Purification and Characterization of an 80-Kilodalton Membrane Protein from *Leishmania donovani*

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Visceral leishmaniasis is caused by the protozoan parasite *Leishmania donovani*. We previously described the development of 16 monoclonal antibodies specific for *L. donovani*. The epitope recognized by one of these monoclonal antibodies, D13, is present at high density on nearly all isolates of *L. donovani* and, along with two other monoclonal antibodies, has been used to develop a sensitive and specific competitive assay for serodiagnosis of visceral leishmaniasis. In this report, we characterize the antigens recognized by D13 by immunoprecipitation of [35S]methionine-labeled promastigotes as two proteins (apparent molecular mass, 72 and 80 kilodaltons). Pulse-chase studies showed no evidence of a precursor-product relationship for the two proteins. We purified the 80-kilodalton protein (p80) to homogeneity by detergent solubilization of promastigote membranes, immunoaffinity chromatography, and ion-exchange chromatography. The epitope on p80 recognized by D13 was completely destroyed by proteolysis but was not affected by periodic acid treatment. P80 did not bind to the radiolabeled lectins concanavalin A, wheat germ agglutinin, and *Ricinus communis* agglutinin. Its apparent molecular mass was not affected by tunicamycin. Thus, it does not appear to be glycosylated. This protein is highly immunogenic and may prove useful for immunoprophylaxis and serodiagnosis of visceral leishmaniasis.

The protozoan parasites of the *Leishmania donovani* complex (*L. donovani, L. infantum,* and *L. chagasi*) cause human visceral leishmaniasis or kala-azar (33, 35, 38). Visceral leishmaniasis classically includes fever, hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia. Untreated visceral leishmaniasis is frequently fatal, primarily because of bacterial superinfections (33, 35). Recent field studies indicate that classical visceral leishmaniasis is but a small portion of a spectrum of disease that includes subclinical and asymptomatic infections (1, 40). Even asymptomatic or latent infection may progress to overt disease with immunosuppression (12).

Conclusive diagnosis of visceral leishmaniasis depends on the demonstration of parasites in material aspirated from spleen, bone marrow, or other tissues either by identification of amastigotes in stained impression smears or by in vitro cultivation of promastigotes. Even in severe disease, parasites may be rare, such that clinicians must resort to either repeated invasive procedures or empiric therapy with potentially toxic drugs (33, 35, 38). Currently available serodiagnostic techniques are sensitive and convenient (2, 3, 20, 33, 35). However, their usefulness is limited by cross-reactions with coendemic diseases (especially Chagas' disease and African trypanosomiasis) and problems in standardization (i.e., results may vary dramatically, depending on the strain of parasite used as an antigen source [2, 3]). Therapy depends on the use of potentially toxic drugs which are not uniformly effective. However, cured infection, whether spontaneous or due to treatment, is associated with protective immunity (reviewed in reference 15). Thus, there is potential for prophylactic immunization.

Numerous investigators have attempted to characterize *L. donovani* antigens that might be useful for serodiagnosis and prophylactic immunization (7, 11, 17, 19, 23, 24, 31, 37, 41, 43). In a previous report, we described the development of 16 monoclonal antibodies specific for *L. donovani* (22). On immunoblot analysis of promastigote membranes, one of these monoclonal antibodies, D13, recognized a major 80-kilodalton (kDa) band, minor bands at 72 and 62 kDa, and a heterodispersed region from 80 to 240 kDa (22). The D13 epitope is present at a high density on nearly all isolates of *L. donovani* when judged by radioimmunoassay (RIA) binding ratios (16, 22). Antibody which binds to the D13 epitope is present in high titer in patients with visceral leishmaniasis (23). D13 and two other monoclonal antibodies have been used to develop a sensitive and highly specific competitive enzyme-linked immunosorbent assay for serodiagnosis of visceral leishmaniasis (23). We found during the purification process that the molecules recognized by monoclonal antibody D13 are proteins which appear on sodium dodecyl sulfate (SDS) gels as 72- and 80-kDa bands. Here we report the purification and initial characterization of the 80-kDa protein (p80) and discuss its possible use in serodiagnosis and immunoprophylaxis.

