## Primary structure of the 170-kDa surface lectin of pathogenic Entamoeba histolytica

(receptor/cellular adherence/carbohydrate binding/cysteine-rich pseudorepeats/amoebiasis)

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The adherence of Entamoeba histolytica to ABSTRACT colonic mucins and to host cells appears to be predominantly mediated by a 170-kDa surface lectin of the amoebae. By using an antiserum to the purified lectin, the corresponding cDNA was isolated from an expression library of the pathogenic E. histolytica isolate HM-1:IMSS. Northern blot analysis indicated a transcript of ≈4 kilobases, and Southern blot analyses suggested that multiple genes may encode the lectin or closely related proteins in HM-1:IMSS trophozoites. The cDNAdeduced amino acid sequence revealed an N-terminal signal peptide and a mature protein of 1270 amino acids corresponding to a molecular mass of 143 kDa, which comprises a short C-terminal cytoplasmic domain with potential phosphorylation sites, a transmembrane region, and a large extracellular portion with nine potential asparagine-linked glycosylation sites. The extracellular portion may be separated into a cysteine-poor domain and a cysteine-rich domain, the latter of which shows in part repetitive structural elements with a low degree of sequence homology to wheat germ agglutinin and to pDd63, a developmentally expressed protein of Dictyostelium discoideum.

The enteritic protozoon *Entamoeba histolytica* infects nearly 10% of the world's population. It causes 50 million cases of colitis or extraintestinal abscesses annually resulting in  $\approx$ 50,000 fatalities (1).

Adherence of *E. histolytica* to colonic mucins and to host cells is considered essential for the colonization of the large intestine and for pathogenic tissue invasion, respectively (2). Both forms of adherence appear to be primarily mediated by a surface lectin of the amoebae, which is inhibitable by galactose and *N*-acetylgalactosamine (3, 4).

The structure, function, and antigenicity of the lectin have been studied in considerable detail (for review, see ref. 2). Its carbohydrate binding specificity was reported to be similar to that of the lectin of the coral tree *Erythrina cristagalli* in that terminal *N*-acetyllactosamine units provide the major binding determinants (5).

The lectin is a membrane-associated glycoprotein with a molecular mass of 170 kDa (6). It is disulfide-linked to one or more units of a 35-kDa protein that was reported to function as a fibronectin receptor (7, 8). The N-terminal amino acid sequence of the lectin has been determined (7). A corresponding oligonucleotide probe was found to hybridize to a 4-kilobase RNA of the amoebae.\*

Five functionally relevant epitopes of the lectin have been defined by studying the binding of monoclonal antibodies and their influence on adherence: antibody binding to two epitopes decreased adherence to mucins and to target cells, binding to another epitope inhibited adherence to target cells but not to mucins, and binding to two further epitopes enhanced adherence to mucins and target cells (9).

Similar effects were observed when antisera of patients with invasive amoebiasis were studied. The antisera either reduced or enhanced the adherence reactions (9). Interestingly, gerbil vaccination studies using the isolated lectin as antigen also revealed two contrary groups of responders; some animals were protected whereas the others showed an increase in morbidity.<sup>†</sup> It was concluded that selected epitopes of the lectin molecule may be candidates for a vaccine against invasive amoebiasis.

To define the location of these epitopes, a detailed structural analysis of the lectin is required. Herein we describe the amino acid sequence and sequence-derived structural domains of the protein.<sup>‡</sup>

## **MATERIALS AND METHODS**

E. histolytica Isolate, Culture Condition, and Harvest. The pathogenic isolate HM-1:IMSS was cultured in axenic medium TYI-S-33 in plastic tissue culture flasks (10). Trophozoites in late logarithmic growth phase were detached by chilling on ice for 10 min, decanted, pelleted by centrifugation at  $500 \times g$  at 4°C for 10 min, washed twice in ice-cold phosphate-buffered saline, and frozen at  $-70^{\circ}$ C.

