Immunoadfinity Isolation and Partial Characterization of the 
Coccidioides immitis Antigen Detected by the Tube Precipitin and 
Immunodiffusion-Tube Precipitin Tests

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The antigen participating in the tube precipitin (TP) serologic test for coccidioidomycosis was isolated from mycelial-phase antigen (coccidioidin) by immunoadfinity and characterized by various analytical procedures. This was accomplished by first preparing the antigen-antibody precipitate by using antigen and human serum positive for TP (immunoglobulin M) antibody and then liberating the antigen by digestion with pronase. This protease destroyed the antibody and left the antigen intact as indicated by immunodiffusion-TP. The coccidioidal antigen was isolated from the proteolytic digest by using size exclusion chromatography. DEAE chromatography of this antigen yielded two fractions with immunodiffusion-TP reactivity which had average molecular sizes of 225 and 140 kilodaltons, respectively. The presence of carbohydrate and amino acids indicated that the antigen(s) is a glycopeptide. Compositional analysis showed that one fraction contained 3-O-methylmannose, mannose, and glucose in a ratio of 8:1:2:1, whereas the second fraction contained 3-O-methylmannose, mannose, glucose, and galactose in a ratio of 1:1:1:1. The amino acids glycine, alanine, serine, threonine, aspartic acid plus asparagine, and glutamic acid plus glutamine constituted 60 to 70% of the amino acids in both glycopeptidcs. Neither antigen could be detected entering the gel in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lectin affinity provided evidence of a high-mannose asparagine-linked glycopeptide in the first peak and an asparagine-linked glycopeptide with a bianternary complex-type structure in the second peak.

The studies of Smith et al. (25, 26) revealed the regular occurrence of two kinds of antibody in coccidioidomycosis. The earlier appearing antibody associated with acute primary coccidioidomycosis, subsequently shown to be immunoglobulin M (IgM) (19, 22), was demonstrable by precipitation when mixed with soluble mycelial antigen (coccidioidin). This represented the tube precipitin (TP) test (Fig. 1). The later appearing antibody, subsequently shown to be IgG (19, 22), was characterized by complement-fixing (CF) activity. The crude antigen active in the TP was water soluble, heat stable, resistant to trypsin, and contained polysaccharide accompanied by amino acids (6, 20). Huppert and Bailey (9) showed that reactions corresponding to the TP and CF could be demonstrated by agar gel (Ouchterlony) immunodiffusion (ID), and the reactions were described respectively as the ID-TP and ID-CF reactions.

Various workers have provided evidence that the water soluble antigen(s) participating in the ID-TP reaction has a molecular mass ranging from less than 100 kilodaltons (kDa) to greater than 200 kDa (4, 32, 37). Certain electrophoretic characteristics have also been described (5).

In attempting to isolate the specific antigen(s), we found that the antigen-antibody precipitate formed in the TP or ID-TP reaction could be solubilized by the proteolytic activity of pronase, which destroyed the antibody without loss of the antigenic activity. This provided a means of immunoadfinity recovery of the specific antigen, which was then subjected to biochemical analysis. This revealed the presence of two antigenic components. These studies are described in the present report.

MATERIALS AND METHODS

Antigen. The antigen employed (F171) was a pooled mycelial-phase filtrate of 22 strains of Coccidioides immitis. This filtrate contains antigens that yield reactions in the ID-CF, ID-TP, and TP tests. The ID-CF activity can be destroyed by heat treatment at 60°C for 30 min, and heated F171 is routinely used to detect IgM precipitins by ID (18). This filtrate has been studied extensively in our laboratory by gel electrophoresis, immunoblotting, and size exclusion chromatography (37, 38). The ID-CF activity is associated with a 48-kDa component in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) involving heated and reduced F171 but in unheated antigen is identifiable with a 110-kDa component (38).

Preparation of antigen-antibody precipitates (TP affinity preparation). Human sera were used throughout these studies. They were heated at 36°C for 30 min to minimize risk of exposure to human immunodeficiency virus.

