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Subunit Structure of the Galactose and *N*-Acetyl-D-galactosamine-inhibitable Adherence Lectin of *Entamoeba histolytica**

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William A. Petri, Jr.‡§¶‖, Martin D. Chapman‡§, Terri Snodgrass‡, Barbara J. Mann‡, Jennifer Broman‡, and Jonathan I. Ravdin**

*From the Departments of ‡Medicine, §Microbiology, **Pharmacology, and the ¶Cancer Center, University of Virginia School of Medicine, Charlottesville, Virginia 22908*

The galactose and *N*-acetyl-D-galactosamine-inhibitable adherence lectin of *Entamoeba histolytica* is a cell surface protein which mediates parasite adherence to human colonic mucus, colonic epithelial cells, and other target cells. The amebic lectin was purified in 100-μg quantities from detergent-solubilized trophozoites by monoclonal antibody affinity chromatography. The adherence lectin was purified 500-fold as judged by radioimmunoassay. The nonreduced lectin had a molecular mass of 260 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and an isoelectric point of pH 6.2. The amebic lectin reduced with β-mercaptoethanol consisted of 170- and 35-kDa subunits. Both subunits could be labeled on the cell surface with ^{125}I, and both were metabolically labeled with [^{3}H]glucosamine. The amino termini of the subunits had unique amino acid sequences, and polyclonal antisera to the heavy subunit did not cross-react with the light subunit. The yield of phenylthiohydantoin derivatives from the second and third positions in the sequence of the heavy and light subunits gave a molar ratio of one 170- to one 35-kDa subunit. Antibodies directed to the heavy subunit inhibited amebic adherence to Chinese hamster ovary cells by 100%, suggesting that the heavy subunit is predominately responsible for mediating amebic adherence.

The understanding of cell-cell recognition and adherence is central to many fundamental areas of biologic investigation, including mammalian fertilization, developmental morphogenesis of tissues and organs, neoplastic metastases and the cell interactions of the immune system. We believe that there is a need for model systems where the processes of cell recognition, adherence, and contact-dependent cytolysis can be studied in the absence of the complexity of cellular reactions occurring at the organismal level.

Entamoeba histolytica is a nonciliated eukaryotic protozoan that is the cause of amebiasis in humans. Colonic colonization with *E. histolytica* trophozoites can be asymptomatic or lead to invasive colitis and/or amebic liver abscesses. It is estimated that 10% of the world's population is infected with *E. histolytica*, resulting in at least 60,000 deaths worldwide each year (1, 2). Adherence of *E. histolytica* trophozoites to intestinal mucus, colonic epithelium, and host inflammatory cells occurs before amebic invasion of the colonic epithelium in animal models of invasive amebiasis (3, 4). Colonic biopsies from patients with amebiasis have also shown amebae adherent to erythrocytes (5) and associated with small focal ulcerations of the surface epithelium of the colon (6, 7).

The sequential steps of *E. histolytica* adherence to and contact-dependent lysis of target cells can be experimentally separated. By using Chinese hamster ovary (CHO)[1] cells as target cells, amebic adherence was studied in the absence of CHO cell lysis at 4 °C. Amebic adherence to CHO cells was mediated by an amebic adhesion protein with carbohydrate-binding properties: galactose (Gal) or *N*-acetyl-D-galactosamine (GalNAc) completely inhibited *E. histolytica* adherence to CHO cells, whereas other simple carbohydrates had no effect (8). Gal or GalNAc also prevented the subsequent contact-dependent lysis of the CHO cells at 37 °C (8–12). This amebic Gal- and GalNAc-inhibitable lectin mediated the adherence of amebae to human colonic mucus glycoproteins and colonic epithelial cells (9, 10) which are the relevant receptors for this colonic parasite. We recently identified the amebic Gal/GalNAc adherence lectin by Gal-affinity chromatography and with adherence-inhibitory monoclonal antibodies (13). Galactose-affinity chromatography of detergent-solubilized amebae revealed proteins of estimated molecular masses of 170 and 35 kDa in reducing SDS-PAGE. A monoclonal antibody which inhibited amebic adherence to CHO cells by 86% recognized only the 170-kDa protein on Western blots (13).

In this report we describe the subunit structure of the native Gal/GalNAc lectin isolated by monoclonal antibody affinity chromatography. The nonreduced lectin consists of a 170-kDa heavy subunit linked by disulfide bonds to a 35-kDa light subunit. Monoclonal and polyclonal antibodies specifically directed against the heavy subunit demonstrate that the heavy subunit predominately mediates amebic adherence.

