INFECTION AND IMMUNITY, Feb. 1990, p. 309–314
0019-9567/90/020309-06$02.00/0

Vol. 58, No. 2

Purification and Characterization of the Extracellular C3d-Binding Protein of *Candida albicans*

ASHIMA SAXENA AND RICHARD CALDERONE*
Georgetown University School of Medicine, Washington, D.C. 20007

Received 8 August 1989/Accepted 20 October 1989

A C3d-binding glycoprotein was purified from the culture filtrate of *Candida albicans* by preparative isoelectric focusing. The protein possessed a pI of 3.9 to 4.1 and could inhibit rosetting of EAC3d (sheep erythrocytes conjugated to C3d) by pseudohyphae of *C. albicans*. When analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and mercaptoethanol, the protein migrated as a doublet with apparent molecular masses of 55 and 60 kilodaltons (kDa) and as a 50-kDa band in nonreducing gels. These results were observed with Aurodye stain for proteins, Western immunoblot, and concanavalin A stain, which indicates that both bands contain carbohydrate as well as antigenic determinants. The treatment of purified glycoprotein with endoglycosidase F but not endoglycosidases H, N, and O resulted in a complete conversion of the doublet into a faster-migrating broad band with an apparent molecular mass of 45 kDa. When the amino acid analysis of the C3d-binding protein was compared with that of the CR2 from B lymphocytes, significant differences were observed. These data indicate that *C. albicans* secretes a C3d-binding protein during growth in vitro which appears to be different from the mammalian C3d receptor.

Several investigators have demonstrated the presence of iC3b- and C3d-binding proteins on the cell surface of *Candida albicans* (3, 5–7, 9, 11). These proteins are found on *C. albicans* and *C. stellatoidea* but not on other *Candida* species (3, 5, 9). Receptors with these specificities are also found on mammalian cells involved in host defense mechanisms. For example, complement receptor 3 (CR3), found on phagocytes and natural killer cells, is specific for iC3b, while CR2, found on B lymphocytes, recognizes iC3b, C3dg, and C3d fragments. Monoclonal antibodies against mammalian CR3, such as OKM-1 and Mo-1, bind to *Candida* cells (5–7), suggesting that the iC3b receptor on *Candida* cells shares epitope homology with its mammalian counterpart. Eigentler et al. utilized OKM-1 to purify proteins of 50, 100, and 130 kilodaltons (kDa) from *C. albicans* by affinity chromatography (6). Each of these proteins presumably has iC3b-binding activity.

The C3d-binding proteins (or CR2) of *C. albicans* have been identified by C3d-ligand affinity chromatography (3) or monoclonal antibody CA-A affinity purification (11). Both procedures yielded proteins of 60 and 70 kDa, although ligand binding was shown to be associated only with the 60-kDa protein (11).

The CR2 of lymphoblastoid cells (Raji) is associated with the cell surface and is released into the culture medium (1, 15). In this study, we sought to determine if *C. albicans* secreted or released its C3d-binding protein and, if so, to characterize it biochemically.

MATERIALS AND METHODS

Organism and culture conditions. *C. albicans* 4918 (12) was used in all experiments. For the cultivation of pseudohyphal cells, 2-liter Erlenmeyer flasks containing 1 liter of Phytone-peptone broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.1% glucose were inoculated with 5×10^7 yeast cells per ml and incubated at 37°C with shaking at 150 rpm. After 18 h, the culture filtrate (CF) was recovered by filtration through filter paper (no. 1, Whatman, Inc., Clifton, N.J.).

For rosetting assays, pseudohyphae of *C. albicans* were cultivated in serum-free RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) at 37°C with shaking at 150 rpm. After 18 h, hyphae were collected by centrifugation at 1,200 × *g*, washed twice with GVB-GVBS buffer (3, 11), and suspended in the same buffer.