ID-TP assays were carried out by double diffusion by a modification of the method of Huppert and Bailey (9) in which antigen (F171) previously heated at 60°C for 30 min was used and sera were concentrated approximately eightfold by evaporation before being tested (18). Unheated antigen was also used to detect CF IgG antibody. Human sera with ID-TP or ID-CF activity were used as controls.

Antigen-antibody precipitates were obtained as a means of isolating the antigen from the crude mixture represented by coccidioidin. Antigen solution (0.2 ml) was mixed with known reactive human serum (0.2 ml) in test tubes (7 by 75 mm) (26, 27). These were incubated at 35°C and observed daily for the formation of a cohesive button (Fig. 1). The supernatant liquid was removed and discarded. The buttons were pooled, washed with phosphate-buffered saline (pH

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fractions dislodged from antigen 7.2) four times, disrupted by repeated expulsion and expulsion through a Pasteur pipette, centrifuged at 400 × g, and suspended in phosphate-buffered saline. This washing and centrifugation was repeated three times to minimize contamination by components of the serum or coccidioidin not participating in the precipitin reaction.

Analytical procedures. Protein determinations were performed either by the method of Lowry et al. (13) with bovine serum albumin as a standard or by determination of A_{280} with IgG as a standard. The anthrone method with mannose as a standard was used to detect hexose in the nondialyzable fractions (24). Fucose determination was performed by the cysteine-sulfuric acid reaction for methyl pentoses (28). The resorcinol-hydrochloric acid method was used to determine sialic acid (10). All samples were concentrated in a Savant Speed-Vac. SDS-PAGE was performed with the discontinuous buffer system of Laemmlli (12) as previously described (37, 38). Gels were stained by the silver stain described by Merrill et al. (14). Protein blot analysis was performed by the method of Towbin et al. (30) as previously described (37, 38).

Proteolytic digestion and antigen isolation. Proteolytic digestion of the antigen-antibody complex was carried out by exhaustive digestion with pronase (Calbiochem-Behring, San Diego, Calif.). Washed antigen-antibody buttons (2 mg/ml) were incubated with 400 μg of pronase per ml in 0.1 M Tris hydrochloride (pH 7.4) containing 0.02 M magnesium chloride (28, 29). The mixture was incubated at 37°C for 72 h and then heated at 60°C for 30 min to terminate the reaction. The mixture was then centrifuged at 500 × g, and the supernatant was tested by ID. It was found to have retained ID-TP activity and no ID-CF activity (Fig. 2).

Controls for comparison with the digested antigen-antibody mixtures included pronase alone, antiseraum alone, antiserum plus pronase, and human IgG (Cappel Laboratories, Dorrington, Pa.) plus pronase. These were subjected to gel filtration, carbohydrate determination, and SDS-PAGE analysis.

In order to separate ID-TP antigen in the proteolytic digestion, the supernatant was chromatographed by using Bio-Gel P60 (100/200 mesh; Bio-Rad Laboratories, Richmond, Calif.) in a gel bed (2.5 by 23 cm). The eluant was 0.02 M potassium phosphate (pH 7.0), and the flow rate was approximately 60 ml/h. Fractions (2 ml) were collected and assayed for protein and carbohydrate. Fractions from the antigen preparation and controls were concentrated approximately 40-fold for testing by ID and SDS-PAGE. The void volume fraction containing ID-TP antigen was pooled from several preparative chromatographic experiments, dialyzed, and concentrated.

The antigen peak was further resolved by ion-exchange chromatography with DEAE-Sephadex (Pharmacia, Inc., Piscataway, N.J.). The antigen was dissolved in 0.05 M Tris hydrochloride (pH 8.8) and eluted with a 0 to 0.5 M linear sodium chloride gradient. Five-milliliter fractions were collected, desalted, and assayed for protein, carbohydrate, and ID-TP activity. Fractions positive for ID-TP activity from several experiments were pooled, dialyzed, and concentrated.