**MATERIALS AND METHODS**

**Monoclonal antibody.** Production of monoclonal antibody D13, monoclonal cultured supernatants, and monoclonal ascites was previously described (22). Polyclonal antiserum was prepared by immunizing New Zealand White rabbits with *L. donovani* membranes and incomplete Freund adjuvant (23).

**Protein assays.** Unless otherwise noted, protein concentrations were determined by the method of Peterson, with bovine serum albumin as the standard (36).

**Parasites.** *L. donovani* LV9 was maintained by twice weekly passage of promastigotes in Schneider's *Drosophila* medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (GIBCO Laboratories, Grand Island, N.Y.). Virulence was maintained by periodically infecting BALB/c mice and harvesting parasites from their spleens.

**Competitive RIA.** Antigenic activity was assessed by a

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competitive RIA modified from the techniques of Kenneth and Williams (29, 44). Briefly, late-log-phase promastigotes were pelleted, washed three times in phosphate-buffered saline (PBS), counted, and resuspended at a concentration of 2 x 10^7/ml. A 50-μl volume containing 10^6 cells was added to each well of 96-well round-bottom polyvinyl chloride plates coated with poly-1,-lysine (P1274; 1 mg/100 ml; Sigma Chemical Co., St. Louis, Mo.), and the plates were centrifuged (1,000 x g, 5 min). To each well was added 50 μl of 0.5% glutaraldehyde. After 15 min of incubation, the plates were emptied, washed twice with PBS, blocked with PBS containing 2% FBS plus 0.02% NaCl (PBS-FBS), and stored at -20°C. Antigens were titrated by serial twofold dilution in PBS containing 0.1% Brij 97 (Ruger Chemical Co., Irvington, N.J.). Diluted antigen (100 μl) was added to D13 antibody ascites fluid (100 μl; diluted 1:4,000 in PBS containing 5% FBS) and incubated (overnight, 4°C). On the next day, promastigote-coated plates were thawed, washed six times with PBS-FBS, and incubated with antibody plus antigen (50 μl per well, 90 min, on ice, duplicate wells). The plates were again washed with PBS-FBS, probed for 60 min with 125I-labeled rabbit F(ab')2, anti-mouse immunoglobulin (2 x 10^6 cpm per well in PBS-FBS), washed six times, dried, and counted on a gamma counter. Antigen units are defined as the reciprocal of the titer giving 50% inhibition of binding times the volume in milliliters.

Antigen purification. The monoclonal antibody was partially purified from D13 ascites by 50% ammonium sulfate precipitation. An affinity resin was prepared by coupling purified antibody to CNBr-activated Sepharose CL-2B (34). Parastyme parasite-enriched fractions were prepared as previously described (26). Briefly, late-log-phase promastigotes (approximately 2 x 10^10) were harvested by centrifugation (500 x g, 30 min), washed twice with PBS, and suspended to a 10-fold dilution in lysis buffer (20 mM Tris hydrochloride [pH 7.3], 10 mM EDTA, 40 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM iodoacetamide) and equilibrated for 10 min at 4°C. The cells were disrupted by nitrogen cavitation (1,500 lb/in², 10 min, 4°C). Cell debris and organs were removed by centrifugation (4,340 x g, 10 min, 4°C). A membrane-enriched pellet was generated by further centrifugation (34,800 x g, 30 min, 4°C) and suspended in 10 ml of lysis buffer (typically 6 to 10 mg of protein per ml by the Bio-Rad protein assay) and stored at -70°C until used. The membrane-enriched fractions were solubilized with Brij 97 (2 mg of detergent per mg of protein) in PBS (pH 7.2)-10 mM EDTA-1 mM phenylmethylsulfonyl fluoride-1 mM iodoacetamide. The material was equilibrated on ice for 30 min and then centrifuged (34,800 x g, 30 min, 4°C). The soluble fraction was diluted in PBS to 0.1% Brij and loaded on a D13 immunoaffinity column. After being washed with 10 column volumes of PBS-0.1% Brij and 1 column volume of 0.5 M NaCl-10 mM phosphate-0.1% Brij (pH 7.2), the column was eluted with 0.1 M formic acid-0.05% Brij (pH 3.5). The eluate was neutralized, concentrated by dialysis with Aquacide IIA (Calbiochem-Behring, La Jolla, Calif.), and dialyzed against 20 mM Tris (pH 8.0). The affinity-purified material was then loaded onto a DE-52 column (Whatman, Maidstone, Kent, England) which had been equilibrated with 20 mM Tris-0.1% Brij (pH 8.0). After extensive washing, antigoigenic activity material was eluted with 1 M NaCl-20 mM Tris (pH 8.0)-0.05% Brij and dialyzed against PBS-0.02% NaCl (pH 7.2).