Concentration of CF. CF obtained from 18-h pseudohyphal cultures was mixed with phenylmethylsulfonylfluoride (Sigma Chemical Co., St. Louis, Mo.) (final concentration, 1 mM), filtered through an HV membrane filter (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.), and concentrated 100-fold by tangential ultrafiltration by using a Pellicon device with a PTGC membrane (cutoff molecular mass, 10,000 kDa) (Millipore). The concentrated CF was lyophilized to yield a powder which was stored at −20°C until used.

Rosetting assay. Samples of EAC3d (sheep erythrocytes conjugated to C3d) were prepared as described previously (3). For the rosetting assay, EAC3d (1.4×10^8/0.1 ml) was mixed with pseudohyphal cells (2×10^6/0.1 ml), incubated for 30 min at 37°C, and kept overnight at 4°C. The percentage of pseudohyphae with adhering erythrocytes was evaluated by light microscopy, as described previously (3). At least 100 pseudohyphae were counted for each treatment. To measure rosette inhibition, EAC3d was incubated first with the purified fraction for 30 min at 37°C and then with pseudohyphae for 30 min at 37°C. The samples were left overnight at 4°C, and the percentage of rosetting with pseudohyphae was evaluated as described above. EAC3d incubated with buffer prior to the addition of pseudohyphae served as a control.

Preparative IEF of CF. CF was fractionated by isoelectric focusing (IEF) with a Rotofor cell (Bio-Rad Laboratories, Richmond, Calif.). The first fractionation was done with 0.6 g of CF in 40 ml of water and 2% Bio-Lyte ampholytes, pH 3 to 10; (Bio-Rad) at 12 W of constant power for 4 h at 4°C. The pH of each fraction was measured. Fractions containing C3d-binding protein (as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] and West-

* Corresponding author.

ern blots [immunoblots]) were pooled, diluted to 50 ml with water, and refractionated under the same conditions. Upon refractionation, the pH of each fraction was measured. Each fraction was also analyzed by Western blots with rabbit antiserum to the C3d receptor (see below) and by an inhibition-of-rosetting assay. Fractions 5 through 7 (between pH 3.9 and 4.1), which contained the C3d-binding protein(s), were pooled and concentrated to 800 μl with Centricon 10 (Amicon Corp., Danvers, Mass.).

Gel permeation chromatography of C3d-binding protein. Partially purified C3d-binding protein, obtained by preparative IEF of pseudohyphal CF, was further purified by gel permeation chromatography on a Protein Pak 300 SW column (7.5 by 30 mm; Waters Associates, Inc., Milford, Mass.) with 50 mM Tris hydrochloride (pH 7.5) containing 100 mM NaCl as the eluant. The column was calibrated by using the following molecular mass standards (Bio-Rad): thyroglobulin (bovine), 670 kDa; immunoglobulin G (bovine), 158 kDa; ovalbumin, 44 kDa; myoglobin (horse), 17 kDa; and vitamin B_{12}, 1.35 kDa.

Sucrose density gradient centrifugation of purified C3d-binding protein. The molecular size of native C3d-binding protein was estimated by sucrose gradient centrifugation, as described previously (13). A linear sucrose gradient containing 10 to 28% sucrose in 50 mM Tris hydrochloride (pH 7.5) containing 100 mM NaCl was prepared in polyallomer tubes, and the sample was overlaid on the gradient. The gradient was centrifuged at 120,000 × *g* for 19 h in a rotor (SW41; Beckman Instruments, Inc., Fullerton, Calif.) and fractionated, and the fractions were analyzed by SDS-PAGE and Western blots. The sedimentation behavior of the C3d-binding protein was compared with the following protein standards (Pierce Chemical Co., Rockford, Ill.): catalase, 240 kDa; aldolase, 158 kDa; bovine serum albumin, 67 kDa; and cytochrome *c*, 12.5 kDa. The positions of the protein standards in the gradient were monitored by measuring A_{280}.

