tr>
<tr>
<td>S. japonicum infection serum</td>
<td>0.314</td>
<td>0.348</td>
<td>0.376</td>
<td>0.433</td>
<td>0.556</td>
<td>0.106</td>
</tr>
<tr>
<td>Normal human serum (no serum)</td>
<td>0.129</td>
<td>0.159</td>
<td>0.098</td>
<td>0.098</td>
<td>0.133</td>
<td>0.072</td>
</tr>
</tbody>
</table>

* *Schistosoma mansoni* freeze-thaw antigen (AFT-1) prepared following Murrell et al. (1974); *S. mansoni* large-molecular-weight antigen (Fx I) purified by ampholine column as described in the present paper.
Figure 4. Fractionation of Fraction I from the Sephacryl gel filtration column by ampholine isoelectric focusing, pH gradient 3-10. Subsequent analysis of this peak by SDS-PAGE revealed a molecular weight of approximately \( \geq 300,000 \).

2) Although the antigen can also be isolated from freeze–thaw preparations (AFT-1), we found no increase in yield to justify the extra effort involved in making AFT-1.

Approximately 4,000 lyophilized adult worms (=approximately 275 mg, dry weight) were resuspended in 25 ml chloroform : methanol (2:1) and disrupted in a ground-glass tissue homogenizer. After 2 hr extraction at room temperature, the mixture was centrifuged at 3,000 g for 30 min and the supernatant removed; a second extraction was done overnight at 4°C. This procedure removed approximately 60 mg of material. The pellet was dried, then resuspended in 5 ml 0.5% Nonidet P-40 (non-ionic) detergent in 0.1 M acetate buffer, incubated for 30 min at 37°C, and centrifuged at 3,000 g for 30 min. The supernatant, which contained approximately 5 mg protein, was dialyzed against two changes of acetate buffer (to remove detergent), then applied to a 1.6 \( \times \) 25-cm Sephacryl S-200 column at a flow rate of 3 ml/hr.

Gel filtration on the Sephacryl S-200 column yielded the elution profile shown in Figure 3. Fraction I consisted of the large-molecular-weight (approximately 300,000) yellowish material that we believe had interfered with initial attempts at preparative affinity chromatography (see discussion). Analysis of this fraction by ampholine isoelectric focusing (Fig. 4) revealed a single peak with an approximate \( p_l \) value of 4.2 (range 4.0–4.4). The component that we sought to purify (molecular weight = 58,000) eluted around the “valley” between Fractions IIa and IIb (Fig. 3). Fractions IIa and IIb (which together contained approximately 2.7 mg protein) were pooled and applied directly to a preparative (0.9 \( \times \) 20 cm) Con A–Sepharose 4B column, washed with acetate buffer, then eluted with 0.45 M borate to yield a single peak.
The eluate from the Con A column (approximately 300 μg) was next dialyzed against distilled water to remove salt, then run on a 110-ml ampholine isoelectric focusing column, pH range 2.5–6.0. As shown in Figure 5, the glycoprotein migrated as a prominent peak, with a pI value of approximately 4.8 (range 4.6–4.9). The minor peaks on this figure probably represent contamination or products of enzymatic degradation that would not have been removed without the ampholine step.

From an initial amount of over 4,000 worms (5 ml packed volume, approximately 275 mg, dry weight), we were able to recover about 40 μg of the Con A-binding glycoprotein and 350 μg of the high-molecular-weight antigen (Table 1). Purity of either final product can be judged by migration as a single peak on the ampholine column (Figs. 4, 5).

**Reactivity with antisera**

Purified antigens were compared to crude freeze-thaw (AFT-1) antigen for their ability to react with antisera in the enzyme-linked immunoassay (EIA). The Con A-binding antigen from *S. mansoni* was found to react with sera from patients infected with *S. mansoni, S. haematobium*, or *S. japonicum* (Table 2). In such a comparison, interpretation must be guarded because quantitative results may simply reflect differences in serum titers. However, it would appear that this antigen is more cross-reactive than the crude (AFT-1) preparation. Similarly, a Con A-binding antigen isolated from *S. japonicum* (Table 2) and the large-molecular-weight antigen isolated from *S. mansoni* (Table 3) were also highly cross-
reactive. Reaction with any of the infection sera was significantly greater than with normal serum.