Size exclusion chromatography. ID-TP positive fractions from ion-exchange chromatography were assayed separately for molecular weight by gel filtration with Sephacryl S-400 (Pharmacia) in a gel bed (2.5 by 48 cm). The column was calibrated by using protein standards ranging from 440 to 25 kDa (Pharmacia). The eluant was 0.02 M potassium phosphate (pH 7.0), and the flow rate was 42 ml/h. Fractions (2 ml) were collected and concentrated 40-fold for ID analysis.

Carbohydrate analysis. Thin-layer chromatography (TLC) was used for the detection of neutral sugars; high-performance liquid chromatography (HPLC) was used for the detection and quantitation of neutral sugars; gas-liquid chromatography (GLC) was used for the detection and quantitation of neutral sugars and amino sugars. For HPLC and TLC, samples were heated in 1 N sulfuric acid at 100°C for 8 h in sealed tubes. Hydrolysates were passed over columns containing AG 50W-X4, 200/400 mesh, hydrogen, connected to AG 1-X8, 200/400 mesh, formate (Bio-Rad) (28, 29). The effluent and washes were pooled and concentrated to dryness. Cellulose Avicel glass plates (Alltech Associates, Los Altos, Calif.) were used for TLC of neutral sugars. The solvent system was pyridine-ethyl acetate-acetic acid-water.
VOL. 27, 1989
PARTIAL CHARACTERIZATION OF C. IMMITTIS ANTIGEN 1761

Alkaline silver nitrate was used for detection of reducing compounds (31), and para-anisidine hydrochloride was used for the specific detection of sugars, in particular 3-O-methylmannose (8, 32, 33). Samples of neutral sugars were dissolved in acetonitrile-water (80:20) for HPLC. Chromatography was performed with a Carbohydrate Analysis column (amine-bonded silica; Waters Associates, Inc., Milford, Mass.) on a Hewlett-Packard liquid chromatograph. The flow rate was 1 ml/min. Neutral sugars were detected by using a postcolumn addition of 0.2% tetrazolium blue in 0.09 N sodium hydroxide, heating the solution for 1 min, and measuring the $A_{560}$ (3, 7, 25).

For GLC of neutral sugars and amino sugars, samples were hydrolyzed in 2.5 N trifluoroacetic acid at 100°C for 4 h and evaporated at 40°C under N$_2$ (2). Sugars were separated as alditol acetate derivatives by the method of Blakeney et al. (2) by using a SP2 100 column on a Varian 3700 gas chromatograph. The column temperature was held at 140°C for 5 min, raised 10°C/min to 200°C, and then held at that final temperature for 25 min. Data were integrated and plotted on a P-E Sigma 10 Delta System. All sugars were identified by relative mobility of standards in each system.

Amino acid analysis. Analysis of separate DEAE peaks 1 and 2 and crude heated mycelial filtrate F171 was performed by using an amino acid analyzer (model 6300; Beckman Instruments, Inc., Fullerton, Calif.) and a standard 24-h hydrochloric acid hydrolysis. Amino acids and amino sugars were identified by relative mobility of standards. Asparagine and glutamine were deaminated by hydrolysis and were detected as aspartic acid and glutamic acid. Aminoguanidino-propionic acid was the internal standard.

Lectin affinity structural assessment. Lectin affinity studies were carried out by using the fractionation scheme of Osaka and Tsuji (17). This makes use of a series of different lectins, the binding specificities of which have been elucidated, to fractionate a precise amount of glycopeptide into the structurally distinct groups of serine-threonine-linked sugar chains and asparagine-linked sugar chains. The latter group can be further divided into three structural groups: high-mannose type, complex type, and hybrid type. Studies were carried out with concanavalin A (ConA)-Sepharose 4B (Pharmacia), which binds to asparagine-linked sugar chains and has a simple sugar specificity of mannosine. Lentil lectin-Sepharose 4B (Sigma Chemical Co., St. Louis, Mo.) has a sugar-binding specificity similar to that of ConA; however, the presence of a fucoside residue is essential for maximum binding of a glycopeptide. Wheat germ agglutinin agarose (E-Y Laboratories, Inc., San Mateo, Calif.) is a simple sugar specificity of N-acetylgalactosamine (GluNAc) and binds to hybrid-type oligosaccharides.