SDS-PAGE and Western blotting. SDS-PAGE was performed by using the buffer system described by Laemmli (10), with or without mercaptoethanol. Samples were electrophoresed in 10% polyacrylamide gels at 200 V for 35 min with a Mini Protean II apparatus (Bio-Rad). After electrophoresis, proteins were transferred to an Immobilon-P membrane (pore size, 0.45 μm; Millipore) with a Trans Blot Cell (Bio-Rad), as described previously (11). The resulting protein blots were either stained with Aurodye (Janssen Life Science Products, Piscataway, N.J.) or blocked with 5% nonfat dry milk in Tris-buffered saline (20 mM Tris hydrochloride [pH 7.5], 0.5 M NaCl) for 1 h at room temperature and incubated overnight at 4°C with rabbit antiserum to the *C. albicans* C3d receptor (1:200 dilution in Tris-buffered saline). The blots were then washed, and bands were detected by the protein A-gold enhancement procedure according to the instructions of the manufacturer (Bio-Rad). The identification of concanavalin A-binding proteins was carried out by the method of Hawkes (8).

Preparation of polyclonal serum to *C. albicans* C3d-receptor. For the preparation of rabbit antisera, 300 μg of the C3d receptor of *C. albicans*, purified as described previously (11), was suspended in saline, emulsified with complete Freund adjuvant, and injected subcutaneously into each of three rabbits. Booster injections of 150 μg of antigen emulsified with incomplete adjuvant were given after 3 weeks. The rabbits were bled after 1 week, and the specificities of the sera were assessed by agglutination, double immunodiffusion, and Western blotting.

Carbohydrate determination. Carbohydrate was determined by the phenol-sulfuric acid method of Dubois et al. (4), with D-mannose as the standard. To determine the neutral-sugar content, the purified glycoprotein was hydrolyzed with 1 N HCl at 100°C for 6 h in a sealed, evacuated tube. The sample was neutralized with AGl-X8 (OH^-) resin and analyzed by high-pressure liquid chromatography (Waters Associates) equipped with a Waters 410 refractive index detector and a single-channel recorder integrator. Samples were chromatographed on an Aminex HPX-87C column (7.8 by 300 mm; Bio-Rad) at 85°C with water as the eluant at a flow rate of 0.6 ml/min.

Endoglycosidase digestion of C3d-binding protein. Peptide:*N*-glycosidase F, endoglycosidases H and F, and endo-β-*N*-acetylgalactosaminidase (*O*-glycanase) were obtained from Genzyme, Boston, Mass. Treatment of purified C3d-binding protein with endoglycosidases were done according to the instructions of the manufacturer. For endo-β-*N*-acetylglucosaminidase H (endo H) digestions, the glycoprotein sample (1.25 mg/ml) was denatured by boiling for 5 min in the presence of 0.5% SDS and 0.1 M β-mercaptoethanol. Samples containing 12.5 μg of protein were diluted with 50 mM sodium phosphate buffer (pH 5.5) and treated with 1 to 4 mU of endo H in a total volume of 25 μl at 37°C overnight. For *N*-glycanase (peptide:*N*-glycosidase F) digestions, the denatured sample (12.5 μg of protein) was diluted with 150 mM sodium phosphate buffer (pH 8.6) containing 20 mM EDTA and 1.25% Nonidet P-40. Samples were digested with 0.5 to 2 U of *N*-glycanase in a total volume of 30 μl at 37°C overnight. Endoglycosidase F digestions (0.06 to 0.24 mU) were carried out similarly, except that the buffer was 0.1 M sodium acetate, pH 6.0. *O*-Glycanase digestions (1 to 4 mU) were also carried out as described above, except that the buffer was 33 mM Tris maleate, pH 6.0. After endoglycosidase treatment, samples were analyzed by SDS-PAGE and Western blots.

Protein determination. Protein was determined by the method of Peterson (14), with bovine serum albumin as the standard.

Amino acid analysis. Purified samples of C3d-binding protein were hydrolyzed in 6 N HCl at 115°C for 18 h. The amino acids were derivatized with phenylisothiocyanate and analyzed with the Pico Tag System (Waters) on a C18 reverse-phase column by the method of Bidlingmeyer et al. (2).