[35S]Methionine metabolic labeling. Promastigotes were harvested from log-phase culture, washed three times in PBS, and suspended in methionine-free Schneider's medium (10/ml). After 1 h of incubation, [35S]Methionine (100 μCi/ml; 1,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) was added and the mixture was incubated (23°C overnight). For some studies, tunicamycin (5-μg/ml final concentration; Sigma) was added 30 min before addition of the radiolabel. Labeled cells were washed twice in PBS, pelleted, snap frozen in a dry ice-ethanol bath, and stored at -70°C. For pulse-chase studies, a sample of cells was removed after 1 h of labeling. The remaining cells were pelleted and suspended in complete Schneider's medium supplemented with 15% FBS. Samples were removed after 1, 2, 4, 8, and 24 h, washed twice in PBS, and snap frozen. Labeled cells were solubilized in PBS-0.5% Brij-10 mM EDTA-1 mM iodoacetamide-1 mM phenylmethylsulfonyl fluoride. After centrifugation (34,800 x g, 30 min), the supernatant was removed. After 10^7 cpm was added to 80 μl of D13 affinity resin or control resin (CNBr-coupled Sepharose CL-2B which does not bind specifically to L. donovani), the mixture was incubated (0°C, 60 min). The resin was washed three times in PBS-0.1% Brij and twice with PBS-0.1% Brij-0.05% SDS and eluted by boiling in SDS sample loading buffer (62.5 mM Tris [pH 6.8], 2% SDS, 20% glycerol, 0.01% bromphenol blue). SDS-polyacrylamide gel electrophoresis. Radiolabeled and unlabeled samples were electrophoresed on 10% polyacrylamide gels with 5% polyacrylamide stacking gels as described by Laemmli (30). Silver staining was performed with a silver staining kit (Bio-Rad Laboratories, Richmond, Calif.). Gels of 35S-labeled proteins were fluorographed by using Autofluor (National Diagnostics, Manville, N.J.).

Immunoblots. Immunoblotting was performed by the method of Towbin et al. (42), with 0.2-μm-pore-size nitrocellulose sheets (Schleicher & Schuell, Inc., Kennes, N.H.). For immunoblots, the nitrocellulose sheets were blocked and washed with PBS containing 3% nonfat milk (25), probed with cell culture supernatants containing monoclonal antibody D13 diluted 1:4 in the wash solution, and subsequently probed with 125I-labeled affinity-purified rabbit F(ab')2 anti-mouse immunoglobulin (2 x 10^6 cpm/ml in PBS containing 3% milk, 60 min, room temperature). Blots were then washed with PBS-3% milk, air dried, and exposed for autoradiography with Kodak X-OMAR film and Cronex Lightning-Plus intensifying screens (Du Pont Co., Wilmington, Del.).

Periodic acid treatment. Promastigote membrane-enriched fractions were suspended in periodic acid (final concentration, 10 mM periodic acid in 0.5 M acetic acid buffer, pH 4.5) and incubated (23°C, 2 h). The reaction was quenched with glycerol. Membrane fractions (periodic acid treated and controls) were then boiled in SDS sample loading buffer for 3 min and analyzed by immunoblotting as described above, except that 8% polyacrylamide separating gels were used.