MATERIALS AND METHODS

Cultivation and Harvesting of E. histolytica and CHO Cells—Axenic *E. histolytica*, strain HM1-IMSS, was grown in medium TYI-S-33 (trypticase and yeast extract, iron and serum) with 100 units/ml penicillin and 100 μg/ml streptomycin sulfate (Pfizer) at 37 °C in 250-ml plastic tissue culture flasks (14). Amebic trophozoites were harvested after 72 h of growth by centrifugation at 150 × *g* for 5 min at 4 °C and washed in 75 mM Tris (Sigma), 65 mM NaCl, pH 7.2 (8).

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‖ A Lucille P. Markey Scholar and to whom all correspondence should be addressed.

[1] The abbreviations used are: CHO, Chinese hamster ovary; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; RIA, radioimmunoassay; PTH, phenylthiohydantoin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Metabolic labeling with [^{3}H]glucosamine was accomplished by adding 1 μCi/ml [^{3}H]glucosamine to the TYI-S-33 medium for the final 24 h of amebic culture. CHO cells were grown in F12 medium (GIBCO) with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate and harvested by trypsinization as described (8).

Adherence of E. histolytica Trophozoites to CHO Cells—The measurement of *E. histolytica* adherence to CHO cells was performed as previously described in medium M199 (GIBCO) supplemented (M199S) with 25 mM HEPES, pH 6.8 (Sigma), 5.7 mM cysteine (Sigma), 0.5% bovine serum albumin (Sigma), and 1% heat-inactivated adult bovine serum (Biofluids) (8). Amebae (1×10^5/ml) were preincubated on ice with serial dilutions of preimmune and immune sera for 60 min. 0.1 ml of amebae were added to 1 ml of CHO cells (2×10^5/ml in M199S) which were then centrifuged together at $150 \times g$ for 5 min and then incubated for 2 h at 4 °C. Adherence was measured as the number of amebae having at least three adherent CHO cells upon vortex resuspension of the cellular pellet, with at least 50 amebae counted per tube. Adherence was expressed as the percent of adherence in paired studies performed in control medium (where 83% of amebae had at least three adherent CHO cells) (8).

Production of Anti-Gal/GalNAc Lectin Polyclonal and Monoclonal Antibodies—The Gal/GalNAc lectin purified by anti-lectin monoclonal antibody H8-5 affinity chromatography (100 μg) (13) was injected in complete and then incomplete Freund's adjuvant into Balb/c mice, with a final boost intrasplenically. Spleen cells from the immunized mice were fused to $S_p2/0$-Ag14 myeloma cells with polyethylene glycol and antibody-producing hybrids cloned as previously described (15). Anti-lectin antibody producing hybridomas were isolated by an enzyme-linked immunosorbent assay to lectin-coated (1 μg/well) microtiter plates. The isotypes of the monoclonal antibodies were determined by an enzyme-linked immunosorbent assay using anti-mouse subclass antisera (HyClone Laboratories, Logan, UT).

Purification of the Gal/GalNAc Adherence Lectin by Monoclonal Antibody Affinity Chromatography—Amebic trophozoites harvested from a 72-h culture were preincubated on ice with a 1:1000 dilution of diisopropylfluorophosphate (Sigma) before solubilization in 150 mM NaCl, 50 mM Tris, pH 8.3, 0.5% Nonidet P-40 (Sigma), 5 mM EDTA (Sigma), and 2 mM phenylmethylsulfonyl fluoride. The solubilized amebae were microfuged for 10 min and the supernatant applied at 4 °C to a monoclonal antibody affinity column consisting of 2 mg each of protein A-purified anti-lectin monoclonal antibodies H8-5, 7F-4, 5B-8, 3F-4, and 6D-2 immobilized on 1–2 ml of Affi-Gel 10 (Bio-Rad). The supernatant was recirculated through the column with a peristaltic pump for 3 h, and the column then extensively washed with solubilization buffer, first with and then without Nonidet P-40. The bound amebic lectin was eluted with 0.2 N acetic acid, pH 2.5, immediately frozen and lyophilized.