RESULTS

Evidence for C3d-binding protein in CF of *C. albicans*. C3d-binding activity in pseudohyphal CF was indicated by the ability of CF to inhibit rosetting of pseudohyphae to EAC3d (2.5 μg of protein was required for 50% inhibition). Furthermore, rabbit antiserum raised against the *C. albicans* C3d receptor also caused 50% inhibition of rosetting at a dilution of 1:1,000. Western blot analysis of pseudohyphal CF with monoclonal antibody (CA-A (11) (results not shown) or rabbit antiserum to the purified C3d receptor (Fig. 1) indicated the presence of proteins in the 55- to 60-kDa region, similar to those characterized previously as C3d-binding proteins (11).

Isolation of the C3d-binding protein from pseudohyphal CF. The C3d-binding protein from CF of *C. albicans* was isolated by preparative IEF followed by gel filtration. CF was initially fractionated with a broad range (pHs 3 to 10) of Biolytes; active material from the first focusing was then refractionated. Most of the C3d-binding protein was localized in three fractions (between pH 3.9 and 4.1) (Fig. 2, lanes

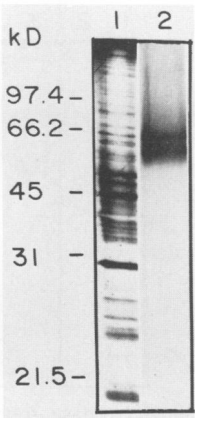

FIG. 1. SDS-PAGE and Western blot analysis of mycelial CF proteins. Electrophoresis was performed with a 10% polyacrylamide gel as described by Laemmli (10), with mercaptoethanol, and separated proteins were transferred onto an Immobilon-P membrane. Lane 1, Aurodye strain for CF proteins; lane 2, immunostain with rabbit antiserum to *Candida* C3d receptor and protein A-gold. Molecular size standards are as follows: phosphorylase *b*, 97.4 kDa; bovine serum albumin, 66.2 kDa; carbonic anhydrase, 31 kDa; and soybean trypsin inhibitor, 21.5 kDa.

5 through 7). The major protein in these fractions had a molecular size of 55 to 60 kDa, as determined by SDS-PAGE and Western blots (Fig. 2). A significant increase in specific activity (calculated as activity per microgram of protein) compared with the crude CF was obtained following IEF. Additional fractionation of the IEF-purified material (between pH 3.9 and 4.1) was obtained by gel filtration on a Protein Pak 300 SW column (Fig. 3). The peak of the biological and antigenic activities coincided with the major protein peak at A_{280}. The data from a typical purification of the C3d-binding protein by IEF and gel filtration are summarized in Table 1. These procedures resulted in a 32-fold increase in activity and an overall yield of 24%.

Molecular size determinations of the purified C3d-bindng protein were made on a 10% acrylamide gel in the presence

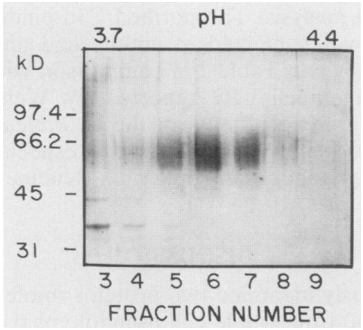

FIG. 2. Preparative IEF of pseudohyphal CF. CF (0.6 g) was diluted in 2% Biolyte ampholyte (between pH 3 and 10) and loaded on the Rotofor cell. Fractions were harvested and analyzed by SDS-PAGE and Western blots. Fractions containing C3d-binding protein were pooled, diluted in water, and refractionated on the Rotofor cell. Refractionated samples were harvested, electrophoresed in the absence of mercaptoethanol with a 7.5% polyacrylamide gel as described by Laemmli (10), and transferred to Immobilon-P membrane as described (11). The blots were stained with Aurodye. Fractions 5 through 7 (pH 3.9 to 4.1) contain the C3d-binding protein.