Isolation of the 170-kDa Surface Lectin. The lectin was isolated from a detergent-soluble membrane extract of a total of  $1.5 \times 10^{10}$  E. histolytica trophozoites by using the following modification of the method described by Petri et al. (6). All buffers contained as proteinase inhibitors 10 µM transepoxysuccinyl-L-leucylamido(4-guanidino)butane (E-64), 6.4 mM benzamidine, and 0.5 mM phenylmethylsulfonyl fluoride (all from Sigma). Membrane extraction and affinity purification of the lectin were performed with 10 samples of  $1.5 \times 10^9$ trophozoites. Membrane proteins were extracted by 2% (wt/vol) *n*-octyl  $\beta$ -D-glucopyranoside (OG; Sigma). Affinity chromatography was performed using a 10-ml column of *p*-aminophenyl  $\beta$ -D-thiogalactopyranoside coupled to Agarose (Sigma) and bound material was eluted with 0.5 M N-acetylgalactosamine (Sigma). The eluate was concentrated ≈250-fold on a Minicon concentrator (Amicon) with a molecular mass cut-off at 15 kDa.

The material pooled from 10 preparations contained 5.3 mg of protein in 2.8 ml. A sample of 1.2 ml was diluted 1:10 in 5 mM Tris·HCl buffer (pH 7.5) containing 0.8% OG and protease inhibitors and subjected to anion-exchange chromatography on a Mono Q HR 5/5 column using the FPLC system

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Abbreviations: OG, *n*-octyl  $\beta$ -D-glucopyranoside; WGA, wheat germ agglutinin.

<sup>\*</sup>Mann, B. J., Snodgrass, T., Kittler, E. L. W., Ravdin, J. I. & Petri, W. A., Jr., 37th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Dec. 4–8, 1988, abstr. 390.

<sup>&</sup>lt;sup>†</sup>Petri, W. A. & Ravdin, J. I., 11th Seminar on Amebiasis, Nov. 15–17, 1989, Mexico City, abstr. 34.

<sup>&</sup>lt;sup>‡</sup>The sequence reported in this paper has been deposited in the GenBank data base (accession no. M60498).

(Pharmacia LKB). The column was washed with 20 mM Tris-HCl (pH 7.5) containing OG and protease inhibitors, and material was eluted with a 20-ml gradient to 1 M NaCl in starting buffer. Fractions of 0.5 ml were collected and analyzed by gel electrophoresis in NaDodSO<sub>4</sub>/PAGE and silver staining.

Antiserum to the isolated lectin was raised in rabbit by repeated intramuscular injections of 100  $\mu$ g of the antigen in 1 ml of isotonic saline emulsified with an equal volume of complete or incomplete (boosts) Freund's adjuvant (GIBCO/BRL).

Amino Acid Sequencing. The purified material was subjected to NaDodSO<sub>4</sub>/PAGE under reducing conditions, transferred to a poly(vinylidene difluoride) membrane (Immobilon; Millipore) using a semidry electroblotting system (Multiphor II; Pharmacia LKB), and stained with Ponceau S (Sigma). Bands were cut out and applied to a modular fluid-phase protein sequencer (model 810; Knauer, Berlin). The method did not allow the identification of cysteine.

**Construction and Screening of the HM-1:IMSS cDNA Libraries.**  $\lambda gt11$  and  $\lambda ZAP$  libraries of the pathogenic *E. histolytica* isolate HM-1:IMSS were used. The construction of the  $\lambda gt11$  library has been described (11). The  $\lambda ZAP$  library was constructed with the ZAP cDNA synthesis kit (Stratagene) by following the instructions of the manufacturer. The library contained 10<sup>7</sup> independent recombinant phages.

A 1:1000 dilution of the rabbit antiserum against the isolated lectin was used to screen  $2 \times 10^5$  recombinant phages of the  $\lambda$ gt11 library by a published method (12). Selected cDNA clones isolated from the  $\lambda$ gt11 library were used to screen the  $\lambda$ ZAP library. Hybridizing phages were isolated, and the plasmids were released according to the instructions of the manufacturer (Stratagene).

**Standard DNA and RNA Technologies.** Nucleotide sequencing, Southern blot, Northern blot, and primerextension experiments were performed according to published procedures (13). For primer extension studies, an oligonucleotide (5'-GTAGTCCATACAAAATGTCTCC) complementary to nucleotides 188–209 of the cDNA sequence (see Fig. 3) was used.

**Computer Analysis.** cDNA and protein sequences were analyzed using the DNASIS program (Pharmacia LKB). Comparisons to published protein sequences (National Biomedical Research Foundation data base, release 6/90) were performed using the FASTA searching program (14).