SDS-PAGE and Western Blots—SDS-PAGE was performed by the method of Laemmli (16) using 7–10% acrylamide running gels. Molecular weight determinations were made using high molecular weight standards from Bio-Rad Laboratories and Boehringer-Mannheim or prestained molecular weight standards from Diversified Biotech, Newton Centre, MA. All protein samples for electrophoresis were boiled in 4% SDS; samples run in reducing conditions also contained 10% β-mercaptoethanol. Autoradiography was accomplished by impregnating the gel with Fluro-Hance (Research Products International, Mt. Prospect, IL) and exposing it to Kodak X-Omat AR film for 3–5 days at 70 °C. The proteins from SDS-PAGE were electrophoretically transferred to 0.1 μM pore size nitrocellulose (Schleicher & Schuell) for Western blots (17). The excess protein-binding capacity of the nitrocellulose was blocked with 5% nonfat dry milk (Richfood, Richmond, VA) in 50 mM Tris, 200 mM NaCl, pH 7.5, for 60 min. The nitrocellulose was then incubated overnight at 4 °C with mouse polyclonal or monoclonal antibodies, washed extensively, and developed with an anti-mouse IgG peroxidase conjugate (Sigma) (13).

Radioimmunoassay of the Gal/GalNAc Lectin—Polyvinyl chloride microtiter plates (Dynatech, Alexandria, VA) were coated with 1 μg/well of anti-lectin monoclonal antibody 3F-4 in 0.1 M bicarbonate buffer, pH 9.6, overnight at 4 °C and residual binding sites blocked with 1% bovine serum albumin in phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20. Affinity-purified Gal/GalNAc lectin (1–50 ng/well) or amebic extracts were incubated in the antibody-coated wells for 2 h at room temperature. After washing the wells, immobilized Gal/GalNAc lectin was quantified by adding 10^5 cpm/well of ^{125}I-labeled anti-lectin monoclonal antibody 7F-4 for 4 h at room temperature. The amount of radioactive 7F-4 bound per well was linearly related to Gal/GalNAc lectin concentration from 1 to 25 ng/well. Protein concentrations were determined by the BCA protein assay (Pierce, Rockford, IL) using bovine serum albumin as the standard.

Cell Surface Iodination of E. histolytica Trophozoites and Immunoprecipitation of Gal/GalNAc Lectin—Amebae (4.5×10^6/ml) in phosphate-buffered saline were labeled with 0.2 mCi of Na[^{125}I] on ice for 10 min using Iodobeads (Pierce) by the manufacturer's instructions. The amebae were removed from the iodobeads and diluted into 30 ml of phosphate-buffered saline containing 5 mM KI. The amebae were pelleted at $150 \times g$ for 5 min and solubilized in 50 mM Tris, pH 8.0, 150 mM NaCl, 10 mM EDTA, 50 mM KI, 0.05% Triton X-100, 20 mM methionine. Amebic viability before solubilization was determined by trypan blue exclusion. The solubilized amebae were microfuged and the Gal/GalNAc lectin isolated from the supernatant by immunoprecipitation with a 50% ammonium sulfate cut of anti-lectin monoclonal antibody H8-5 or an unrelated monoclonal antibody ascites (as control). The immune complexes were precipitated with protein A-agarose beads (Bio-Rad).

Isoelectric Focusing—Preparative isoelectric focusing was performed in a flat bed of Ultradex (Pharmacia LKB Biotechnology Inc.) in an LKB Multiphor. Amebic trophozoites solubilized in 50 mM β-D-octylglucoside (Sigma) were mixed into a gel slurry containing 4 g of Ultrodex and 4% v/v carrier ampholytes, pH 3–10 (Pharmacia) in 100 ml of distilled water and applied to the Multiphor (15). The gel slurry was air-dried to 70% of its initial weight and focused at 8 watts for 16 h at 10 °C. Gel segments were eluted with 2 ml of distilled water and assayed for pH, protein content, and Gal/GalNAc lectin by radioimmunoassay.

Gel Filtration—Gel filtration was performed using Sephacryl S-300 HR (Pharmacia). Solubilized amebic trophozoites were applied to the Sephacryl S-300 column in 50 mM sodium phosphate buffer, pH 7.0, 250 mM NaCl, 0.1% Triton X-100, and 10 mg/ml galactose. Column fractions were assayed for the Gal/GalNAc lectin by radioimmunoassay.