Each coccidioidal oligosaccharide was initially quantitatively assayed for activity by ID reaction to obtain a precise, limited amount of antigen. The antigens were diluted in 0.01 M Tris hydrochloride (pH 7.4) containing 0.001 M calcium chloride and 0.001 M manganese chloride for ConA studies. The lectin was washed four times with the same buffer. The antigen was mixed with the lectin for 30 min at room temperature and centrifuged. The supernatant and three subsequent lectin washes with Tris buffer were pooled for the nonbound fraction. The lectin was then incubated for 30 min at room temperature with 15 mM methyl-alpha-D-mannoside (Sigma) in the same buffer to release bound saccharide. Again the supernatant and three separate gel washes were pooled for the loosely bound fractions that were released by the 15 mM mannoside competitor. Finally, the same incubation and wash were carried out with 200 mM mannoside for the fraction tightly bound to ConA.

The lentil lectin affinity step was carried out by using the same buffer and 200 mM competitive mannoside for elution. Affinity of the antigen for wheat germ agglutinin was assayed in phosphate-buffered saline (pH 7.2) using 200 mM GluNAc as competitive eluant. All fractions were desalted by using Sephadex G-25M (PD-10 columns; Pharmacia) and concentrated by dryness. They were rehydrated with 50 µl of water for ID analysis.

RESULTS

Immunoaffinity preparation of antigen. The TP and ID-TP reactivity of coccidiodal antigen was liberated from tube precipitin antigen-antibody precipitate by exhaustive proteolytic digestion (Fig. 1 and 2). The ID-TP reaction obtained was specific; neither pronase alone (which contains less than 2% carbohydrate and would not contribute significant amounts of glycopeptide to the digest) nor any other controls reacted in the ID test (Fig. 3). SDS-PAGE of the pronase digests did not reveal any bands originally present in the crude antigen, as detected in an 11% acrylamide gel even after the gel was overloaded with material (Fig. 4). SDS-PAGE analysis of the digest using a 5% running gel and a 3% stacking gel showed two areas of stained material, one of which was at the top of the stacking gel and the other of which was at the top of the running gel (Fig. 5). Thus, the antigen after digestion was still of such a molecular conformation as to preclude entry into even these gels with large pore sizes.

Control SDS-PAGE of pronase-treated serum showed the digestion to have reduced the immunoglobulin to peptides smaller than 20 kDa (Fig. 4).

Size exclusion and ion-exchange chromatographic purification. Chromatography of the digest on Bio-Gel P60 to separate antigen from proteolytic digest material revealed one peak in the void volume which contained ID-TP reactivity (Fig. 6, peak A). This fraction contained 130 µg of protein and 100 µg of carbohydrate per ml. No other fractions were ID-TP positive. Control preparations of pro-
nase alone, pronase plus antisera, and pronase plus IgG revealed no detectable carbohydrate in equivalent fractions. Pools of the positive fraction were dialedyzed and applied to DEAE-Sephadex. Two separate ID-TP-positive fractions were resolved by using this ion-exchange medium (Fig. 7 and 8). One fraction was not bound to DEAE under the column conditions used, and a second fraction was bound and then released by the salt gradient. These fractions were labeled peak 1 (DEAE unbound) and peak 2 (DEAE bound). There were no other ID-TP-positive fractions. Additional non-IDTP reactive fractions were eluted as carbohydrates with peaks at 100 and 250 ml. Neither ID-TP-positive peaks 1 or 2 yielded any bands when tested by SDS-PAGE or by immunoblotting with ID-TP or ID-CF sera followed by anti-IgG or anti-IgM (data not shown). This was probably related to the earlier demonstrated failure of the antigen to enter the polyacrylamide gel.