## RESULTS

Isolation of the 170-kDa Surface Lectin, Amino Acid Sequencing, and Production of an Antiserum. The lectin was isolated from cell membranes of  $1.5 \times 10^{10}$  trophozoites of the pathogenic E. histolytica isolate HM-1:IMSS. Membrane components were extracted by 2% OG and subjected to affinity chromatography on immobilized p-aminophenyl  $\beta$ -Dthiogalactopyranoside. Bound material was eluted with 0.5 M N-acetylgalactosamine and further purified by anionexchange chromatography. Upon NaDodSO<sub>4</sub>/PAGE of the isolated material under reducing conditions, a major protein of 170 kDa was found. Sequencing of the N-terminal amino acids revealed a sequence similar to the one described by Petri et al. (7) (Fig. 1). A rabbit antiserum to the isolated material reacted on immunoblots of E. histolytica membrane extracts with a 170-kDa antigen, when the gel was electrophoresed under reducing conditions.

Isolation and Characterization of cDNA Clones Coding for the 170-kDa Surface Lectin. The antiserum to the isolated lectin was used to screen 200,000 recombinant phages of a  $\lambda$ gt11 cDNA library derived from the pathogenic *E. histolytica* isolate HM-1:IMSS. After tertiary screening, 11 reactive clones were isolated. The cDNA inserts were released and

Previous data (Ref. 7): Protein sequencing	NH2-G	ĸ	L	N	E	F	s	A	D	I	D	¥	¥	D	L			
Present data: Protein sequencing	NH2-D N	Q K	v	N	E	F	s	λ	D	I	D	Y	Y	D	L	?	?	м
<b>CDNA</b> deduced sequence	D	ĸ	L	N	E	F	s	λ	D	I	D	Y	¥	D	L	G	I	M

FIG. 1. N-terminal amino acid sequences obtained for the isolated 170-kDa surface lectin of E. histolytica compared to the corresponding sequence deduced from cDNA analysis.

subjected to Northern blot analyses. One cDNA insert, designated gt-Eh170/5, hybridized to an E. histolytica RNA of  $\approx$ 4 kilobases (kb). A 35-mer antisense oligonucleotide probe derived from the N-terminal amino acid sequence of the isolated lectin hybridized to the RNA at the same position of 4 kb. Nucleotide sequence analysis of gt-Eh170/5 revealed an open reading frame spanning the entire insert of  $\approx 2.4$  kb. The lack of a 5'-initiating ATG, the absence of a poly(A) tail, and the finding that the hybridizing RNA was substantially larger than the gt-Eh170/5 insert indicated that the 5' and 3' ends of the mRNA were not represented in the cDNA clone. To obtain longer cDNA sequences, gt-Eh170/5 was used to screen a  $\lambda$ ZAP library also derived from E. histolytica isolate HM-1:IMSS. Sixty out of 100,000 recombinant phages hybridized to the probe. Four of them were purified and the cDNA inserts designated ZAP-Eh170/1 to 4, were sequenced. The sequences of all four inserts contained identical ends plus poly(A) tails but differed in their 5' ends, 3' suggesting that they were independent cDNA clones derived from the same mRNA species. The amino acid sequences deduced from the longest cDNA clones ZAP-Eh170/3 and 4 contained a nucleotide sequence that corresponded to the N-terminal amino acid sequence of the isolated lectin as it was obtained by protein sequencing and an additional stretch of hydrophobic amino acids. However, the inserts lacked an in-frame initiation codon at the appropriate position. Primerextension experiments revealed an extension product of 20 nucleotides 5' to ZAP-Eh170/4, suggesting that the fulllength coding sequence contains a few additional amino acids.

A ROUNT REF B 9.4 -6.6 -3.5 -2.4 -1.1 -1.0 -B

FIG. 2. Southern and Northern blot analyses. (A) Southern blot. Approximately 10  $\mu$ g of HM-1:IMSS genomic DNA was digested to completion with restriction enzymes as indicated, submitted to electrophoresis, blotted, and hybridized to ZAP-Eh170/4 under high stringency. Sizes in kb are indicated on the left. (B) Northern blot. Five micrograms of HM-1:IMSS total RNA was submitted to electrophoresis, blotted, and hybridized to ZAP-EH170/4 under low stringency.

