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Characterization of a Soluble Protein of *Coccidioides immitis* with Activity as an Immunodiffusion-Complement Fixation Antigen

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A 48-kilodalton (kDa) electrophoretically distinct antigen from *Coccidioides immitis* mycelial- and spherule-endospore-phase filtrates was previously associated by immunoblotting with the immunodiffusion band that corresponds to complement-fixing activity (ID-CF). To characterize this antigen and its precursor, both mycelial- and spherule-endospore-phase filtrates were fractionated by size exclusion chromatography, lectin affinity chromatography, and nondenaturing electrophoresis. By size exclusion chromatography, most of the protein and carbohydrate of the crude filtrates eluted in a peak of average molecular size less than 30 kDa, although other components were detected. ID-CF activity was associated with the component at a relative mobility of 110 kDa. Fractions containing the ID band that corresponded to tube precipitin activity occurred from 200 to 40 kDa. The appearance of the 48-kDa band in denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) specifically coincided with the fractions containing ID-CF activity. Nondenaturing PAGE of filtrates showed silver-stainable and immunoblot-reactive bands in the region of 110 kDa. Prior treatment with pronase destroyed this electrophoretically separable antigen, whereas periodate had no effect. Trypsin did not affect the 110-kDa band in unheated or unreduced antigen. Mycelial filtrates were chromatographed on lentil lectin or concanavalin A-Sepharose 4B to deplete them of glucose- or mannose-containing carbohydrate. The effluent fraction contained ID-CF activity and, upon denaturing electrophoresis, the 48-kDa antigen. The 110-kDa protein represents the ID-CF antigen which is heat labile and denatured to a 48-kDa band by sodium dodecyl sulfate-PAGE.

Coccidioides immitis, the etiologic agent of coccidioidomycosis, grows in hyphal-arthroconidial form in nature and under usual laboratory conditions. In the host, infectious arthroconidia become rounded and enlarge to form spherules, which contain many endospores. At maturity the spherules rupture, releasing endospores which initiate a new cycle. The spherule-endospore (SE) cycle, which can be maintained in vitro under certain conditions, presents a defined sequence of both cellular and immunologic development and antigens released into the culture medium (8, 14, 15, 30, 31).

Precipitin antibodies, primarily immunoglobulin M (IgM), are usually indicative of a recent coccidioidal infection (21, 24). These are detected by either tube precipitin, latex particle agglutination, or immunodiffusion (ID-TP) under appropriate conditions. Complement-fixing antibodies, also demonstrable by ID (ID-CF), appear later in the course of infection and are primarily IgG (21, 24). The correlation of severity of disease with CF antibody titer is well documented (27). A mycelial-phase culture filtrate-autolysate, coccidioidin, has customarily been used for serodiagnosis, but the SE-phase filtrates also contain serologically active antigens.

Using denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analyses, we previously showed at least 15 separable antigens in both SE-phase extracellular protein and soluble mycelial-phase (coccidioidin) antigen (30, 31). Protein bands common to both SE and mycelial preparations were noted at 48 and 18 kilodaltons (kDa). The 48-kDa band was strongly reactive with CF-positive human serum demonstrated by immunoblot reaction with IgG. Prior heat treatment of the antigens at 60°C for 30 min, which destroys CF and ID-CF activity, altered or destroyed the component usually detected as the 48-kDa band. The present study was undertaken to characterize this electrophoretically distinct antigen present in both SE-phase and culture filtrate coccidioidin. Chromatographic fractionation by size exclusion and lectin affinity, followed by ID and denaturing SDS-PAGE, provided a means of characterizing this antigen in its native conformation, as did nondenaturing electrophoretic patterns. Treatment with proteolytic enzymes and sodium periodate further assisted in the characterization of this antigen.

MATERIALS AND METHODS

Mycelial-phase antigens. Two mycelial-phase culture antigens were studied. Both were produced in 2% glucose–1% yeast extract broth (22). The first was a filtrate (SF) derived from strain Silveira (ATCC 28868) (6). This antigen is used routinely in our laboratory to detect ID-CF antibody in human and other sera. This antigen also contains ID-TP activity. The second antigen, F171, was a pooled filtrate of 22 strains of *C. immitis*. This filtrate also contains ID-CF and ID-TP activities. ID-CF activity could be destroyed by heat treatment at 60°C for 30 min in both antigens. Heated F171 is routinely used to detect IgM precipitins by ID (20). All antigens were concentrated in a Savant Speed-Vac to a protein concentration between 3.0 and 5.5 mg/ml.