Gel filtration and subsequent ID analysis of each separate DEAE peak showed DEAE peak 1 (unbound) to have a molecular size range of 66 to 220 kDa (average size, 140 kDa). DEAE-bound peak 2 had a size range of 110 to 340 kDa (average size, 225 kDa).

Pools of each separate peak prepared from several experiments and concentrated for compositional analysis contained an approximate carbohydrate-to-protein ratio of 5:1. A UV spectrum of each pool (measured from 200 nm to 320 nm) revealed an absorbance maximum of 200 nm.

Carbohydrate compositional analysis. Carbohydrate compositional analysis by all methods showed the DEAE-separable antigens to be composed of the neutral sugars mannose, 3-O-methylmannose, glucose, and galactose. DEAE peak 1 (unbound) contained a 3-O-methylmannose, mannose, and glucose in a ratio of 8:1:2:1. Galactose was detected in only trace amounts. DEAE peak 2 (bound), however, contained a 1:1:1:1 3-O-methylmannose, mannose, glucose, and galactose in a ratio of 1:1:1:1 (Fig. 9).

FIG. 4. Silver stain of SDS-PAGE of tube precipitin immunofinity preparation pronase digests and controls (11% acrylamide). Lanes: 1, Low-M, standards; 2, heated mycelial antigen F171 only; 3, pronase-treated TP preparations (no bands present); 4, pronase-treated serum (no antigen); 5, serum only (no antigen or pronase). Molecular size standards (in kilodaltons) are indicated on the left.

FIG. 5. Silver stain of SDS-PAGE of pronase digests (5% acrylamide running gel; 3% stacking gel). Lanes: 1, Pronase-treated TP preparations (silver-stainable material has not entered the running gel); 2, high-M, standards. Molecular size standards (in kilodaltons) are indicated on the right.

No amino sugars were detected by GLC. Fucose was not detectable by cysteine sulfuric determination nor by GLC, HPLC, or TLC. Sialic acid was not detected by the resorcinol hydrochloric acid test.

Lectin affinity analysis. The ID-TP activity of DEAE peak 1 was bound by ConA-Sepharose. The antigen was not released by elution with 15 mM mannose but was released by elution with 200 mM mannose, indicating a strong affinity for the lectin. The antigen was not bound by wheat germ agglutinin. This pattern of affinity indicated an asparagine-linked carbohydrate. The results of compositional analyses confirmed this tentative structure (see above) as one of the high-mannose variety.

FIG. 6. Size exclusion chromatography of pronase-treated ID-TP antigen on BioGel P60. All ID-TP activity is in peak A, which corresponds to the column void volume.
The ID-TP reactivity of DEAE peak 1 was likewise bound by ConA-Sepharose. In contrast to DEAE peak 1, however, the activity of peak 2 was released from the lectin upon elution with 15 mM mannoside. This pattern indicated an asparagine-linked oligosaccharide with biantennary complex-type glycopeptide structure, which was confirmed by compositional analysis. The antigen was bound by lentil lectin, suggesting the presence of fucose residues. However, no fucose was detected by compositional analysis.

**Amino acid analysis.** Amino acid analysis indicated that glycine, alanine, serine, threonine, aspartic acid plus asparagine, and glutamic acid plus glutamine constituted 60 to 73% of the amino acids in DEAE peaks 1 and 2 (Table 1). Proline was 3.7 and 4.9% of the amino acid content, respectively. In contrast, analyses of the crude heated mycelial filtrate showed threonine and proline to be 19.8 and 19.2%, respectively, of the amino acid content. Glutamic acid plus glutamine, glycine and alanine were the other major amino acids present. GluNAc was not detected in any antigen, although a minor peak, possibly representing N-acetylgalactosamine, was present in DEAE peak 2.