Reaction of *S. mansoni* infection serum with the Con A-binding glycoprotein could be inhibited by the addition of any of several monosaccharides (Table 4). Inhibition was obtained using 10% fucose, mannose, or α-methylmannoside, but not with glucose. Reduction of antibody binding also occurred with galactosamine and glucosamine. Inhibition occurred only at very high concentrations, and in no instance was 100% inhibition of antibody binding achieved.

**Inhibition of Con A surface binding**

Fluorescein isothiocyanate-conjugated concanavalin A (FITC–Con A) was found to bind the surface of *S. mansoni* adult worms. The reaction was greatest after 30 min incubation with 25 μg/ml FITC–Con A; a weaker positive reaction could also be obtained using a concentration of 2.5 μg/ml. At either concentration, addition of the 58,000-molecular-weight glycoprotein to the incubation medium was found to reduce surface binding of the labeled lectin (Table 5). Although we
Table 4. Modified enzyme-linked immunoassay (EIA) to detect inhibition of antibody binding by various carbohydrates. Data expressed as absorbance at 405 nm after 30-min incubation with p-nitrophenyl phosphate substrate.*

<table>
<thead>
<tr>
<th>Sugar</th>
<th>0</th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>1,000</th>
<th>10,000 (1%)</th>
<th>100,000 (10%)</th>
<th>Serum blank (no antigen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glc</td>
<td>0.521</td>
<td>0.458</td>
<td>0.463</td>
<td>0.546</td>
<td>0.540</td>
<td>0.563</td>
<td>0.531</td>
<td>0.031</td>
</tr>
<tr>
<td>glcNH</td>
<td>0.593</td>
<td>0.525</td>
<td>0.449</td>
<td>0.496</td>
<td>0.572</td>
<td>0.499</td>
<td>0.268</td>
<td>0.036</td>
</tr>
<tr>
<td>glcNAc</td>
<td>0.520</td>
<td>0.445</td>
<td>0.423</td>
<td>0.537</td>
<td>0.480</td>
<td>0.432</td>
<td>0.349</td>
<td>0.035</td>
</tr>
<tr>
<td>gal</td>
<td>0.561</td>
<td>0.559</td>
<td>0.492</td>
<td>0.505</td>
<td>0.498</td>
<td>0.466</td>
<td>0.397</td>
<td>0.032</td>
</tr>
<tr>
<td>galNH</td>
<td>0.565</td>
<td>0.507</td>
<td>0.534</td>
<td>0.522</td>
<td>0.492</td>
<td>0.527</td>
<td>0.247</td>
<td>0.041</td>
</tr>
<tr>
<td>galNAc</td>
<td>0.538</td>
<td>0.523</td>
<td>0.519</td>
<td>0.472</td>
<td>0.497</td>
<td>0.475</td>
<td>0.335</td>
<td>0.037</td>
</tr>
<tr>
<td>fuc</td>
<td>0.456</td>
<td>0.398</td>
<td>0.371</td>
<td>0.423</td>
<td>0.346</td>
<td>0.281</td>
<td>0.211</td>
<td>0.015</td>
</tr>
<tr>
<td>man</td>
<td>0.552</td>
<td>0.521</td>
<td>0.401</td>
<td>0.506</td>
<td>0.389</td>
<td>0.427</td>
<td>0.224</td>
<td>0.028</td>
</tr>
<tr>
<td>Me-α-man</td>
<td>0.512</td>
<td>0.476</td>
<td>0.382</td>
<td>0.338</td>
<td>0.445</td>
<td>0.454</td>
<td>0.174</td>
<td>0.029</td>
</tr>
<tr>
<td>Antigen blank (no serum)</td>
<td>0.036</td>
<td>0.037</td>
<td>0.031</td>
<td>0.027</td>
<td>0.029</td>
<td>0.030</td>
<td>0.033</td>
<td>0.032</td>
</tr>
</tbody>
</table>


did not have sufficient purified antigen to perform a complete titration series, Con A binding was clearly inhibited at the antigen and lectin concentrations examined.