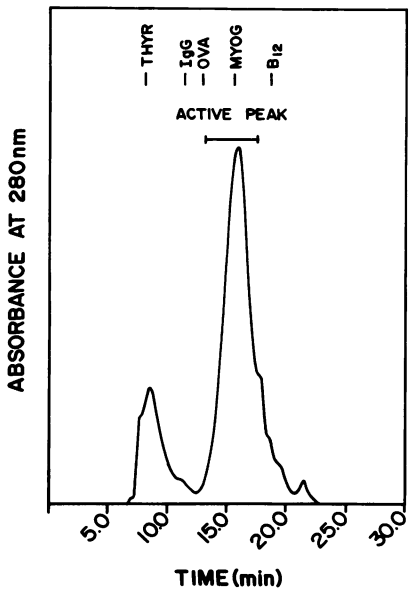

FIG. 3. Gel permeation chromatography of C3d-binding protein. Partially purified C3d-binding protein obtained by preparative IEF of pseudohyphal CF was applied to a Protein Pak 300 SW column (7.5 by 30 mm) equilibrated with 50 mm Tris hydrochloride (pH 7.5) containing 0.1 M NaCl. The flow rate was 0.8 ml/min, and the fraction size was 0.8 ml. The optical density at 280 nm was monitored during elution, and 10 μl of each fraction was analyzed for C3d-binding protein by SDS-PAGE, Western blots, and inhibition-of-rosetting assay. Fractions containing C3d-binding protein indicated by the bar were pooled and concentrated. THYR, thyroglobulin; IgG, immunoglobulin G; OVA, ovalbumin; MYOG, myoglobulin; B_{12}, vitamin B_{12}.

and absence of mercaptoethanol (Fig. 4). Under reducing conditions, the protein migrated as a doublet with apparent molecular masses of 60 and 55 kDa for the upper and lower bands, respectively. However, under nonreducing conditions, the protein migrated as a diffuse band in the 50- to 55-kDa region. These results were observed with Aurodye stain (Fig. 4, lanes 1 and 2), Western immunoblot (Fig. 4, lanes 3 and 4), and concanavalin A stain (data not shown).

To determine subunit composition of the C3d-binding protein, an attempt to determine the molecular size of the native protein was made. Gel permeation chromatography was not successful (Fig. 3), since the protein eluted with an apparent molecular size of 14 kDa. This result was probably due to nonspecific interaction of the protein with the gel filtration medium. As an alternative, sucrose density gradient centrifugation following IEF was used. By this technique, the molecular size of the C3d-binding protein was estimated to be approximately 240 kDa (Fig. 5).

Glycoprotein nature of C3d-binding protein. The glycoprotein nature of C3d-binding protein was indicated by its ability

TABLE 1. Purification of the C3d-binding protein from *C. albicans*

Source of fraction	Total protein (mg)	Activity[a]	% Recovery
Culture supernatant	380	2.5	100
IEF	4	0.1	26
Protein Pak 300 SW	3	0.08	24

[a] Amount of protein (in micrograms) required for 50% inhibition of rosetting of pseudohyphae with EAC3d.

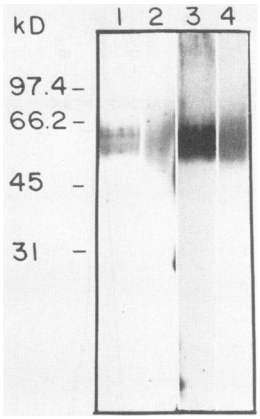

FIG. 4. SDS-PAGE of purified C3d-binding protein. Electrophoresis was performed with a 10% polyacrylamide gel, and separated proteins were transferred onto an Immobilon-P membrane. Lanes 1 and 2 were stained with Aurodye, whereas lanes 3 and 4 were immunostained with rabbit antiserum. Lanes 1 and 3, C3d-binding protein in the presence of mercaptoethanol. Lanes 2 and 4, C3d-binding protein in the absence of mercaptoethanol.

to bind concanavalin A (11). The amount of carbohydrate was found by the phenol-sulfuric acid method to be about 30%. To characterize the sugar residues of C3d-binding protein, purified protein was subjected to hydrolysis with 1 N HCl for 6 h. Analysis of this sample by high-performance liquid chromatography on a Aminex HPX-87C column resolved two peaks corresponding to *N*-acetylglucosamine (11.4%) and mannose (18.5%).