SE-phase antigen. The SE-phase antigen was prepared as previously described (31). Briefly, the Silveira strain of *C. immitis* was grown in synchronous SE-phase culture by the method of Levine et al. (14). At 120 h after inoculation, cultures were centrifuged at 400 × *g* for 15 min. The supernatants were filtered through 0.45-μm (pore size) filters. Phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 1 mM

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to inhibit endogenous proteases. Samples were concentrated in an Amicon pressure cell with a UM 10 ultrafilter (Amicon Corp., Lexington, Mass.) to a protein concentration of 2.25 mg/ml. This filtrate contained both ID-CF and ID-TP activity. As with mycelial antigens, the former activity could be destroyed by heat.

Protein and carbohydrate determination. Protein determinations were done either by the method of Lowry et al. (16) with bovine serum albumin as a standard or by A_{280} with IgG as a standard. The anthrone method with mannose as a standard was used to detect hexose in the nondialyzable fractions (26).

ID. ID reactions were performed with pooled human sera that are routinely used as positive controls in diagnostic testing for either ID-TP or ID-CF coccidioidal antibodies.

Gel chromatography. Gel filtration was done with BioGel P200 (100/200 mesh; Bio-Rad Laboratories, Richmond, Calif.) in a gel bed (2.5 by 52.5 cm). The column was calibrated by using protein standards ranging from 440 to 25-kDa (Pharmacia LKB Inc., Piscataway, N.J.). Samples of mycelial antigen SF or F171 or the 120-h SE-phase antigen, each containing 3.0 to 5.5 mg of protein, were applied to the column. The eluant was 0.02 M potassium phosphate (pH 7.0), and the flow rate was 12 ml/h. Fractions (2 ml) were collected and assayed for protein and carbohydrate. Fractions for ID tests and for denaturing SDS-PAGE were concentrated approximately 20-fold.

Gel electrophoresis and protein blot analysis. Denaturing SDS-PAGE was performed with the discontinuous buffer system of Laemmli (13) as previously described (31). Nondenaturing PAGE was performed similarly, except that all reagents were free of β-mercaptoethanol and SDS, and the samples were not heated. Gels were stained by the silver stain described by Merril et al. (17). The relative mobility standards ranged from 200 to 14 kDa (Bio-Rad).

Protein blot analysis was performed by the method of Towbin et al. (28) as previously described (31). Pooled human serum was used in protein blot analysis. The ID-CF pool was negative for ID-TP reactivity, and the ID-TP pool was negative for ID-CF activity. Pooled normal serum consisted of 50 serum specimens negative for coccidioidal antibodies after approximately eightfold concentration and testing by ID. Sections of each blot were also stained with 0.1% amido black in 45% methanol–10% acetic acid to correlate bands observed in blots and stained gels.

Affinity chromatography. Affinity chromatography was performed with either lentil lectin-Sepharose 4B (Sigma) or concanavalin A-Sepharose 4B (Pharmacia). Samples of the mycelial antigens were equilibrated in 0.01 M Tris hydrochloride (pH 7.4) containing 0.001 M calcium chloride and 0.001 M manganese chloride. These were initially applied to a column containing 5 ml of the pre-equilibrated gel and later mixed with the gel in a batch method. Nonbound components were eluted at a flow rate of 20 ml/h. Bound components were eluted in the same buffer containing 0.2 M methyl α-D-mannoside. All fractions were assayed for protein, for serological activity by ID, and for protein bands by denaturing SDS-PAGE. Bound fractions were not assayed for carbohydrate because of the presence of the eluting mannoside. Nonbound fractions were later pooled and subjected to size exclusion chromatography.

Enzyme and sodium periodate treatment. Antigens were diluted to a protein concentration of 2 mg/ml in 0.1 M Tris hydrochloride (pH 7.4) containing 0.02 M magnesium chloride. Separate aliquots were incubated with either (i) pronase (Calbiochem-Behring, San Diego, Calif.) at a final concentration of 400 μg/ml, (ii) trypsin (Calbiochem) at a final concentration of 1 mg/ml, or (iii) sodium periodate (J. T. Baker Chemical Co., Phillipsburg, N.J.) at a final concentration of 0.05 M periodate (12.7 mg/ml). All dilutions were made in the Tris-magnesium chloride buffer. Incubation with enzymes was for 2 h at 37°C; treatment with periodate was for 18 h at 4°C (7, 18). Following periodate oxidation, the antigen preparations were extensively dialyzed against 0.02 M potassium phosphate (pH 7.0) for 48 h at 4°C. All samples were immediately lyophilized following digestion and dissolved in the appropriate electrophoresis sample buffer to a final concentration of 1 mg of protein per ml immediately prior to electrophoresis. Antigens incubated without the enzymes and the enzymes alone were run in parallel.