RESULTS

Monoclonal Antibody Affinity Purification of the Gal/GalNAc Lectin—A monoclonal antibody affinity column was

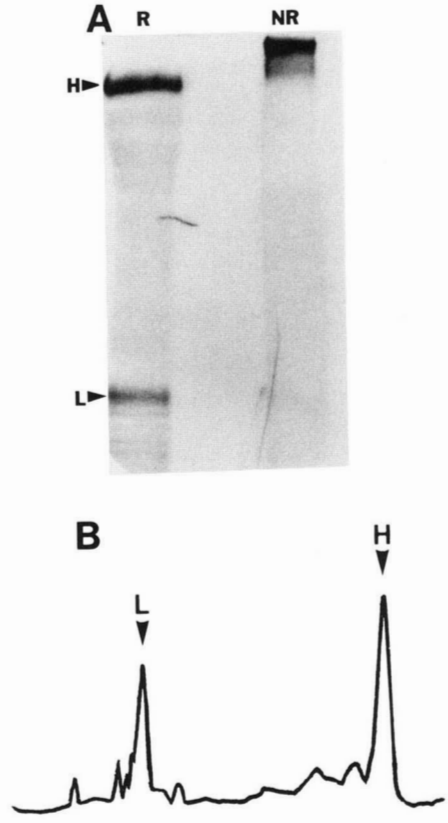

FIG. 1. **Coomassie Blue-stained SDS-PAGE of monoclonal antibody affinity purified Gal/GalNAc lectin.** *A*, SDS-PAGE of the adherence lectin reduced (*R*) with 10% β-mercaptoethanol or nonreduced (*NR*). Relative migrations of the reduced heavy (170-kDa) and light (35-kDa) subunits are indicated. *B*, laser densitometry of the reduced lectin stained with Coomassie Blue.

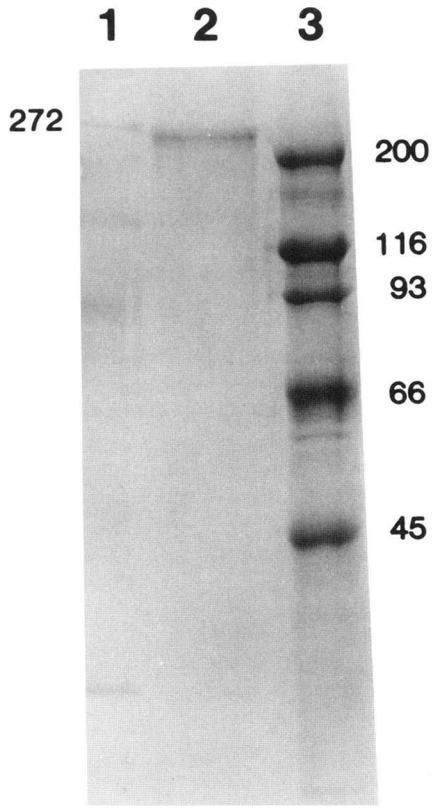

FIG. 2. **Coomassie Blue-stained gel of the nonreduced amebic adherence lectin boiled in SDS and separated in a 7% polyacrylamide gel.** *Lane 1*, jack bean urease (Sigma); *Lane 2*, nonreduced lectin; *Lane 3*, Bio-Rad high molecular weight standards.

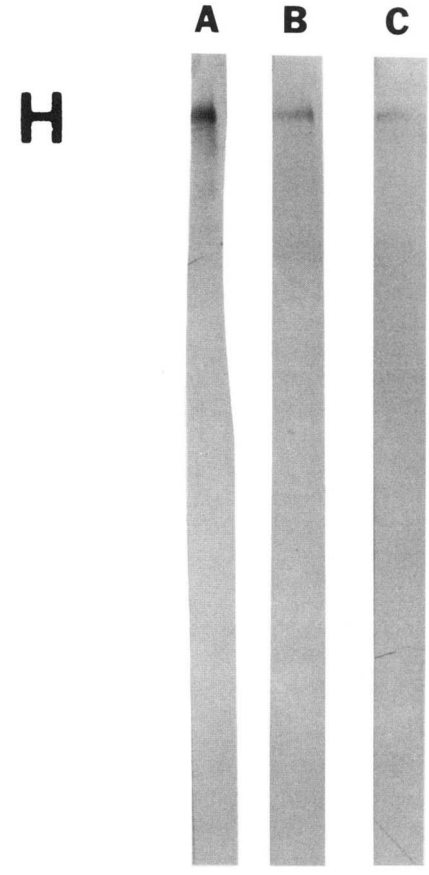

FIG. 3. **Western blots of total amebic proteins probed with antibodies to the adherence lectin of *E. histolytica*.** Amebic protein electrophoresed in 10% SDS-PAGE and transferred to nitrocellulose were probed with (*A*) mouse anti-lectin antisera (1:1000 dilution) or hybridoma cell culture supernatants from monoclonal antibodies (*B*) 8A3 and (*C*) 4F4. Western blots were developed with a secondary peroxidase-conjugated goat anti-mouse immunoglobulin G antibody.