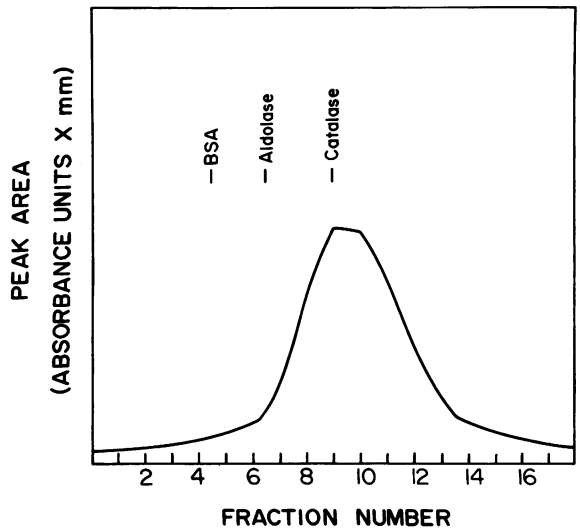

FIG. 5. Sucrose-density gradient centrifugation pattern for purified C3d-binding protein. Approximately 0.5 mg of purified C3d-binding protein in 0.1 ml was layered on a linear sucrose gradient containing 10 to 28% sucrose in 50 mM Tris hydrochloride (pH 7.5) containing 100 mM NaCl. The gradient was centrifuged at 120,000 × *g* for 19 h in a Beckman SW41 rotor and fractionated, and the fractions were analyzed by Western blot. The blots were scanned with an Ultroscan XL laser densitometer to determine the area of the peak corresponding to C3d-binding protein. The reference proteins catalase, aldolase, and bovine serum albumin (BSA) were run on separate gradients, which were fractionated; the fractions were assayed by measuring the optical density at 280 nm. The peak positions for reference proteins are marked.

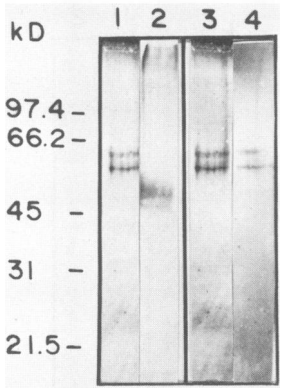

FIG. 6. Effects of endoglycosidase treatment on C3d-binding protein. Samples of purified C3d-binding protein before (lanes 1 and 3) and after (lanes 2 and 4) treatment were electrophoresed on 10% gels in the presence of mercaptoethanol and analyzed by Western blotting with rabbit antiserum. Lane 2, C3d-binding protein after treatment with 0.12 mU of endo F. Lane 4; C3d-binding protein after treatment with 4 mU of *O*-glycanase.

The glycoprotein nature of the C3d-binding protein was characterized further with endoglycosidases. The treatment of purified protein with endoglycosidase F, which cleaves biantennary, complex N-linked oligosaccharides (16), increased the electrophoretic mobility of the protein, resulting in a complete conversion of the doublet to a 45-kDa species (Fig. 6, lanes 1 and 2). The broad band formed following digestion with endo F was immunoreactive and still able to bind concanavalin A (results not shown), indicating that it contained carbohydrate. Treatment with *N*-glycanase, which cleaves all classes of N-linked oligosaccharides or endoglycosidase H, did not result in a complete conversion of the doublet to the 45-kDa species. The activities of all the endoglycosidases were confirmed by cleavage patterns of the proteins fetuin and ovalbumin.

The possibility of O-linked oligosaccharides was investigated by treatment of purified protein with *O*-glycanase, which cleaves the core disaccharide linked to serine or threonine residues. Treatment of purified C3d-binding protein with up to 4 mU of *O*-glycanase did not alter the electrophoretic mobility of the protein (Fig. 6, lanes 3 and 4).