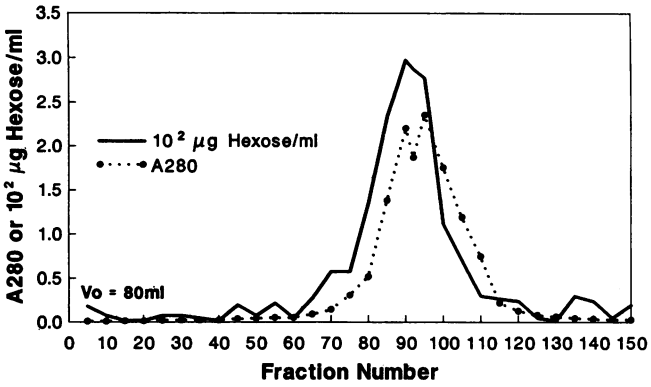

FIG. 1. Protein and carbohydrate contents of fractions obtained by gel filtration from mycelial antigen SF. All of the ID-CF antigen eluted in fractions 30 to 40 with an average molecular size of 110 kDa. These fractions yielded the 48-kDa band upon denaturation. Fractions containing ID-TP activity occurred from 200 to 40 kDa (fractions 5 through 55). Vo, Void volume.

RESULTS

Gel chromatography. All of the ID-CF activity contained in a representative mycelial filtrate antigen (SF) eluted in a peak of 110 kDa average (fractions 30 to 40) which showed little protein or carbohydrate (Fig. 1). On the other hand, virtually all of the protein and carbohydrate eluted in a peak of less than 30 kDa average. This peak had no demonstrable serological activity, and any pigmented material present in the antigen eluted with this peak. Fractions containing the ID band that corresponded to the ID-TP activity occurred in a broad range extending from 200 to 40 kDa found in fractions 5 through 55.

Denaturing SDS-PAGE of these gel filtration fractions showed that the 48-kDa band was present in fractions 30 through 40 and specifically coincided with all ID-CF activity (Fig. 2). The 18-kDa band, which was present in different fractions having an average elution of 44 kDa, showed no ID-CF activity.

Similar data were obtained for mycelial antigen F171 and for the SE-phase antigen (not shown). Fractions of 110-kDa average size yielded a 48-kDa band upon denaturing gel electrophoresis. ID-CF activity specifically correlated with undenatured 110-kDa and denatured 48-kDa fractions.

Gel electrophoresis and protein blot analysis. Reducing and nonreducing PAGE analyses of the representative antigen, mycelial-phase SF, using previously heated and unheated preparations, were done with an acrylamide concentration of 15% (Fig. 3). Under nondenaturing conditions, this unheated

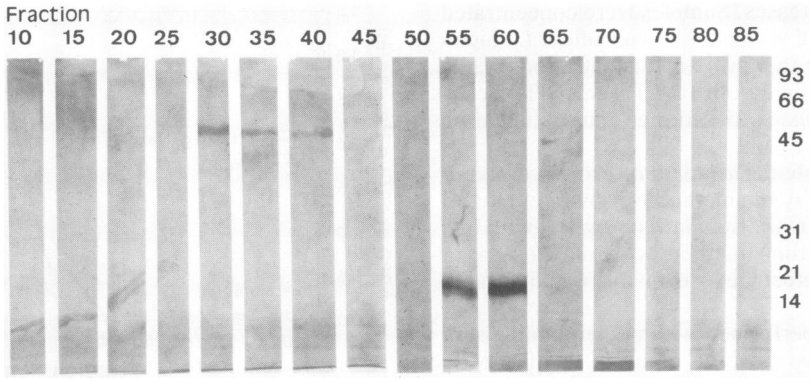

FIG. 2. Denaturing SDS-PAGE of gel filtration fractions of mycelial antigen SF. The 48-kDa band, present in fractions 30 to 40, specifically coincided with fractions containing ID-CF activity. The 18-kDa band appeared in fractions 55 and 60. Molecular size standards in kilodaltons are on the right.

coccidioidin had a rather diffuse staining pattern from 60 to 80 kDa. Definite bands were seen at 31 and 25 kDa. Under these same conditions, previously heated SF (lacking ID-CF activity) showed no diffuse staining but did show a distinct 34-kDa band not seen in unheated SF. Similar results were obtained for the F171 mycelial filtrate. Presented in comparison and described previously (31), electrophoresis of both mycelial antigens under denaturing conditions revealed bands at 48, 38, and 18 kDa. Heating SF completely destroyed the 48- and 38-kDa bands.