**Discussion**

Despite their potential for improved serodiagnosis and vaccine development and their importance in basic immunological research, there has been little progress in isolating and purifying surface antigens from *S. mansoni*. Nash et al. (1977) isolated a circulating proteoglycan secreted by the gut of adult worms, and soluble egg antigen (SEA) has been well defined (Boros and Warren, 1970; Pelley et al., 1976; Carter and Colley, 1978). However, attempts to harvest antigens from the tegument have yielded very heterogeneous mixtures containing protein, glycoprotein, and lipid (Murrell et al., 1974). The procedure described in this paper represents the first quantitative isolation of a single surface membrane or tegumental antigen from *S. mansoni* adult worms.

**Technical considerations**

As shown in the flow diagram in Figure 2, the Con A-binding glycoprotein may be isolated from fresh, frozen, or lyophilized worms. However, yields are comparatively poor, because the glycoprotein represents less than 1% (by weight) of

Table 5. Inhibition of concanavalin A surface binding* by incubation of *Schistosoma mansoni* adult worms with fluorescein-labeled lectin (FITC-Con A) and purified glycoprotein antigen.

<table>
<thead>
<tr>
<th>Concentration of antigen</th>
<th>0 μg/ml (control)</th>
<th>180 μg/ml</th>
</tr>
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<tbody>
<tr>
<td>25 μg/ml</td>
<td>FITC-Con A</td>
<td>++++</td>
</tr>
<tr>
<td>2.5 μg/ml</td>
<td>FITC-Con A</td>
<td>+</td>
</tr>
</tbody>
</table>

* Surface binding scored as: positive reaction (+), stronger reaction (++), very strong reaction (+++), extremely strong reaction (++++) or no reaction (−).
the water-soluble proteins extracted by either freeze–thaw or detergent. Initial attempts to isolate the 58,000-molecular-weight antigen by affinity chromatography were fraught with difficulties. When approximately 20 mg of unlabeled freeze–thaw (AFT-1) antigen was applied directly to a 0.9 × 20-cm Con A column, the α-methylmannoside eluate appeared as a very low broad peak (Fig. 6A, Fraction II). As an alternative, we eluted the column using borate buffer, because borate inhibits Con A binding by forming complexes with the 4- and 6-hydroxyls of nonreducing pyranosides in glycoproteins (Svensson et al., 1970; Kennedy and Rosevear, 1973). Although increasing concentrations of borate buffer eluted more material from the column (Fig. 6A, Fractions III and IV), SDS gels of this material revealed numerous protein bands, suggesting nonspecific adsorption of freeze–thaw antigen by Sepharose and elution due to an increase in ionic strength of the buffer. Washing the column with 500 ml acetate buffer before elution reduced the yield (Fig. 6B), but the eluate was comprised primarily of the 58,000-molecular-weight antigen. A final modification of the protocol, without α-methylmannoside elution, is shown in Figure 6C. After washing with 0.1 M acetate buffer, an increase in ionic strength (to 0.45 M acetate) removes additional impurities. Fraction IV represents material eluted by interaction with the borate ion.

However, results were complicated by nonspecific and irreversible binding of crude antigen to Con A-Sepharose. When one of the preparative (0.9 × 20 cm) Con A columns was used a second time, the yield was markedly diminished. After three or more sample applications, the column took on a permanent yellow-brown color; successive washings with sodium acetate (pH 4.5) to “regenerate” the column failed to elute the contaminant. Both chloroform: methanol extraction and gel filtration remove yellow-colored material so that a more defined sample may be applied to the Con A column.