Amino acid analysis. The purified C3d-binding protein of *C. albicans* was subjected to amino acid analysis. These results are shown in Table 2 in comparison with an analysis of a mammalian-cell CR2 reported by Weis et al. (18). Substantial differences between the *C. albicans* and mammalian-cell proteins were observed, especially with the amino acids glutamic acid-glutamine, leucine, methionine, and tyrosine.

DISCUSSION

We previously identified two proteins (molecular masses, 62 and 70 kDa) from whole-cell pseudohyphal extracts of *C. albicans* that bind the C3d fragment of C3 (3). These proteins, purified by C3d affinity chromatography, were found to be mannosylated and were sensitive to heat and protease treatments (3). However, when a monoclonal antibody, CA-A, was used for the isolation of the C3d receptor from pseudohyphal extracts, this activity was found to be associated only with the 60-kDa protein (11). This report describes the isolation and characterization of the C3d-binding protein of *C. albicans* released during growth in liquid culture.

TABLE 2. Comparison of amino acid profiles of mammalian and *C. albicans* CR2

Amino acid	% of total amino acids in CR2	
	Mammalian[a]	*C. albicans*[b]
Asp/Asn	8.0	5.5
Thr	5.7	3.6
Ser	8.9	14.6
Glu-Gln	11.1	29.8
Gly	12.0	15.2
Ala	5.5	6.1
Cys	ND[c]	ND
Val	5.9	3.6
Met	1.6	0.6
Ile	4.4	2.4
Leu	6.6	3.0
Tyr	6.3	1.2
Phe	3.7	1.2
His	2.4	2.4
Lys	5.4	5.5
Arg	4.4	2.4
Pro	8.0	2.4
Trp	ND	ND

[a] Data from reference 18.
[b] Determined as described in Materials and Methods.
[c] ND, Not determined (Cys and Trp are destroyed by acid hydrolysis).

Although CF from *C. albicans* appeared to contain a large number of proteins, it served as a good source for the large-scale isolation of the C3d-binding protein. Considerable purification was achieved by preparative IEF of CF, and an additional, smaller purification was achieved by gel permeation chromatography. When analyzed by SDS-PAGE in the presence of mercaptoethanol, the purified protein migrated as a doublet of 60 and 55 kDa. The respective band patterns were identical for more than five separate purifications, and the patterns did not change upon storage at −20°C. Therefore, we do not believe that the multiple banding pattern is due to proteolysis. Both bands were recognized by rabbit antiserum to the *C. albicans* C3d receptor as well as by concanavalin A. The CF form of C3d-binding protein contained 30% carbohydrate by weight, which was composed of *N*-acetylglucosamine and mannose. The oligosaccharide component of the protein was susceptible to endo F and less so to endo H and *N*-glycanase, which suggests that these N-linked oligosaccharides are of the high-mannose and biantennary-complex type. The molecular mass of endo F-treated protein was 45 kDa. However, the endo F-treated protein was still able to bind concanavalin A, indicating that it still contained carbohydrate. In contrast, the mammalian CR2 has been reported to contain the complex type of N-linked oligosaccharides only (17). As with the mammalian CR2, the CF form of the C3d-binding protein from *Candida* spp. was found to lack *O*-linked oligosaccharides. These results also suggest that the 55- and 60-kDa bands could be results of differential glycosylation of the 45-kDa species.

From data described in this study, several additional characteristics of the C3d-binding protein which suggest that the protein is different from the mammalian CR2 were found. The molecular size of the native protein as estimated by sucrose density centrifugation was 240 kDa, which suggests the existence of subunits in the protein. However, more rigorous studies are needed to confirm this result. The protein focused between pH 3.9 and 4.1, which is different from the pI of 8.2 reported for mammalian CR2 (18). The amino acid composition of C3d-binding protein was found to be different from that of mammalian CR2. The high glutamic acid content (29% of total amino acids) was consistent with the low pI of this protein. Although the C3d-binding protein from *Candida* spp. appears to be different from mammalian CR2, similarities in specific domains, especially those involving the ligand-binding site of the protein, cannot be ruled out.