**DISCUSSION**

The antigen reactive in the TP test, which is known to contain polysaccharide (6, 20), was still reactive with antibody after pronase digestion, thus making possible the immunoaffinity preparation with TP buttons. A general first step in the elucidation of the structure of the carbohydrate units of glycoproteins is digestion of the material with pronase (29). In order to separate the antigenic glycopeptides in the digests from other peptide material, gel filtration was undertaken. We chose Bio-Gel P60, since our previous work (37) had shown the whole ID-TP antigen to have an average molecular mass of 120 kDa, which is greater than the exclusion mass of 60 kDa for the P60 gel. Such an antigen would not be retained by the P60 gel, and the void volume would be devoid of components of <60 kDa.

Chromatography of the pronase-digested antigen-antibody precipitate with P60 provided evidence of a coccidiodal polysaccharide of greater than 60 kDa. Chromatography of a pronase digest of the control immunoglobulin (without antigen) showed no carbohydrate in the void volume. Thus, there was no polysaccharide from the immunoglobulin in the pronase-digested coccidiodal precipitin buttons. In addition, our SDS-PAGE of the pronase-digested precipitin buttons revealed no peptide bands (Fig. 4, lane 3). That the immunoglobulin was thus reduced by the pronase to peptide fragments of <20 kDa was evident also by SDS-PAGE of control immunoglobulin (without antigen) treated with pronase (Fig. 4, lane 4). The control undigested immunoglobulin showed the expected pattern on SDS-PAGE (Fig. 4, lane 5). An effective immunoaffinity preparation of specific glycopeptide C. immitis TP antigen was thus obtained.

DEAE chromatography provided two distinct glycopeptides differing in molecular weight. Each gave a reaction

### Table 1. Percent amino acid content in heated mycelial filtrate F171, DEAE peak 1 (unbound), and DEAE peak 2 (bound)

<table>
<thead>
<tr>
<th>Amino acid(s)</th>
<th>Heated F171</th>
<th>DEAE peak 1</th>
<th>DEAE peak 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid plus asparagine</td>
<td>5.82</td>
<td>9.83</td>
<td>10.26</td>
</tr>
<tr>
<td>Threonine</td>
<td>19.79</td>
<td>8.58</td>
<td>9.91</td>
</tr>
<tr>
<td>Serine</td>
<td>6.26</td>
<td>11.93</td>
<td>9.83</td>
</tr>
<tr>
<td>Glutamic acid plus glutamine</td>
<td>11.31</td>
<td>9.07</td>
<td>9.17</td>
</tr>
<tr>
<td>Proline</td>
<td>19.16</td>
<td>4.86</td>
<td>3.70</td>
</tr>
<tr>
<td>Glycine</td>
<td>10.61</td>
<td>15.28</td>
<td>10.89</td>
</tr>
<tr>
<td>Alanine</td>
<td>9.44</td>
<td>12.41</td>
<td>13.10</td>
</tr>
<tr>
<td>Valine</td>
<td>5.86</td>
<td>6.12</td>
<td>6.22</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.29</td>
<td>0.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.68</td>
<td>4.11</td>
<td>3.83</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.87</td>
<td>6.78</td>
<td>7.23</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.13</td>
<td>—</td>
<td>2.90</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.90</td>
<td>3.20</td>
<td>3.34</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.38</td>
<td>4.11</td>
<td>5.36</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.94</td>
<td>1.11</td>
<td>1.06</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.56</td>
<td>2.49</td>
<td>3.18</td>
</tr>
</tbody>
</table>

* — Present but not quantitated due to contaminating peak.
FIG. 9. HPLC of neutral sugars (left graphs) and GLC of alditol acetate derivatives of neutral sugars (right graphs) of the two DEAE-purified coccidioidal ID-TP glycopeptides. DEAE peaks 1 (unbound) and 2 (bound). 1, 3-O-Methylmannose; 2, mannose; 3, glucose; 4, galactose; IS, inositol (internal standard for GLC).