**Biochemical characterization of the Con A-binding antigen**

Throughout the text, we have described the Con A-binding antigen as having a molecular weight of 58,000. This value can only be taken as a rough approximation based upon relative mobility in SDS-PAGE. It is well documented that the carbohydrate moieties of glycoproteins can greatly alter the intrinsic net charge of the molecule (Poretz and Pieczenik, 1981) and cause migration abnormalities of up to 33% in SDS gels (Glossmann and Neville, 1971; Weber and Osborn, 1975). Because we have not yet determined the total amount or identity of carbohydrates in the glycoprotein, it would be premature to speculate about its actual molecular weight.

The ability of the glycoprotein to bind concanavalin A but not *Lens culinaris* (lentil) lectin is problematic, because it is generally assumed that both lectins react with α-D-glucosyl or sterically related carbohydrate groups. Although a number of glycopeptides will react with both Con A and *L. culinaris*, there are significant differences in association constants between the two (Young and Leon, 1974). It appears that the actual binding site of Con A accommodates structures as large as di- or trisaccharides; concanavalin A has greatest affinity for “mannobiosyl-N-acetylglucosamine,” while “N-acetylglucosaminylmannobiose” is the structure recognized by *L. culinaris* (Toyoshima et al., 1972; Young and Leon, 1974). Because these structures are similar, we would expect at least some binding of the glycoprotein with *L. culinaris* as well as with Con A. Failure of the antigen
to react with both may be due to our use of immobilized lectins. As observed by Kennedy and Rosevear (1973), "molecules of soluble Con A are free to move in relation to each other . . . . Immobilization of the protein would prevent this movement and consequently only carbohydrates with a particular distance between nonreducing ends would interact with the maximum possible strength . . . . In the immobilized case, molecules may be fractionated according to differences in the length of branches or the distribution of branch points which are not apparent when the complexation is carried out in solution." Thus, separation of the 58,000-molecular-weight Con A-binding glycoprotein from the 94,000-molecular-weight lentil lectin-binding glycoprotein (compare Fig. 1A and B) may reflect differences in the steric arrangement of the lectin molecules bound to Sepharose beads rather than major differences in exposed carbohydrate residues of the glycoproteins.

We believe that mannose, or a structurally similar saccharide, is present as a terminal or penultimate carbohydrate in the Con A-binding antigen. Reactivity with Con A but not with wheat germ agglutinin suggests terminal glucosamine but not N-acetylglucosamine. Metabolic labeling using tritiated precursors revealed significant incorporation of galactose in the 58,000-molecular-weight antigen; detection of other labeled sugars has not yet been conclusive. Elucidation of the complete carbohydrate composition and sequence of branch chains in the glycoprotein must await further analysis.

**Immunoochemical characterization of the Con A-binding antigen**

Localization of the glycoprotein on the surface of the parasite is inferred by previous studies using fluorescein-labeled lectins, which showed that Con A binding was restricted to the surface (Simpson and Smithers, 1980). Inhibition of Con A binding by the glycoprotein (Table 5) argues strongly that it is a surface antigen. Further support is gained from isolation of the glycoprotein by detergent extraction, because low concentrations of non-ionic detergent appear to remove only tegumental material (Brink et al., 1980; Oaks et al., 1981; Hayunga and Murrell, 1982). Conclusive proof of surface localization would require electron-microscopic histochemistry.

Cesari (1974) and Bennett and Seed (1977) speculated that Con A-binding material from adult worms might represent a tegumental alkaline phosphatase. The glycoprotein isolated in this study failed to give a colorimetric reaction when incubated with p-nitrophenyl phosphate substrate at alkaline pH; either it was not a phosphatase or enzymatic activity was lost as a result of the isolation procedure.