Proteinase digestion. Nitrocellulose paper (0.45-μm pore size; Schleicher & Schuell) was loaded into a dot blot apparatus (Bio-Rad). Rabbit polyclonal antiserum to L. donovani promastigote membranes was partially purified by ammonium sulfate precipitation. Antibody (80 μg of protein) was added to each well of the dot blot apparatus and incubated (4°C overnight). Purified p80 was incubated in the presence or absence of proteinase K (Boehringer-Mannheim; 1 μg/ml; 37°C). After incubation for 0 min, 30 min, 1 h, and 20 h, the reaction was quenched by addition of phenylmethylsulfonyl fluoride to each tube (final concentration, 5 mM). p80 (1 μg of protein from each reaction mixture) or the equivalent volume of PBS-FBS was added to each antibody-coated well and incubated (30 min on ice). The
wells were drained by gravity and then washed three times with PBS-FBS. The nitrocellulose was then blocked with PBS containing 3% nonfat milk and probed with D13 monoclonal supernatants and 125I-labeled rabbit F(ab')2 anti-mouse immunoglobulin as described above for immunoblots. After the final wash, the nitrocellulose sheets were air dried and exposed for autoradiography. Sections from the remaining portions of the nitrocellulose sheets were counted in a gamma counter.

**Lectin blots.** Wheat germ agglutinin, concanavalin A, and *Ricinus communis* agglutinin (RCA120) were iodinated with chloramine-T in the presence of inhibitory sugars (N-acetyl-D-glucosamine, α-methyl-β-mannoside, and D-galactose, respectively) as previously described (5). The reaction was quenched with sodium sulfite (5). Radiodinated lectins were combined with PBS containing 1% periodic acid-treated bovine serum albumin (BSA-P) (13) and separated from the inhibitory sugars on Sephadex G-25 columns. For lectin dot blots, purified p80 (5 μg), ovalbumin (0.2 to 5 μg), promastigote membranes (50 μg of protein), or PBS containing 3% BSA-P was added directly to nitrocellulose paper in the dot blot apparatus. In addition, whole parasite membranes, purified p80, and ovalbumin were separated by SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose as described above for immunoblots. Both lectin blots and dot blots were probed with PBS containing 3% BSA-P. (13) Wash solution for the lectin blots consisted of PBS plus 1% BSA-P, 1 mM CaCl2, 1 mM MgCl2. The nitrocellulose was incubated with each lectin in the presence or absence of 40 mg of inhibitory sugar per ml (90 min, room temperature, 2 × 103 cpm/ml in wash solution). Blots were then washed three times, dried, and exposed for autoradiography. In addition, sections of the dot blot sheets were counted in a gamma counter.

**Amino acid analysis.** The amino acid composition of the purified p80 was determined with a Beckman model 7300 amino acid analyzer with a norleucine standard in the laboratory of Ken Williams at the Yale Medical School protein analysis facility. For routine analysis, purified p80 (5 to 10 μg) was hydrolyzed in 6 N HCl containing 2% phenol (16 h, 115°C). Cysteine content was determined by performic acid oxidation of p80 (10 to 20 μg) before hydrolysis (14). For tryptophan determinations, methanesulfonic acid hydrolysis was used (10 to 20 μg of protein) (14). The results are the mean of three blank-corrected determinations.