produced by immobilizing 10 mg of anti-lectin monoclonal antibodies onto 1–2 ml of Affi-Gel 10. The anti-lectin monoclonal antibodies used were designated H8-5 (produced from a previous fusion of mice immunized with whole trophozoites) (13)), and 7F-4, 5B-8, 3F-4, and 6D-2 which recognized only the 170-kDa heavy chain of the Gal/GalNAc lectin on Western blots (26). Amebae were pretreated with the protease inhibitor diisopropyl fluorophosphate before solubilization with nonionic detergent in the presence of additional protease inhibitors. The supernatant of solubilized amebic proteins was recirculated over the monoclonal antibody affinity column for at least 3 h before washing the column and eluting it with 0.2 N acetic acid, pH 2.5.

A monoclonal antibody-based two-site radioimmunoassay (RIA) for the Gal/GalNAc lectin was used to follow its purification. The supernatant of solubilized amebae applied to the monoclonal antibody column contained 2.2 μg of Gal/GalNAc lectin/mg protein as determined by RIA. A typical yield was 300 μg of affinity-purified lectin from 200 mg of solubilized amebic proteins and represented a 400–500-fold purification of the lectin.

Subunit Structure of the Purified Gal/GalNAc Lectin—The Gal/GalNAc adherence lectin purified by monoclonal antibody affinity chromatography was analyzed by SDS-PAGE followed by Coomassie Blue staining of the gel. The SDS-treated nonreduced lectin migrated as a broad band of estimated molecular mass 260 kDa (Figs. 1*A* and 2). Reduction of the lectin with β-mercaptoethanol prior to SDS-PAGE demonstrated two major subunits of estimated molecular masses of 170 and 35 kDa (Fig. 1*A*). In addition several minor protein bands which may be related to proteolytic breakdown of the subunits were evident (Fig. 1, *A* and *B*). Laser densitometry of the Coomassie-stained gel of the reduced lectin gave an estimated molar ratio of two light (35-kDa) to one heavy (170-kDa) subunit (Fig. 1*B*). However, a more accurate determination of the subunit stoichiometry was made by comparing the yield of PTH-derivatives from the second and third positions in the amino-terminal sequence of the heavy and light subunits (see below). The native adherence lectin

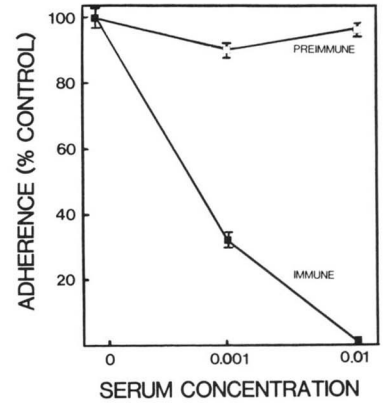

FIG. 4. **Effect of antiserum directed against the Gal/GalNAc lectin on amebic adherence to CHO cells.** *E. histolytica* trophozoites were preincubated with preimmune or immune mouse sera for 1 h at 4 °C before measuring amebic adherence to CHO cells (+ S.E., $n = 6$).

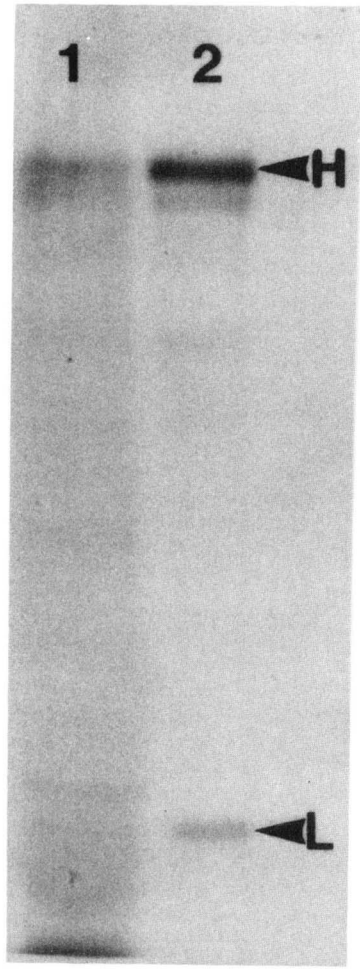

FIG. 5. **SDS-PAGE autoradiograph of the Gal/GalNAc lectin metabolically labeled with [^{3}H]glucosamine.** Amebic trophozoites were labeled for 24 h with 1 μCi/ml [^{3}H]glucosamine in TYI medium. *1*, total amebic proteins; *2*, Gal/GalNAc lectin immunoprecipitated with mouse polyclonal antisera.