This precipitinogen gave a line of identity in reference ID-TP analysis. This antigen was not isolated in the same manner as the TP glycoprotein described in the present paper but may represent the same antigen or partial component thereof. The isolation of antigen by TP and demonstration of its identity with antigen producing the ID-TP reaction provided evidence that the same antigen is responsible for the reaction in the fluid as well as in the gel milieu.

An initial structural assessment using lectin affinity was performed with the two glycopeptides. These studies revealed that both were bound by ConA, indicating the presence of an asparagine-linked oligosaccharide. The three general types of asparagine-linked oligosaccharides are high-mannose, hybrid, and complex type. In polysaccharides
from other sources, these three groups share a common pentasaccharide core structure but differ in their outer branches. High-mannose type oligosaccharides typically have two to six additional mannose residues linked to the core. Complex-type structures may be modified by extra branches on the mannose residues (biantennary, triantennary, etc.), by addition of extra sugar residues that elongate the outer chains, or by an additional GluNAc or fucose residue linked to the core. Hybrid molecules have features of both high-mannose and complex-type oligosaccharides (11).

Coccidioidal DEAE peak 1 (unbound) had a strong affinity for ConA and no affinity for wheat germ agglutinin. This indicated a high-mannose type of carbohydrate. It is composed mainly of 3-O-methylmannose and mannose, lending credence to the high-mannose structure. On the other hand, DEAE peak 2 (bound) had a weak affinity for ConA plus an affinity for lentil lectin, indicating a complex-type biantennary carbohydrate with a possible fucose residue. However, no trace of fucose was found by any analytical means. The lectin affinity pattern of methylated mannose groups has not, to our knowledge, been identified; perhaps the presence of a methyl residue on the mannose confers an affinity pattern similar to that of the methyl pentose fucose.

Our previous SDS-PAGE immunoblotting experiments with periostral and spherule-endoспорule-phase antigens, including the F171 used in this study, showed several immuno reactive bands that were not stainable with silver. These included a 46-kDa band and three doublet bands in the 50- to 65-kDa area which were highly reactive in immunoblots, particularly with ID-TP serum detected by anti-IgM (37). In repeated trials, the ID-TP reactive glycopeptide(s) isolated in the present study did not, however, enter a large-pore acrylamide running gel, despite overloading of antigenic material on the gel. This may have been the result of the large proportion of neutral carbohydrate precluding SDS binding and thus restricting the necessary negative charge for electrophoretic mobility. We thus concluded that the ID-TP-reactive antigens isolated in the current study may not represent antigens previously studied by us or Calhoun et al. (4).

Peaks 1 and 2 showed a carbohydrate-to-protein ratio of approximately 5:1. In previous studies using ethanol fractionation of coccidioidin, a similar content (15 to 20%) of peptide material was noted (20). Amino acids detected in that earlier preparation by paper chromatography had mobility characteristics of alanine, aspartic acid, glycine, proline, serine, threonine, and tyrosine. In the present study, a pronase digestion might have removed protein that was not protected by the carbohydrate in these antigens. A comparison with other antigens may not be warranted. However, it is evident that the amino acid composition is more complex than previously described (20) and that the major amino acids present are alanine, aspartic acid plus asparagine, glutamic acid plus glutamine, glycine, serine, and threonine (Table 1).

The lectin affinity studies and the aspartic acid-plus-asparagine concentration (10%) suggests that these two glycopeptides (DEAE peak 1 and peak 2) are N-linked through asparagine. However, generous concentrations (approximately 10% each) of serine and threonine were also detected in peaks 1 and 2 (Table 1). These amino acids may be associated with the presence of O-linked oligosaccharides in the same glycoprotein. This structure would not be unique; for instance, the well-characterized mannose-containing glycoprotein from Saccharomyces cerevisiae contains both O- and N-linked groups (15, 16). Certainly, further chemical and enzymic analysis are in order before definite assignment of structure can be made. Investigations to determine further the structure of as well as production of antibody to the two glycopeptides described are currently in progress.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