**RESULTS**

**Purification.** p80 was purified to homogeneity in a four-step procedure involving isolation of promastigote membranes, detergent solubilization, affinity purification, and ion-exchange chromatography. The preparation of parasite membranes removes cytosolic and nuclear proteins. Antigen activity was present only in the fractions containing parasite membranes (23). Only about one-third of the membrane protein was solubilized in detergent (Table 1). This is consistent with studies by Dwyer under similar conditions which showed that most of the detergent-insoluble material in *L. donovani* membranes is composed of subpellicular microtubules with small amounts of integral membrane proteins (8). No contaminating bands were seen on silver-stained gels after affinity purification (Fig. 1). However, a 72-kDa band was present in the high-salt wash. With ion-exchange chromatography, the effluent contained 80-kDa material that was not antigenically active by immunoblots or competitive RIA. This suggests that some of the p80 did not renature after acid elution of the affinity column. The antigenically active eluate from the DE-52 column contained 0.3 to 0.5% of the total membrane protein (Table 1) and appeared on silver-stained gels as a single 80-kDa band (Fig. 1, lane d). The band seen on silver-stained gels comigrated with the antigenic activity as assessed by immunoblots (Fig. 1). It is not of note that the heterodispersed region that is reproducibly represented on immunoblots of crude membranes (Fig. 1, lane h; 22, 24) is diminished after solubilization. Instead, the antigen appears as a discrete band. This heterodispersed pattern is not affected by periodic acid digestion (Fig. 1, lane i). Thus, the heterogeneity may be due in part to aggregation or association with other membrane components.

Since the competitive RIA used to quantitate antigenic activity could be performed optimally only on soluble antigens, solubilized membranes are regarded as the starting material for calculations of yield and purification. Yields of the antigenic activity of the solubilized membranes in purified p80 ranged from 40 to 70%. Calculation of antigenic activity per microgram of protein showed that the final product represents 40- to 50-fold purification of the solubilized membranes (Table 1).

**Amino acid analysis.** The amino acid composition of purified p80 exhibits no unusual features except for the apparent

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### Table 1. Purification of p80

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg)</th>
<th>Antigen U (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antigen U/μg of protein</th>
<th>% Purification</th>
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<tr>
<td>Crude membranes</td>
<td>63.6</td>
<td>3,290 (100)</td>
<td>0.13</td>
<td></td>
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<tr>
<td>Solubilized</td>
<td>25.0</td>
<td>1,584 (48)</td>
<td>4.95</td>
<td>37.6</td>
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<tr>
<td>membranes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affinity-purified</td>
<td>p80</td>
<td>0.32</td>
<td>1,411 (43)</td>
<td>6.25</td>
</tr>
<tr>
<td>DE-52-purified</td>
<td>p80</td>
<td>0.226</td>
<td>1,411 (43)</td>
<td>6.25</td>
</tr>
</tbody>
</table>

<sup>a</sup> These results are from a representative preparation.

<sup>b</sup> Percentage of antigenic units from the solubilized membranes remaining in a fraction.

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*FIG. 1. Purification of p80. Lanes a to d show a silver-stained gel, lanes e to i show autoradiographs of immunoblots probed with monoclonal antibody D13. Lanes: a and h, crude membranes; b and e, solubilized membranes; c and f, eluate from the D13 affinity column; d and g, eluate from the DE-52 column; i, periodic acid-treated membranes. Note that the percentage of polyacrylamide was decreased to 8% for the gel of crude membranes (lanes h and i) to better demonstrate the heterodispersed pattern in the high-molecular-mass (M<sub>r</sub>) region.*
absence of tryptophan (Table 2). There are 10 cysteine residues which may form a maximum of five disulfide bonds.

Metabolic labeling. Our initial characterization of the antigen focused on determining whether the monoclonal antibody recognized a carbohydrate or peptide epitope. Immunoprecipitation of promastigotes metabolically labeled with [35S]methionine yielded two major bands at 72 and 80 kDa. Several bands in the 40- to 60-kDa range appeared with control resins and thus are nonspecific (Fig. 2). When labeling was performed in the presence of 5 μg of tunicamycin per ml, a concentration previously shown to inhibit N-linked glycosylation of leishmanial proteins markedly (4, 6, 32), no change was noted in the molecular masses of the bands immunoprecipitated with D13 affinity resin (Fig. 2).