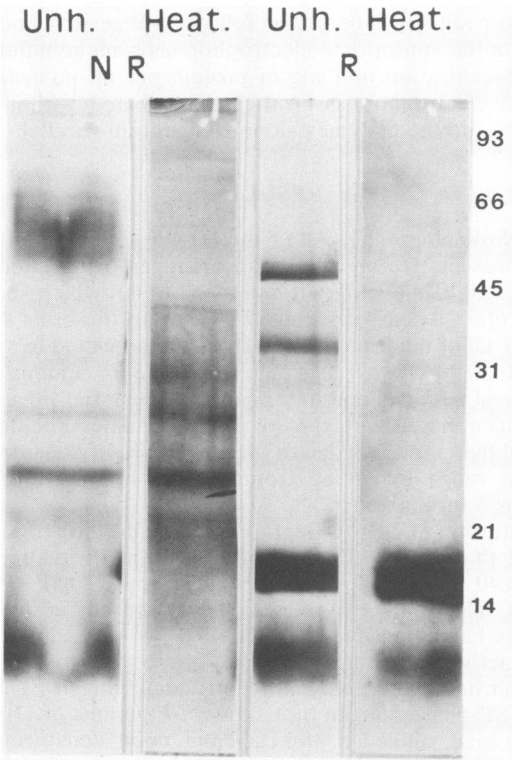

FIG. 3. Reduced (R) and nonreduced (NR) PAGE of mycelial antigen SF using previously heated and unheated preparations. The acrylamide concentration was 15%. The unheated nonreduced gel showed diffuse staining (60 to 80 kDa) and bands at 31 and 25 kDa. Heating destroyed the 48- and 38-kDa bands shown in the reduced unheated preparation. Molecular size standards in kilodaltons are on the right.

To determine whether larger-molecular-size polymers in these filtrates were present but precluded from entering a 15% gel, electrophoresis was performed with 5% acrylamide gels (Fig. 4). Both unheated coccidioidins showed a diffuse staining that extended approximately from 150 to 100 kDa. This suggests the presence of a large-molecular-size polymer that did not enter smaller-pore (15% acrylamide) gels. This band was absent from both of the heated mycelial antigens.

The nonreduced PAGE of the 120-h SE-phase filtrate is shown in lane U (right panel) of Fig. 5. This filtrate showed at least three major bands in the 90- to 115-kDa region. Major bands were also observed at 46 and 20 kDa. Heating this preparation eliminated all of these bands (data not shown). Presented in comparison in lane U (left panel) of Fig. 5 and described previously (31) is electrophoresis of the 120-h filtrate under denaturing conditions. This filtrate showed at least 17 bands, including an intensely staining triplet present at 50 to 48 kDa, the latter corresponding to the 48-kDa band described above for the mycelial antigens.

Heated and unheated filtrates were immunoblotted following nondenaturing electrophoresis (Fig. 6). At least one of the major bands, greater than 100 kDa and described above as stainable with silver, appeared to be immunoreactive.

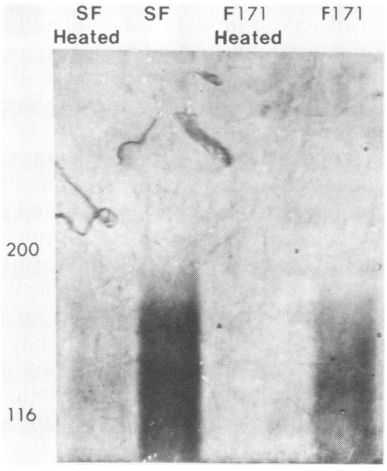

FIG. 4. Nondenaturing PAGE of mycelial antigens SF and F171 using previously heated and unheated preparations. The acrylamide concentration was 5%, permitting detection of fractions in the unheated preparation that retained ID-CF activity. Molecular size standards in kilodaltons are on the left.