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Southern and Northern Blot Analyses. Genomic DNA from trophozoites of the pathogenic *E. histolytica* HM-1:IMSS was digested with various restriction enzymes and hybridized to ZAP-Eh170/4 under high-stringency conditions. In each digest, several hybridizing fragments were found (Fig. 2A).

Northern blot analysis of total RNA isolated from amoebae of the same isolate showed an RNA species of  $\approx 4$  kb hybridizing to the cDNA probe (Fig. 2B). This result was obtained under high- as well as under low-stringent washing conditions.

**Primary Structure of the 170-kDa Surface Lectin.** The amino acid sequence deduced from the nucleotide sequence of cDNA ZAP-Eh170/4 (Fig. 3) suggests that the protein consists of several structural domains as shown in Fig. 4. A hydrophobic segment of 29 residues near the C terminus was

found that is characteristic for membrane-spanning domains. Approximately 95% of the protein is located on the N-terminal side of this segment and appears to reside extracellularly.

The most N-terminal amino acids are exclusively hydrophobic, indicating that they form a signal peptide. The remaining sequence constitutes a mature protein of 1270 amino acids with a molecular mass of 143 kDa. The sequence contains nine potential asparagine-linked glycosylation sites, all of which are located within the extracellular part of the molecule.

The first domain of the mature protein comprises residues 1-372. This region was designated "cysteine-poor" because of its low cysteine content of 1.6%. The cDNA-deduced N-terminal amino acids of the mature protein corresponded