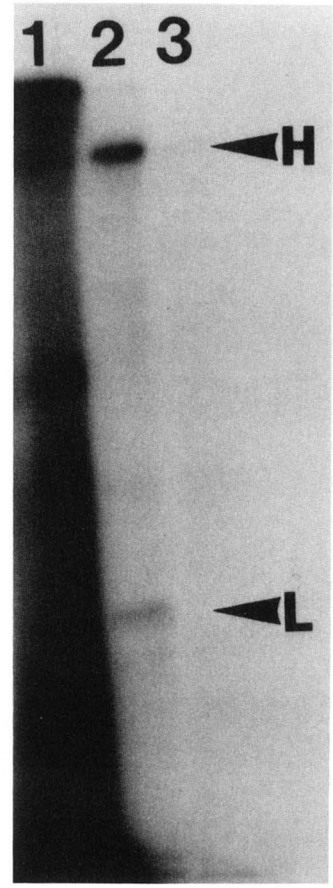

FIG. 6. **Autoradiograph of ^{125}I-surface-labeled trophozoite proteins separated by SDS-PAGE.** *1*, total amebic proteins; *2*, Gal/GalNAc lectin immunoprecipitated with anti-lectin monoclonal antibody H8-5; *3*, control immunoprecipitation with unrelated monoclonal antibody.

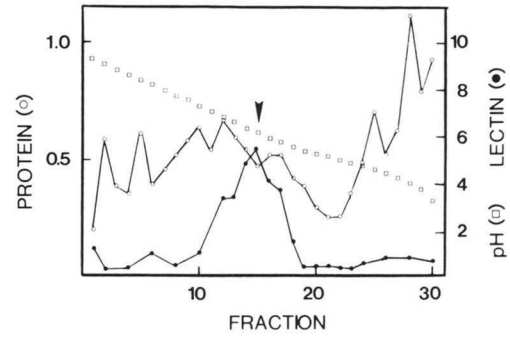

FIG. 7. **Isoelectric focusing of the Gal/GalNAc lectin.** Amebae solubilized in β-D-octylglucoside were focused in an LKB Multiphor using pH 3–10 ampholytes. The lectin (●) and protein (○) concentrations and pH (□) of each fraction are shown.

was sequenced three separate times. At position two, the ratios of PTH-derivatives was 11:11 pmol, ?:35 pmol, and 20:14 pmol on the first, second, and third sequencing runs, respectively (it was not possible to quantitate the PTH-lysine derivative on the second sequencing run). At position three, the ratios were 16:17, 48:42, and 24:23, respectively. On the basis of the yields of PTH-derivatives, there is a 1:1 molar ratio of heavy to light subunits in the native adherence lectin.

Immunization of Mice with the Amebic Gal/GalNAc Lectin—In order to produce polyclonal anti-lectin antisera as well as additional monoclonal antibodies, mice were immunized with the purified lectin. Intramuscular immunization with 100-μg quantities of the lectin in complete followed by incomplete Freund's adjuvant yielded a strong antibody response against the heavy but not the light subunits of the Gal/GalNAc lectin (Fig. 3*A*). Representative Western blots with anti-lectin monoclonal antibodies produced from the immunized mice are shown in Fig. 3, *B* and *C*. None of the 13 anti-lectin monoclonal antibodies isolated was directed against the light chain of the Gal/GalNAc lectin (26).

Effect of Anti-Lectin Antiserum on Amebic Adherence—We had previously shown that a monoclonal antibody specific to the heavy chain of the Gal/GalNAc lectin (designated F14) inhibited amebic adherence to CHO cells by 86% (13). The inhibitory effect of antiserum from the amebic lectin immunized mice was similarly tested. Amebic trophozoites were preincubated for 60 min at 4 °C with preimmune and immune sera before measuring adherence to CHO cells. Amebic adherence was inhibited by 67% at a 1:1000 dilution and 100% at a 1:100 dilution of the anti-lectin immune mouse sera. Preimmune mouse sera had no significant effect on adherence at these concentrations (Fig. 4).