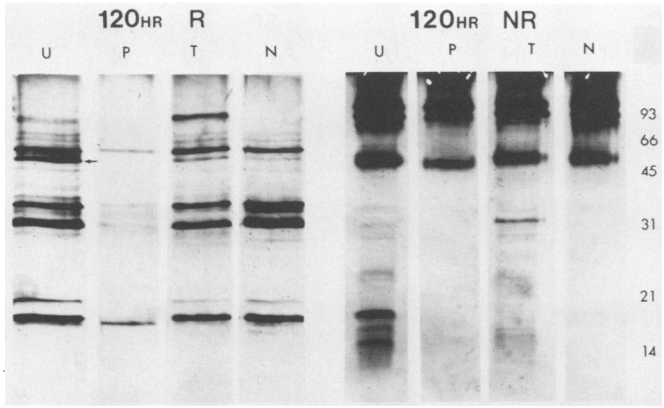

FIG. 5. Silver stain of denaturing SDS-PAGE (R) and nondenaturing PAGE (NR) of the 120-h SE-phase antigen following enzyme and sodium periodate treatment. U, Untreated; P, pronase; T, trypsin; N, sodium periodate. Arrow indicates 48-kDa band removed by pronase under reducing conditions. Molecular size standards in kilodaltons are on the right.

(The relative mobility appeared to be slightly higher in this gel because of the decreased concentration of acrylamide not constraining migration of the separated material.) The 110-kDa band was strongly reactive with CF serum when anti-IgG was the detecting antibody, but this reactivity was eliminated by heating the antigen before electrophoresis. In contrast, blots of the mycelial filtrates under nondenaturing conditions in 5 or 10% gels revealed no specific immunoreactive bands of molecular size greater than 100 kDa; rather, the entire nitrocellulose strip generally reacted very quickly upon substrate addition regardless of the detecting antibody or anti-immunoglobulin (data not shown).

TABLE 1. Anti-IgG immunoblot reactivity of the mycelial SDS-PAGE 48-kDa band following enzyme or periodate treatment

Antigen treatment	Antigen reactivity[a]		
	SF	F171	Heated F171
Untreated	+	+	Altered
Pronase	−	−	−
Trypsin	−	+	−
$NaIO_4$	+	+	+

[a] +, Retained; −, lost.

Effect of pronase, trypsin, and sodium periodate on antigens. Treatment with pronase and trypsin was done to determine the effect of proteases on immunoblot reactivity of the mycelial 48-kDa SDS-PAGE band. Treatment with periodate was to determine whether it would alter the reactivity of the same band by its effect on carbohydrate (Table 1; Fig. 7). Pronase treatment eliminated the ability of any electrophoretically distinct mycelial antigen to bind to antibody in protein blots. Trypsin had no effect on the reactivity of unheated coccidioidin. In contrast, trypsin treatment of previously heated F171 (which gives an altered