	ר <mark>ר ⊫</mark> צבף-170 ( ר בי בי די 1 אז אמז אנד אמז אוד	/3 TGCA A
:	2 GATAMACTTAATGMATTITCAGCAGATATTGATTATTATGACCITGGATATATGTCICGGGAAGAATGCAGGTTCATGGTAATCATTCITACACTCACCATATGAGATTITCTATTATTIAGCTATGCAACCATGGAAGCATT 1 <u>D K L N E F S A D I D Y Y D L G I M</u> S R G K N A G S W Y H S Y T H O Y D V F Y Y L A N O P W R H F	IGTA V
14	2 TGGACTACTTGTGAMCAACCAAGGGTAATAAGAATGTTATAATGATAATATTAATCAATGAAGATCATAACCTTGACAATGAATATGCATATGCATATGCAATATGCATAT 1 W T T Ç E T T K G N K E Ç Y K Y I I N E D H N L N A Q Q L N N I K N L D K Q D F Ç Q K E Y A Y P I	GAA E
3: 10	2 AMATATGAAGTIGATIGGGACAATGTICCAGTIGATGAAGAAAATTGAAAGTIGTATAATGGAAAATTIGTITTAAATATGCAGCTAAAGACCATIGGCTTATGTIAATTAAATAGAAAATGGAACAAA 1 K Y E V D W D N V P V D E O O I E S V D I N G K T Ç F K Y A A K R P L A Y V Y L N T K N T Y A T K	ACT
48 15	2 GAAGCATATGATGTTIGTAGAATGGATTICATTGGGGGAGGAGAATGAATGAGATCATTIFAACAATGGAATAAAGGCTITATTGGTGATAATAGCAAAAAGGTACGATGATGATGATGATGATGATGATGATGATGATGATGATG	MT N
63 20	2 ACACATCTIGGANTIATICTIGGIATIACTGATICIACAGTGATIANATGACTICIAGAGAATITATCICITITAAGTGAACAGIGAAGAGGAAGACTGATACTGATAGTGATGATAGGAACGATTGATAAGAT I T H L A I I L G I T D S T V I K S L O E 🚆 L S L L S O L K O S R V T L Y Y L K D D S Y A T D N I K	TTA L
78 25	2 AMAGATITGAMATATGAMACACITGTCAMATACACAGCAGGACAAGGACAAGGTGATCCACTGTTATGCAGAAGAATCAACTATGCATGATGATGAGAAATCAAMAGAGGAACTATGGTTGGTTAATGGACAAT I K D L K Y E T L V K Y T A G O G O V D P L V H O A K H D L F K H I S D K K I K R G T H V V L H D H	GCA A
93 30	? СТЯGATCAGAATTTAATGCAGAACAGAATTTGATAGAAAGAATATTCAGTTCATAGTATTAGAATAAAGACTCAAAATTACATGTTGAACTGTTTGAACTGTTTGAACTGTTTGAACTGTTTGAACTGTTTGAACTGTTTGAACTGTTTGAACTGTTTGAACTGTTTGAACTGTTTGAACTGTTTGAACTGATTAGAATAAGAATTAGAATTAGAATTAGATTAGATTAGATTAGATTAGATGAACTG   LGSEFNAETEFDRKLISVNTVVLNRNKDSKITYSALKLVSLGPNYNEFT	NGT S
108 35	E MATAGTGAMGTATCAMCTACTATTGATGAMCTITTCAMAGGAMTTAGAGCTAATTTAACAGAMAGATGTGATAGAGATAMATGTTCAGGTITTGTGATGCAMTGATAGAATGCCAATGTCCAATGTGTTGTGAGAATGATTGTTTC NSEVSTTID ———————————————————————————————————	TAT Y
123 40	ACATECTETERATGTAGAACAGGATCATETATTCCATGGCCTAAAGCTAAAGCTAAAGCAAAGAATGTCCAGCAACATGTGTAGGATGTATATAAGAATGTAAAGAATGTTAGAAGA	iaa E
138. 451	CCAMMAGTGAAATGCTAGCTAATTGTGATGATGATGATAATATGTAAAGGAAGTATGTAAAAGCAAAGTGTAATGGTAGCAAGTATGCAAGTATGTGGAGTTATACATGTGATGATGATGATGATGATGATGATGATGATGATGAT	GT Ç
1532 501	MGMATATAMACACGGMATTIGIGIACAGGAMMACTACTAACIGICAAGAATATGITIGIGATTCAGAACAAGATGIACIGITCAAGAATGIGIAMAACATCACCATACATTGAAATGITATGITATGIGIAAGA K K Y K N G N L C T G K T T N C O E Y Y C D S E O R C T V O E K Y C V K T S P Y L E N S C Y V A K	GT C
1682 551	MATCTCAATACAGGTATGTGTGAGAACAACTAATCATGTGGTGAACATACTCATCATGTGGTGGGAGATTCTAACGGATGGAGTGATGCAATGATGCTAACTGATGTAATGGTAATGAATGATAATGGTAATGATAATGGTAATGATAATGGTAATGATAATGGTAATGATAATGGTAATGAATGATAATGGTAATGAATGATAATGGTAATGATAATGGTAATGAATGATAATGGTAATGATAATGGTAATGAATGATAGTAATGATAATGGTAATGAATGATAATGGTAATGAATGAATGAATGATAATGGTAATGAA	AT D
1832 601	ТСТАВТАААСАТБАААТТТВГВАТТАТАСТБСАБАТАМАССАЛМАТБТАТТГСТСГААЛБАТАСАВАДАТСТТВТТАВАДАТБСТТАТТАВДАЛБТААЛААСААДТАЛААСААДТАТАТБСБАСАЛАТТБСБАСАЛАТТВСТ S S K H E I Ç D Y T G D K P K Ç I V S E Ç T E D L V R D G Ç L I K R Ç M K T S K T T Y W E N V D Ç	CA S
1982 651	AACACTAAGATTGATTGCTCAAGATGGTAAATCTGAAACTATGTGTAAACCATATTACTCAGCTACATGTTGAATGGACAATGGTGTGTTCAAGCAGTGGGATATTGCTGGATATTGCTGAATGGAACAG N T K I E F A Q D G K S E T N Ç K P Y Y S A T Ç L N G Q Ç V V Q A V G D V S N V G Ç G Y Ç S N G T I	ו <b>ד</b> ו
2132 701	ANTGTTATTACATATCATATCATGATGATGTGATTCACGTANATCACAATGGGGANCTTTATGGTANGGGTGATCATGCGAGTAATGTATGTGTATTGGGATTATGGTATTGGATTGTGGTATTGGGATAATGGATAATGGATATGGGATAATGGATATGGATGAT	T
2282 751	GANTGTICTAGTTIANCATGTCCAGCTGATACTACATACATACAGAGACATATGACTCAMMACAGGAACATGTIANGCCAACACCATCATGTICAGTATGCGAMMAGGAMATTIGTAGAGAMATGGAMAGATC E C S S L T C P A D T T Y R T Y T Y D S K T G T C K A T Y K P T P S C S Y C E K G K F Y E K S K D G	A
2432 801	AMATAGAAGGTAAAGTCACTITAGAAGATGGAAAGAATATCAATACCAATATTCCAAAGAATGGATGG	A
2582 851	CCATGTCAGCATATGTTACAGCTACCTATCATTCATTTATCATTTATCAATTTAACAGCATGATTATCACCAGAGGAATATGGAGGAGGAGGAAGGA	r
2732 901	AAAACATGTICATTAACTGAAACTAAAGAAAAAGTAGAAGAAATGATTIGTIGCGCAGAAGAACTAAGAATGAGGGGGGGCTGCCATTGAATGCAAACAATGGATTGGATGGTAGATGGATG	a ' 1
2882 951	ΑΤΤΟΑΛΟΛΟΑΤΤΟΤΤΑΤΤΑCΛΟΛΛΑΝΟΑΤΟGAATAGAACAACATCITAAACATGOTACAAAAACAACATGOTACAATAAGAAGAATAGAAGATGOCGGTAAAGCAATTCATGOAAGGAATGOGGAATGO Ι Ο Ε Ι V Ι Τ Ε Κ D G Ι Κ Τ Τ Τ C Κ D G T Κ Τ Τ C D T N N K R I E D A R K A F I E G K E G I E O V E	' 1
3032	TOTOCANGTACTOTTTGTCAANATGATAATAGTTGTCCAATTATTGCTGATGATAGAAAATGTAATGAAGGAGTGGTGATGGATG	
3182	NETGATGATCCATCATTAGTAGTGAGCATTTAGAGCTAAATGGGGAGTTGAACTTAACAATGCTGTTTTAGAGTAAGGAGAAGGGAGGG	t t
1101	ΊΤΓΟΝΤΕCAMARICEMIGUANTECHTGEGAMETECAMENTETERTEATEGEAMEATEGEAMARTATTACEAMAGUMATEATETTETTEGEATETTETTEGEATETTECT Ι D P K P R N P C E T A T C D O T T G E T Ι Y T K K T C T V S E E F P T I T P N O G R C F Y C O C S	, T
1151	NTETHOLOGITEATEMITETAETATEMITETAETATEMEGAAGAAGAATATATATATEATETTATEGATETGEGATEGEGTTIGGATEGAAGAGAGAGAGAGAGAGAGAGA D G S S V L T N Y G E T D K E Y Y D L D A Ç G N Ç N V M M O T D R T G O L N M N T E Ç I L A G E	r F
1201	1 + 1 + 2 + 3 + 1 + 4 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1	s
1251	E N A E Y V G A D N E A T N A A T Y N G ° ZAP-EN170/4 44 ZAP-EN170/4 44 ZAP-EN170/1 24	te a