When used as a coating antigen for enzyme-linked immunoassay (EIA), both the Con A-binding glycoprotein and the large-molecular-weight antigen from *S. mansoni* reacted with sera from patients infected by *S. haematobium* or *S. japonicum* as well as those infected by *S. mansoni*. Similarly, the glycoprotein isolated from *S. japonicum* adult worms (Hayunga et al., 1982) also reacted with all three antisera. Thus, in terms of practical application, purified antigens were for the most part as sensitive as crude antigen (AFT-1) in EIA, but none of them could be considered a species-specific antigen for serodiagnosis. In a previous study, the Con A-binding glycoprotein from *S. mansoni* schistosomula was found
to have a molecular weight significantly different from 58,000 (Taylor et al., 1981), which suggests that the glycoprotein isolated from adult worms may represent a stage-specific antigen.

Inhibition of glycoprotein–antibody binding by monosaccharides was significant only at concentrations of 1% and 10% (Table 4). Although high, these concentrations are comparable to those used to inhibit lectin binding to the tegument of *S. mansoni* (Bennett and Seed, 1977) and to inhibit both lectin binding (Goldstein et al., 1965; Young and Leon, 1974) and antibody binding (Cisar et al., 1974, 1975) to dextran. The results described in this paper suggest that antibody-binding sites on the 58,000-molecular-weight glycoprotein most likely contain moieties that are structurally similar to mannose, fucose, glucosamine, and galactosamine. In contrast, the lack of inhibition by glucose indicates that there are strict steric requirements for antibody binding. Fucose (6-deoxy-galactose) was a more efficient inhibitor than galactose. The 2-amino sugars were more efficient than were their N-acetyl derivatives, suggesting steric hindrance of an amino group important in recognition. Both the amino and N-acetyl sugars were more efficient inhibitors of antibody binding than were their parent sugars. Doubtless, the actual antibody-binding sites on the glycoprotein are complex, involving a number of saccharides in a precise sequence and linkage. There may be several different antibody-binding sites on the antigen, and binding sites for antibodies may not necessarily be identical to those for lectins. Monoclonal antibodies derived from hybridomas would be invaluable tools to elucidate the number and types of binding sites on this antigen.

Recently, Hillyer and Sagramoso de Ateca (1979) have isolated a Con A-binding glycoprotein from the tegument of *Fasciola hepatica* that not only reacts with *S. mansoni* infection serum but that gives partial protection against murine schistosomiasis. With an apparent molecular weight of 60,000 and pI value 4.0–4.4, this antigen bears a striking resemblance to the Con A-binding glycoprotein that we have isolated from *S. mansoni* adult worms. Preliminary experiments in our laboratory suggest that the *S. mansoni* glycoprotein may also have some effect in protecting mice against infection. Clearly, further characterization is needed for what appear to be common surface antigens in two distantly related trematodes.

**Isolation of other antigens**

As a by-product of our efforts to purify the Con A-binding glycoprotein from *S. mansoni*, we also isolated a large (approximately 300,000)-molecular-weight antigen and an uncharacterized lipid fraction. The large-molecular-weight antigen was found to have a pI value of approximately 4.2 (range 4.0–4.4) as determined by ampholine isoelectric focusing (Fig. 4), and it reacted strongly with infection serum (Table 3). The nominal molecular weight of 300,000 is a rough approximation based on gel filtration; the antigen barely penetrated the stacking gel in SDS-PAGE. A slight, but statistically significant, reduction of antibody binding (data not shown) was found with each of the nine monosaccharides used for the inhibition test, which is suggestive of a large glycoprotein with complex arrangement of saccharide chains. Thus, the antigen may correspond to one of the glycoproteins of similar molecular weight found in preparations of culture antigens (Murrell et al., 1974), membrane antigens (Murrell et al., 1977), or detergent
extracts of the tegument (Hayunga et al., 1979). Further analysis of this antigen and the lipid fraction is currently under way.

Acknowledgments

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Literature Cited


Erratum

The surname of the first author was misspelled for the paper "*Vampirolepis schmidtii* sp. n. (Cestoidea: Hymenolepididae) from *Triaenops persicus* (Hippodideridae) of Tanzania," Proc. Helminthol. Soc. Wash. 50:135–137. The name should read "Jensen." It is hoped that this correction will eliminate the possible confusion in the citing of this paper.