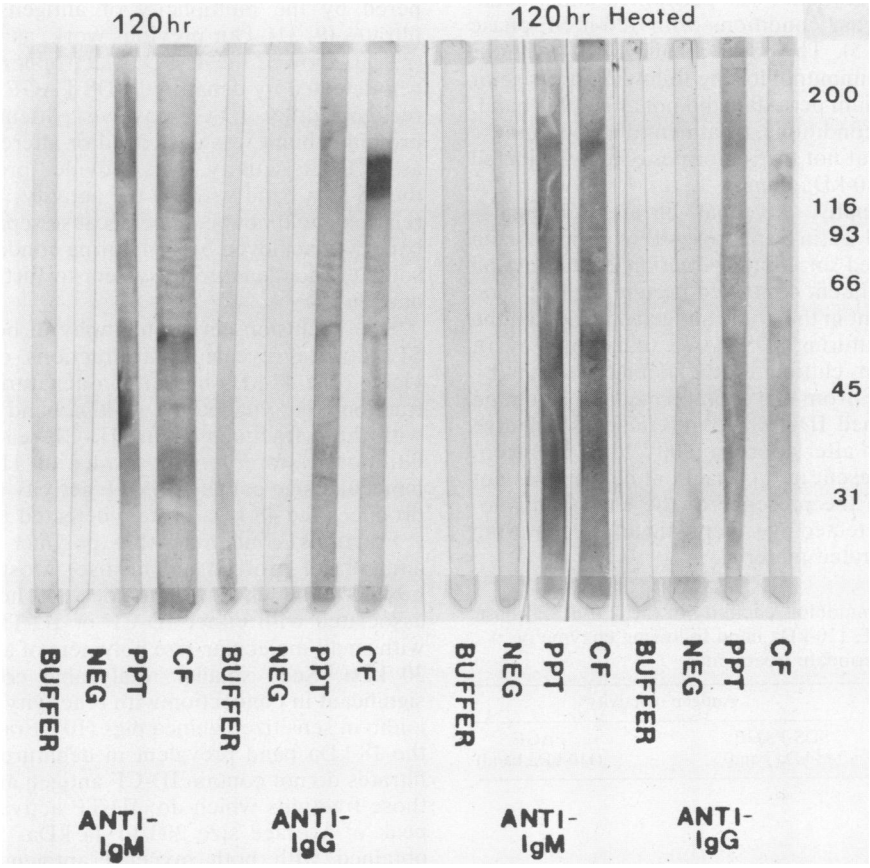

FIG. 6. Nondenaturing PAGE immunoblot of 120-h SE-phase filtrate. CF, CF serum; PPT, precipitin serum; NEG, negative serum. Molecular size standards in kilodaltons are on the right.

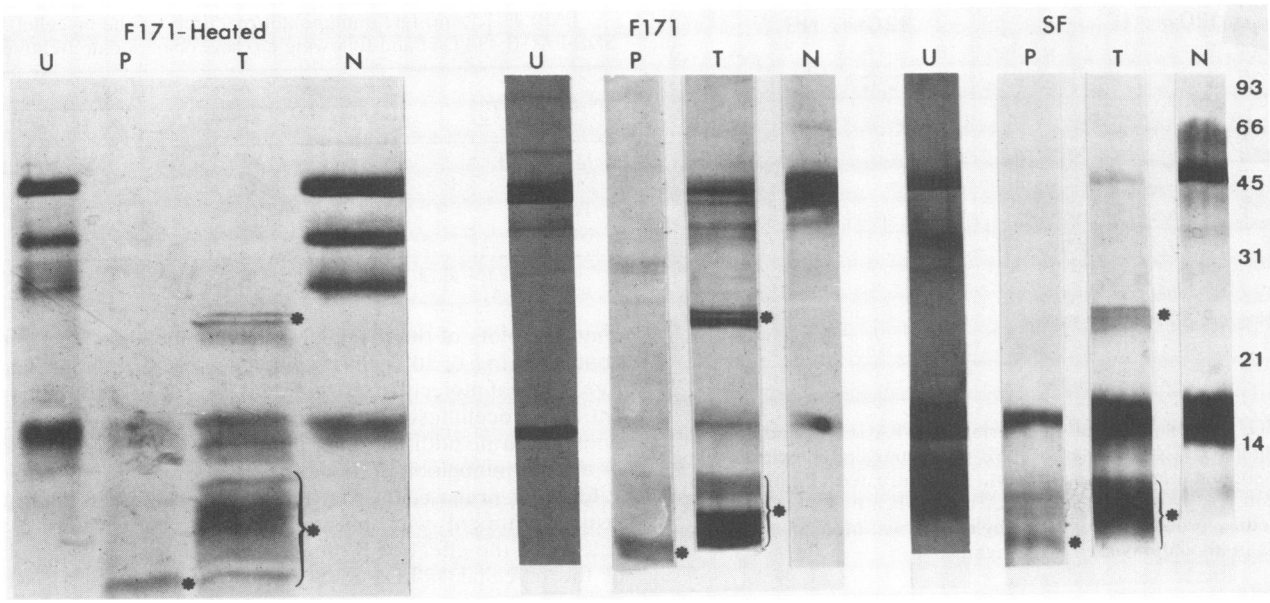

FIG. 7. Silver-stained SDS-PAGE pattern of mycelial filtrates following enzyme or sodium periodate treatment. The 48-kDa band was eliminated by pronase. Trypsin destroyed this band only after heating. U, Untreated; P, pronase; T, trypsin; N, sodium periodate; *, bands present in enzyme controls. Molecular size standards in kilodaltons are on the right.

48-kDa band in SDS-PAGE) completely inhibited any antigenic activity of the altered band. Periodate had no effect on the immunoblot reactivity of the 48-kDa band of either mycelial antigen.