cDNA derived amino acid sequence of the 170-kDa surface lectin of E. histolytica. The sequence is incomplete by 20 nucleotides at the 5' end. The beginning and the end of each of the cDNA clones ZAP-Eh170/1 to -4 are indicated by arrows. Positive numbering of the amino acids starts at the N erminus of the mature protein. Thin underline shows the position of the amino acid sequence deternined by protein sequencing. The outative transmembrane region is inderlined by a heavy bar. The stop codon and cysteine residues are marked by asterisks, and poential asparagine-linked glycosylation sites are indicated by solid squares.

FIG. 3. cDNA sequence and



FIG. 4. Structural domains of the 170-kDa surface lectin of *E. histolytica*. Domain structure was based upon sequence properties. Boxes within the shaded area represent pseudorepeats. Potential asparagine-linked glycosylation sites are indicated by dots.

to those found by sequencing the N terminus of the isolated lectin. Identity to the cDNA deduced sequence may be obtained if the two sequences obtained by protein chemistry are combined (Fig. 1).

The second domain spans residues 373-1203. It was termed "cysteine-rich" because of its high cysteine content of 10.9%. Certain repetitive elements were identified in the sequence between residues 373 and 648. They may be aligned to form nine contiguous pseudorepeats of  $\approx 30$  amino acids, each of which contains four cysteines and several other conserved amino acids (Fig. 5).