Proteinase digestion. Nitrocellulose was coated with polyclonal rabbit antiserum to L. donovani promastigote membranes in an antigen-trapping assay. Rabbit antiserum to L. donovani promastigote membranes binds both carbohydrate and peptide epitopes (27). Thus, oligosaccharide epitopes which might not otherwise bind to nitrocellulose after proteolysis of associated peptides could be trapped and quantitated. When purified p80 was treated with proteinase K before antigen trapping, antigenic activity as assessed by binding of the D13 monoclonal antibody was rapidly destroyed, with 95% of the antigenic activity destroyed after 30 min (Fig. 3). After overnight incubation, no antigenic activity remained and the radioactivity bound was equivalent to that found in negative controls. The molecules recognized by D13 are proteins in that they incorporate radiolabeled methionine. The epitope recognized by D13 appears to be associated with the peptide chain, since it is sensitive to proteolysis but not affected by periodic acid treatment.

Lectin binding. Radiiodiated lectins were used to detect any carbohydrate residues that might be present on the purified p80. The lectins used were wheat germ agglutinin, which recognizes N-acetyl-D-glucosamine and N-acetylglycospine.
for the iodinated controls and ovalbumin for concanavalin A. The negative controls were BSA-P. The numerical values are binding ratios determined by counting pieces of nitrocellulose paper in a gamma counter. Values are expressed as binding ratios calculated as follows: (mean cpm of sample — background cpm)/(mean cpm of negative control — background cpm).

Subsequently, frozen samples were solubilized and equal counts of labeled material were immunoprecipitated with D13 affinity resin, washed, and eluted by being boiled in SDS sample buffer. The initial sample showed bands at 72 and 80 kDa in approximately equal proportions. Both bands persisted for 8 h in the same relative proportions (Fig. 5). After 24 h, both protein bands could still be detected but were labeled very weakly. Thus, there is no evidence of a precursor-product relationship for these two proteins.

**FIG. 4.** Lectin dot blots. Autoradiograph of p80 with positive and negative controls probed with iodinated lectins, concanavalin A above and RCA120 below. The positive controls were parasite membranes for RCA120 and ovalbumin for concanavalin A. The negative controls were BSA-P. 

**FIG. 5.** Pulse-chase studies. Parasites were metabolically labeled with a 1-h pulse of [35S]methionine, chased with cold medium, and sampled at various time points before immunoprecipitation with D13 affinity resin. An autoradiograph of a single gel is shown.

**DISCUSSION**

In recent years, much attention has been focused on *L. donovani* antigens which might be useful for serodiagnosis and immunoprophylaxis. Since protective immunity follows cure of *L. donovani* infection, immunoprophylaxis appears to be possible (15). A defined molecular vaccine for visceral leishmaniasis would be an important advance. No purified antigen has been used for prophylactic immunization against *L. donovani*. The only purified leishmanial antigens reported to be successfully used in experimental immunization are the *L. major* glycoconjugate (18), the 63-kDa glycoprotein and glycoconjugate of *L. mexicana* (39), and the 46-kDa glycopeptide of *L. amazonensis* (submitted for publication). The relevance of these observations for human visceral leishmaniasis is unclear, since cross-protection from non-donovani leishmanial strains is limited (15). Thus, it may be important to include *L. donovani*-specific antigens in the search for vaccine candidates.

In approaching this problem, our initial focus was on purification of the *L. donovani* antigen recognized by monoclonal antibody D13. The D13 epitope is present at a high density on nearly all isolates of *L. donovani* as judged by RIA binding ratios (16, 22). It is present on both promastigotes and amastigotes by immunofluorescence (unpublished data). Antibody, which binds to this epitope as assessed by competitive RIA, is present in high titer in both humans (23) and dogs (unpublished data) infected with *L. donovani* but not in uninfected controls (23). In pilot studies, titers of antibodies that compete for binding of this monoclonal antibody appear to correlate with the parasite burden in experimentally infected dogs. In this report, we describe the purification of the 80-kDa *L. donovani* protein recognized by monoclonal antibody D13. p80 represents about 1% of the total promastigote membrane protein. Whereas its biologic role has yet to be established, p80 is certainly a major component of the parasite membrane and highly immunogenic. Preliminary data suggest that low doses of p80 can reduce the parasite burden in mice challenged with *L. donovani* promastigotes (unpublished data). Similarly, reports on T-cell blotting from other laboratories suggest that amastigote antigens in the 57- to 84-kDa range (which includes p80 as well as a number of other proteins) are involved in proliferative responses that might be associated with protective immunity (28). Thus, with the optimal dose, route of administration, and adjuvant, p80 may prove useful for prophylactic immunization.