The same set of experiments was performed under denaturing and nondenaturing conditions with 120-h SE-phase antigen (Table 2; Fig. 5). The 48-kDa band in SDS-PAGE was not detectable by immunoblotting following pronase or trypsin treatment. Sodium periodate did not affect this band. Under nondenaturing conditions, treatment of the SE-phase antigen with pronase but not trypsin or periodate eliminated the reactivity of the 110-kDa band.

Affinity chromatography. Mycelial filtrates chromatographed on either lentil lectin-Sepharose 4B or concanavalin A-Sepharose 4B yielded an effluent fraction containing all ID-CF activity. This effluent contained protein, hexose, and pigment material present in the crude antigen and yielded the 48-kDa band upon denaturing SDS-PAGE (data not shown).

Competitive gradient elution of the bound fraction by methyl α-D-mannoside from the affinity medium liberated material which contained ID-TP activity and was negative for ID-CF antigen even after approximately 20-fold concentration. Protein was present in this fraction; hexose was not determined because of the presence of the eluting mannoside. No bands were detected by silver stain after denaturing SDS-PAGE of the liberated material.

TABLE 2. Anti-IgG immunoblot reactivity of the 120-h SE-phase 48-kDa band or PAGE 110-kDa band following enzyme or periodate treatment

Antigen treatment	Antigen reactivity[a]	
	SDS-PAGE (48-kDa band)	PAGE (110-kDa band)
Untreated	+	+
Pronase	−	−
Trypsin	−	+
$NaIO_4$	+	+

[a] +, Retained; −, lost.

DISCUSSION

Detection of the ID-CF antibody in patients with coccidioidomycosis has well-established clinical value. Study of the precise antigen involved in this reaction has been hampered by the multiplicity of antigens present in culture filtrates (9, 31). Our previous work, as well as that of others (2, 12), showed the 48-kDa electrophoretically distinct antigen detected by denatured SDS-PAGE to be significant and recognized by ID-CF-positive patient sera. The 48-kDa protein subunit was destroyed or altered by heat treatment, as is ID-CF activity. This provided probable correlation of the 48-kDa band with ID-CF activity, but unequivocal correlation could not be made because separation of the 48-kDa band was achieved by denaturing conditions. Therefore, we sought a nondenaturing system to further characterize this antigen.

Size exclusion chromatography of both the mycelial and SE filtrates revealed that fractions of 110 kDa average yielded the 48-kDa band upon denaturing SDS-PAGE. The fractions that yielded the 48-kDa band specifically coincide with those fractions having ID-CF reactivity. Thus, by gel filtration, a forerunner polymer of 110 kDa approximate molecular size contains ID-CF activity and, upon reduction, produces the 48-kDa protein detected in SDS-PAGE.

Fractions containing this polymer also contain small amounts of protein and hexose. Most of the protein and hexose in the whole filtrate does not, however, appear to be associated with either ID-CF or ID-TP activity but rather with small-molecular-size polymers of average size less than 30 kDa. Such smaller, dialyzable components appeared significant in connection with reactivity or anergy to coccidioidin in sensitized guinea pigs (10). Fractions which yielded the 18-kDa band prevalent in denatured SDS-PAGE of all filtrates do not contain ID-CF antigen and are separate from those fractions which do. ID-TP activity eluted in a broad peak of average size 200 to 44 kDa. Similar results were obtained with both mycelial antigens and with the SE antigen.

Nondenaturing electrophoresis in 15% acrylamide gels of

mycelial filtrates gave a rather diffuse staining pattern. Electrophoresis in 5% acrylamide gels, however, showed that heat-labile, stainable material of approximate size 100 to 150 kDa was present. The molecular sizes obtained in nondenaturing electrophoresis correlated with those obtained by gel chromatography. Immunoblots of nondenatured mycelial antigen were generally obscured by the large amount of carbohydrate present; these antigens have a carbohydrate:protein ratio of up to 70:1 (31).

In contrast, the 120-h SE-phase antigen has a carbohydrate:protein ratio of 1:1 and protein blots were relatively better defined. Nondenaturing electrophoretic studies revealed a heat-labile polymer in the 90- to 125-kDa region. This polymer was silver stainable and specifically reactive with ID-CF serum when probed with anti-IgG (heavy chain specific). These data again correlate with our gel chromatography results and with the results of Calhoun et al. (2). The latter workers eluted regions of a nondenatured PAGE of coccidioidin and subjected these to SDS-PAGE. The nondenatured 130- to 170-kDa region yielded seven antigens after denaturation, including a 45- to 48-kDa peptide.