The third domain, which may be located between residues 1204 and 1232, constitutes a putative transmembrane region. It is followed by the presumed cytoplasmic domain of 38 residues, which extends to the C terminus of the protein. It contains 11 candidate phosphorylation sites at serine, threonine, and tyrosine residues.

Computer searches revealed low-degree sequence homologies to other proteins, which were found to lie within the cysteine-rich region of the molecule. Sequence identities exceeded 20% of the residues for wheat germ agglutinin (WGA) isolectin 1 and pDd63, a developmentally expressed protein of *Dictyostelium discoideum* (15, 16) (Fig. 6).

## DISCUSSION

The structure, function, and immunogenicity of the 170-kDa surface lectin of *E. histolytica* have been studied in considerable detail by Ravdin (2), who termed the molecule galactose/*N*-acetylgalactosamine-inhibitable adherence lectin. By using an affinity purification proposed by these investigators (6), we isolated a protein from amoeba membranes that was considered to be this molecule because (*i*) the same method was applied for the isolation, (*ii*) the protein migrated with an apparent molecular mass of 170 kDa in NaDodSO<sub>4</sub>/PAGE under reducing conditions, and (*iii*) the N-terminal amino acid sequence of the protein reported by Petri *et al.* (7) is identical to the sequence presented here except for the first three residues; the differences may be due to sequencing errors or to polymorphisms of the polypeptide.

An antiserum raised against the isolated lectin was used to identify a corresponding cDNA clone in an expression library of pathogenic *E. histolytica*, which subsequently was used to isolate a nearly full-length cDNA clone. This clone was considered to represent the lectin because (i) an antiserum to

the isolated lectin was used for the initial screening, (*ii*) the N-terminal amino acid sequence determined for the isolated lectin was found within the cDNA deduced amino acid sequence at an appropriate position, and (*iii*) the molecular mass of 143 kDa calculated for the mature protein from the cDNA-deduced amino acids is in good agreement with the molecular mass of 170 kDa experimentally determined for the isolated lectin. The difference between the two values may be due to multiple glycosylations of the native protein. Previous findings indicated that the lectin is a glycosylated protein (2), and the cDNA-deduced amino acid sequence revealed a total of nine potential asparagine-linked glycosylation sites. Glycosylation at several or all of these sites may increase the mass of the molecule to 170 kDa.

The N-terminal amino acid sequence of the mature protein as it was deduced from the cDNA sequence shows a combination of the two sequences obtained by protein chemistry. Although sequencing errors cannot be excluded, the data may be interpreted as an indication of polymorphisms in the polypeptide chain. These may relate to the finding that the corresponding cDNA hybridized to multiple fragments in genomic Southern blots (Fig. 2A). The complexity of the hybridization pattern could be due to intervening genomic sequences or to gene multiplicity. Since no intervening sequences have so far been found in the genome of E. histolytica, the results of the Southern blots more likely suggest that the HM-1: IMSS amoebae contain multiple genes coding for the 170-kDa surface lectin or closely related proteins. Polymorphisms of the protein may, therefore, result from polymorphisms among concomitantly expressed genes. If they exist, then the RNA blot indicates that the sizes of the transcripts do not vary substantially (Fig. 2B).

The architecture of the protein is characteristic for a cell surface receptor. The N-terminal sequence codes for a typical signal peptide (18). The putative transmembrane region of  $\approx$ 30 amino acids resembles other known membrane anchors (19). Like in most other membrane proteins, the cytoplasmic domain is located toward the C terminus. In this molecule it is rather small but contains 11 amino acids with hydroxyl groups, suggesting that signal transduction may occur by way of phosphorylation.

Approximately 95% of the molecule appears to be located extracellularly. According to the cysteine content, the extracellular part of the molecule may be separated into two domains. The N-terminal domain is cysteine-poor. Second-



FIG. 5. Alignment of nine pseudorepeats found within the cysteine-rich domain of the 170kDa surface lectin of *E. histolytica*. Residues that are conserved in at least four out of nine repeats are boxed. Conserved cysteine residues are surrounded by a double line. For optimal alignments, gaps were introduced into the sequences. The number of the first residue of each repeat is indicated on the left.