Metabolic Labeling of the Gal/GalNAc Lectin with [^{3}H] Glucosamine—The total amebic proteins labeled metabolically with [^{3}H]glucosamine and separated on SDS-PAGE are shown in Fig. 5, *lane 1*. The mouse anti-lectin antiserum was used to immunoprecipitate the amebic lectin in *lane 2*. Both the heavy and light chains were metabolically labeled with

TABLE I

Amino-terminal amino acid sequences of the subunits of the Gal/GalNAc adherence lectin

Approximately 40 pmol of the electroeluted heavy chain and 100 pmol of the electroeluted light chain were subjected to sequence determination. At each step, the yield of PTH-derivatives for the heavy chain was 8 ± 2 and for the light chain 21 ± 3 pmol (S.D.).

Heavy (170-kDa) subunit

Gly-Lys-Leu-Asn-Glu-Phe-Ser-Ala-Asp-Ile-Asp-Tyr-Tyr-Asp-Leu

Light (35-kDa) subunit

Lys-Thr-$\frac{\text{Asn}}{\text{Gln}}$-Asp-$\frac{\text{Asn}}{\text{Gly}}$-$\frac{\text{Arg}}{\text{Lys}}$-Asp-Gln-$\frac{\text{Phe}}{\text{Leu}}$-Ser-Pro-Asn-Tyr-Pro-Tyr-Gly-Lys

TABLE II

Amino acid compositions of the subunits of the Gal/GalNAc adherence lectin

Residue	Heavy subunit	Light subunit
	residues/100 amino acids	
Asp	13.7	7.0
Glu	11.6	9.2
Cys	ND[a]	ND
Ser	8.5	7.3
Gly	10.5	8.5
His	2.0	1.9
Thr	8.2	7.7
Ala	5.7	6.8
Arg	3.2	6.9
Pro	3.5	5.9
Tyr	3.7	6.6
Val	5.0	5.1
Met	2.0	1.5
Ile	4.2	4.4
Leu	5.5	5.8
Phe	3.5	6.0
Lys	9.0	9.4
Trp	ND	ND

[a] ND, not determined, as cysteine and tryptophan are destroyed by acid hydrolysis.

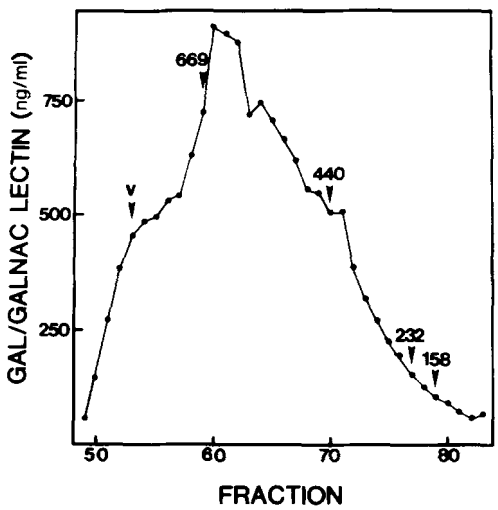

FIG. 8. **Gel filtration of the nonreduced Gal/GalNAc lectin.** Amebae solubilized in Triton X-100 were applied to a Sephacryl S-300 HR column. The lectin (●) concentrations of each fraction are shown as are the elution peaks of blue dextran 2000 (*V*), thyroglobulin (*669* kDa), ferritin (*440* kDa), catalase (*232* kDa), and aldolase (*158* kDa).

[^{3}H]glucosamine, suggesting that both are glycoproteins.

Cell Surface Iodination—The Gal/GalNAc lectin has been identified on the cell surface by indirect immunofluorescence using a heavy chain specific monoclonal antibody (13). To confirm the presence of both subunits on the cell surface, trophozoites were surface ^{125}I-labeled. Trophozoite viability was 94% after labeling as judged by trypan blue exclusion. An autoradiograph of the ^{125}I-labeled amebic proteins and the Gal/GalNAc lectin immunoprecipitated with monoclonal antibody H8-5 is shown in Fig. 6. Both the heavy and light chains of the amebic adherence lectin were labeled by this procedure.

Isoelectric Point of the Native Adherence Lectin—Total amebic proteins solubilized with β-D-octylglucoside were focused in pH 3–10 ampholytes at 8 watts for 16 h. Gel segments were assayed for pH, protein concentration, and lectin concentration (as determined by radioimmunoassay) (Fig. 7). The Gal/GalNAc lectin focused to a single peak with an isoelectric point of pH 6.2

Amino-terminal Sequences and Amino Acid Compositions of the Subunits—The subunits of the affinity-purified Gal/GalNAc lectin were electroeluted and the amino termini sequenced by sequential Edman degradation by the Protein and Nucleic Acid Sequencing Center of the University of Virginia. The subunits had unique amino termini (Table I); the amino termini of the subunits did not share any close homologies with other sequenced proteins in the National Biomedical Research Foundation data bank. The amino terminus of the light subunit displayed microheterogeneity in a 1:1 molar ratio for residues 3, 5, 6, and 9 (Table I). The amino acid compositions determined for both subunits are listed in Table II.