In the present work, pronase effectively eliminated detection of the 48-kDa band in SDS-PAGE from both SE and mycelial-phase preparations. In nondenatured immunoblots of SE-phase antigen treated with pronase, the 110-kDa band was no longer reactive. These experiments indicate the protein nature of the 110-kDa antigen.

The effects of trypsin were varied. The 48-kDa band was diminished in mycelial-phase antigen SF but not in the other unheated mycelial antigen, F171, a pooled filtrate of 22 strains. The immunoblot of this latter antigen was also unaffected by trypsin treatment. Prior heat treatment of F171 altered the SDS-PAGE pattern of the 48-kDa band. Trypsin treatment of the heated antigen completely eliminated all of the altered bands. In SE-phase antigen, the reactivity of the 110-kDa nondenatured band, forerunner of the 48-kDa band, was unaffected by trypsin; but the 48-kDa denatured band was deleted by trypsin.

Trypsin hydrolyzes peptide bonds on the carboxylic side of arginine and lysine residues. Most native disulfide-bond-containing "tight" proteins, such as lysozyme, are not susceptible to trypsin (3). The experiments with single-strain mycelial and SE-phase filtrates suggest that the nonreduced 110-kDa polymer has residues buried in its conformation that become exposed and susceptible to trypsin when the 48-kDa band is separated from the rest of the polymer by reduction of the disulfide bond and baring of arginine and lysine. In unheated mycelial pooled filtrate F171 there may be several *C. immitis* strain-dependent reactive polymers migrating to the 48-kDa area in SDS-PAGE. Evidently, one of these peptides is not susceptible to tryptic digestion. Prior heat treatment at 60°C for 30 min, however, destroys the conformation so that the appropriate residues become available for cleavage. It is of interest that endogenous protease(s) with activity present in the SE-phase filtrates does not destroy the ID-CF or ID-TP activity (23, 29).

A small amount of hexose is present in ID-CF antigen-positive fractions remaining after lectin treatment and after size exclusion chromatography. Studies with both lectin affinity chromatography and periodate oxidation showed that neither treatment affected ID-CF reactivity as detectable by immunoblotting. Periodic acid oxidizes cyclic 1,2 diols to straight-chain aldehydes. Failure of the periodate to affect immunoreactivity may reflect a lack of carbohydrate in the epitope(s) reactive in ID-CF. An alternative possibility is suggested by finding with *Salmonella* species certain polysaccharides which contain different linkages among mannose and glucose. In exhaustive periodate oxidation of these carbohydrates, glucose was largely destroyed yet mannose was unaffected (11). Thus, the possibility exists that periodic acid did not oxidize one of the major sugars of *C. immitis* filtrates (22). Since some of the mannose is methylated at position 3, a further speculation might be that a 1,2 oxidation site was protected by steric hindrance (1, 25).

The lack of affinity of the ID-CF polymer for the concanavalin A and lentil lectins also reflects a lack of reactive glucose or mannose residues in the antigen. Both lectins preferentially bind to asparagine-linked sugar chains but not serine-threonine-linked sugar chains. An unmodified hydroxyl group at C-3 of the glucopyranosyl residue is essential for the interaction of concanavalin A with a sugar (19). Both the lectin chromatography data and periodate oxidation data indicate that if a carbohydrate is a structural part of the ID-CF polymer, it must lack sterically accessible glucose or unmodified mannose. It probably does not contain accessible asparagine peptide-carbohydrate linkages, but it may contain others.

Cox et al. (5) isolated an antigen reactive with ID-CF antiserum. They produced an antiserum to heat-stable components in a concanavalin A effluent fraction and removed those components by immunoaffinity chromatography with the resultant monospecific antiserum. This antigen was assayed by two-dimensional immunoelectrophoresis and corresponded to antigen 3 in the reference system of Huppert et al. (9). Using the same immunoelectrophoresis techniques, Cox and Britt (4) reported that coccidioidin-derived antigens, including one reactive by ID-CF, are identical to immunoelectrophoresis precipitinogens of spherulin. The data presented herein also show the presence in coccidioidin and SE filtrates of a similar ID-CF antigen.

We conclude that a 110-kDa protein polymer is responsible for ID-CF activity. Upon SDS-PAGE denaturation, this polymer yields a 48-kDa peptide detected by its specific reactivity with ID-CF-positive serum in immunoblots.

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