Eh170 WGA1	CKDLEGCVVTKYNASCEPKVKCM VPYCDDDNNLKE QRCGEQGSNMECPNNLCCSQYGYCGMGGDYCGK	434-468 1- 33
pDd63	CDDITGCCNTPINVDDNN PCT VDACTKSTGVTHT	376-409
Eh170	VCKQKANCEADQK PSSDGYCWSYTCDETTGFCKKYKH	469-505
pDd63	PVNVDDNNKCTIDASDK CNEASCC DGVC TLKP	408-441
Eh170	GNLCTGKTTNCQEYVCDSEQRCTVQ EKVCVKTSPYIEM	506-543
pDd63	ISCPKPKNKCOVAKCDLIKGCTVS NVVCDDGNACTED	442-478
Eh170	SCYV AKCNL NTGMCENRLSCDTYSSCGGD STGS	544-576
WGA1 pDd63	G S C G L G S E F C G G G C Q S G A C S T D K P C G G D A G G R S C C S D T G K C Q F E P I K L P K N K N K C I I S K C D P I K G T I T N S	108-139 479-516
Eh170	V CKCD ASTGNOCKCNKVENGNYCDSSKHEI CDYT	577-610
WGA1	V CT NNYCCSKGGSCG IGPG YCGAGCQSGGCDA	140-171
pDd63	TVNCECDDLCNIGEC CEDTGKCNY RQKD CDDN	517-548

FIG. 6. Sequence homology of the cysteine-rich domain of the 170-kDa surface lectin of E. histolytica (Eh170) with WGA isolectin 1 (WGA1) and the DIF-induced prestalk protein of D. discoideum (pDd63). On the right, the residue numbers are indicated for the respective protein. Identical residues are boxed; conserved cysteine residues are doubly boxed. For optimal alignments, gaps were introduced into the sequences. In the overlap of  $\approx 170$ amino acids, 22% (Eh170/WGA1) and 24% (Eh170/pDd63) of the residues are identical. The amino acids of WGA that are considered to be involved in ligand binding are Asp-29, Ser-62, Tyr-73, Glu-115, Ser-148, and Tyr-159 (17); only Tyr-159 is conserved in the sequence of the amoeba lectin.

ary structure predictions (20) suggest that the protein folds predominantly into  $\alpha$ -helices and  $\beta$ -sheets. No repetitive elements were identified. Computer searches did not reveal significant homologies to any other protein sequences listed.

The extracellular domain adjacent to the transmembrane region has a cysteine content of >10%. In the N-terminal part of this domain, evidence was found for repetitive structural elements that may be aligned to form nine contiguous pseudorepeats (Fig. 5). In addition, this part of the sequence has a low degree of homology to two proteins with cysteine-rich repetitive structures (Fig. 6). The similarity to WGA was of particular interest because WGA is also a lectin and its tertiary structure has been determined by X-ray crystallography (15, 21). However, no reasonable assumptions could be made concerning the tertiary structure of the amoeba lectin since it lacks several cysteine residues that appear to form disulfide bonds critical for the stability of the WGA molecule (21). Furthermore, no conclusions could be drawn with regard to the carbohydrate binding site of the amoeba lectin since only one of the six amino acids that are believed to form the carbohydrate binding site in WGA was found to be conserved (22). It should be noted, however, that the carbohydrate ligands of WGA, which are N-acetylglucosamine and N-acetylneuraminic acid, differ from those of the amoeba lectin (17)

In conclusion, the location of the carbohydrate binding site remains unclear. No attribution to a distinct class of lectins could be made on the basis of consensus sequences (23). It cannot even be predicted that the carbohydrate binding site is located within the cysteine-rich domain because there are examples of lectins that contain a cysteine-rich domain but bind to the carbohydrate ligand in a cysteine-poor region (17).

The availability of cDNA clones coding for the 170-kDa surface lectin of E. histolytica will allow the recombinant expression of selected parts of the molecule. Thereby, it may be possible to identify functionally relevant regions such as the carbohydrate binding site. Furthermore, recombinant technology will possibly allow the selective expression of

those regions of the molecule that were found to elicit a protective immune response.

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