Our preliminary characterization demonstrated that the D13 monoclonal antibody binds to two bands of 72 and 80 kDa which are readily labeled with [35S]methionine and are not affected by tunicamycin. These results were confirmed...
by demonstrating that the antigenic activity of p80 is destroyed by proteinase K, that binding of D13 to promastigote membranes is not affected by periodic acid treatment, and that p80 does not bind radioiodinated lectins. Thus, p80 appears to be a protein that is not glycosylated.

p80 appears to be a membrane protein in that it was isolated from membrane-enriched fractions and, in R1As, D13 binds to the surface of glutraldehyde-fixed whole parasites and membrane-enriched fractions but not to soluble fractions (23). However, the estimated hydrophobicity of p80, based on the amino acid composition, is not consistent with values seen for traditional integral membrane proteins (9). This may be a reflection of the limitations of hydrophobicity scales, especially when applied to total amino acid composition rather than sequence data. The p80 might contain a relatively small hydrophobic region or be anchored to the membrane by a lipid linkage, as demonstrated for other leishmanial membrane antigens (10).

Several groups have previously investigated L. donovani antigens, primarily in attempts to identify immunologically active molecules for use in a serodiagnostic assay for visceral leishmaniasis. A number of reports have identified antigens in the 65-kDa range that react with sera from patients with visceral leishmaniasis (7, 19, 31, 37). Jaffe and Zalis recently described the purification of two L. donovani membrane antigens recognized by sera from patients with visceral leishmaniasis (24). One antigen was a glycoprotein doublet of 70 and 72 kDa. The second antigen, termed dp72, had a apparent molecular mass of 72 kDa and was not sensitive to periodate.

We purified p80 from solubilized L. donovani membranes by acid elution of a D13 affinity column and ion-exchange chromatography. The dp72 described by Jaffe and Zalis was purified from the same strain of L. donovani by alkaline elution of a D13 affinity resin. Thus, it is antigenically related to p80. Bands at both 72 and 80 kDa were identified by immunoblots of promastigote membranes in the original characterization of this monoclonal antibody (22). In addition, we demonstrated both 72- and 80-kDa bands from solubilized, [35S]methionine-labeled parasites immunoprecipitated with D13 affinity resin (Fig. 2). Jaffe and Zalis showed only immunoblots of their purified dp72 made by using a radioiodinated probe (24). Because of the limitation of the resolution of this technique, it is impossible to tell whether their dp72 represents one or both bands. It is unclear why the purification technique we used resulted in isolation of only the 80-kDa protein. We noted a 72-kDa band in the high-salt wash of our affinity column. Thus, it is possible that the 72-kDa band has a lower affinity for our resin. Alternatively, the 72-kDa protein may be the product of limited proteolysis. In pulse-chase studies, we were unable to show any change in the proportions of the 72- and 80-kDa bands immunoprecipitated with D13. Thus, these two molecules do not appear to be related as simply a precursor and a product, as it is possible that they represent different forms of one molecule, as described for the soluble acid phosphatase of L. donovani (4), or even different molecules with a shared epitope.

We have described the purification and characterization of the L. donovani p80. It is a major component of the membrane of L. donovani and is highly immunogenic. It appears to be useful as an antigen for serodiagnosis. It is potentially an important antigen for immunoprophylaxis. Work is in progress to define these roles further.

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LITERATURE CITED


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