Gel Filtration of the Native Gal/GalNAc Lectin—Solubilized amebic trophozoites were applied to a Sephacryl S-300 column and the Gal/GalNAc lectin content of the eluted fractions determined by RIA. The Gal/GalNAc lectin eluted as a broad peak at approximately 600 kDa with a shoulder at 440 kDa, suggesting that the adherence lectin solubilized from the trophozoites existed as aggregates of two to three molecules (Fig. 8).

DISCUSSION

Monoclonal antibody affinity chromatography proved to be an effective and high yield single-step method for the purification of 100-μg quantities of the *E. histolytica* Gal/GalNAc adherence lectin. The inclusion of protease inhibitors and the rapidity of the purification procedure minimized proteolytic breakdown of the purified lectin. The ability to purify relatively large quantities of the Gal/GalNAc lectin enabled us to begin the biochemical and biological characterization of this cell surface adherence protein.

Our data demonstrate that the Gal/GalNAc-inhibitable adherence lectin of *E. histolytica* is a complex molecule composed of a 170-kDa heavy chain and a 35-kDa light chain. The two subunits are covalently attached to each other by disulfide bonds. The apparent molecular mass of 440–600 kDa of the Gal/GalNAc lectin on Sephacryl S-300 gel filtration indicates that the nonreduced protein exists as dimers or trimers when solubilized in nonionic detergents. The *E. histolytica* Gal/GalNAc adherence lectin is far larger than other eukaryotic galactose-binding lectins such as the 67-kDa elastin receptor (18), the rabbit asialoglycoprotein receptor complex of 40- and 48-kDa polypeptide chains (19) or the 25–27-

kDa galactose-binding hemagglutinins of the cellular slime mold (20). The amebic lectin's size and complexity are more similar to other cell adhesion molecules including the leukocyte adhesion receptor Mo 1, which consists of 155- and 95-kDa noncovalently linked subunits (21) and the neural and liver cell adhesion molecules (22).

The ability of heavy chain-specific antisera to completely inhibit amebic adherence to CHO cells is strong evidence for a predominant role of the heavy subunit in mediating adherence. The contribution of the light subunit to adherence or amebic contact-dependent cytotoxicity cannot at present be assessed in the absence of light subunit-specific antibodies. Interestingly, Balb/c mice immunized with the native Gal/GalNAc lectin mounted an antibody response only to the heavy subunit. It is not known if this was due to masking of the light chains by the heavy chain or to an inherent lack of antigenicity of the light chains.

The microheterogeneity present in the Gal/GalNAc lectin light chain amino-terminal sequence most likely reflects the presence of at least two different light chain genes in *E. histolytica*. The amino acid substitutions at the heterogeneous positions were conservative as to the relative polarity, charge, and hydrophilicity of the substituted amino acid residues, as is generally the case for other proteins which demonstrate microheterogeneity (23). Understanding the functional significance of having more than one Gal/GalNAc lectin light chain gene must await a more detailed knowledge of the role of the light chain in *E. histolytica* adherence and cytolysis.

The heavy and light subunits appear to be structurally dissimilar as evidenced by their lack of antigenic cross-reactivity, dissimilar amino acid compositions, and unique amino-terminal amino acid sequences. However it is not possible with the present data to rule out the possibility that the light subunit is derived from the heavy subunit. The presence of a 1:1 molar ratio of the light and heavy subunits suggests that they could be derived from a single large precursor protein, as has been demonstrated for the insulin receptor (24), but it is also possible that they are assembled after the translation of separate mRNAs, for which the acetylcholine receptor is an example (25). The polyclonal antisera and amino terminus sequence data described in this report are being used in our laboratory to isolate the gene(s) for this amebic cell adherence molecule. Already, oligonucleotide probes based on the heavy subunit's amino-terminal amino acid sequence have been shown to specifically hybridize to a 4.4-kilobase *E. histolytica* RNA molecule, which is the predicted size for the heavy subunit mRNA based on its molecular weight and amino acid composition (27). The isolation and sequencing of the gene(s) for the amebic Gal/GalNAc adherence lectin will improve our understanding of the subunits' assembly, and perhaps offer clues to the functions of this complex molecule which plays a central role in the pathogenesis of invasive amebiasis.

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