In summary, the results of this study suggest that the CF form of C3d-binding protein is different from that of mammalian cells. These results are in agreement with earlier studies by Eigentler et al. (6) and Edwards et al. (5), who observed that anti-CR2 monoclonal antibodies did not bind to *C. albicans* pseudohyphae or hyphae when assayed by immunofluorescence. Edwards et al. (5) were, however, successful in blocking C3d binding to *C. albicans* with high concentrations of an anti-CR2 polyclonal antibody at a low ligand density.

The data presented also demonstrate the successful purification of the C3d-binding protein in milligram quantities. The purified protein can be used for obtaining monoclonal and monospecific antibodies, sequence determination, and structural studies.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant AI25738 from the National Institutes of Health to R.A.C.

We thank Stephanie Coleman and Margaret Sieber for typing the manuscript. We are grateful to Ray Fields (Laboratory of Cellular Development and Oncology, National Institute of Dental Research, Bethesda, Md.) for the amino acid analysis.

LITERATURE CITED

1. **Berger, M., T. A. Gaither, and M. M. Frank.** 1982. Complement receptors. Clin. Immunol. Rev. **1**:471–545.
2. **Bidlingmeyer, B. A., S. A. Cohen, and T. L. Tarvin.** 1984. Rapid analysis of amino acids using pre-column derivatization. J. Chromatogr. **336**:93–104.
3. **Calderone, R. A., L. Linehan, E. Wadsworth, and A. L. Sandberg.** 1988. Identification of C3d receptors on *Candida albicans*. Infect. Immun. **56**:252–258.
4. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Reber, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
5. **Edwards, J. E., T. A. Gaither, J. O'Shea, D. Rotrosen, T. J. Lawley, S. A. Wright, M. M. Frank, and I. Green.** 1986. Expression of specific binding sites on *Candida* with functional and antigenic characteristics of human complement receptors. J. Immunol. **137**:3577–3583.
6. **Eigentler, A., T. F. Schulz, C. Larcher, E.-M. Breitwieser, B. L. Myones, A. L. Petzer, and M. P. Dierich.** 1989. C3bi-binding protein on *Candida albicans*: temperature-dependent expression and relationship to human complement receptor type 3. Infect. Immun. **57**:616–622.
7. **Gilmore, B. J., E. M. Retsinas, J. S. Lorenz, and M. K. Hostetter.** 1988. An iC3b receptor on *Candida albicans*: structure-function and correlates for pathogenicity. J. Infect. Dis. **157**:143–146.
8. **Hawkes, R.** 1982. Identification of concanavalin A-binding proteins after sodium dodecyl sulfate-gel electrophoresis and protein blotting. Anal. Biochem. **123**:143–146.
9. **Heidenreich, F., and M. P. Dierich.** 1985. *Candida albicans* and *Candida stellatoidea*, in contrast to other *Candida* species, bind iC3b and C3d but not C3b. Infect. Immun. **50**:598–600.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Linehan, L., E. Wadsworth, and R. A. Calderone.** 1988. *Candida albicans* C3d receptor, isolated by using a monoclonal antibody. Infect. Immun. **56**:1981–1986.

12. **Manning, M., and T. G. Mitchell.** 1980. Strain variation and morphogenesis of yeast- and mycelial-phase *Candida albicans* in low-sulfate, synthetic medium. J. Bacteriol. **142:**714–719.
13. **Martin, R. G., and B. N. Ames.** 1961. A method for determining the sedimentation behavior of enzymes: application to protein mixtures. J. Biol. Chem. **236:**1372–1379.
14. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. Anal. Biochem. **83:**346–356.
15. **Ross, G. D.** 1986. Opsonization and membrane complement receptors, p. 87–114. *In* G. D. Ross (ed.), Immunobiology of the complement system. Academic Press, Inc., New York.
16. **Tarentino, A. L., T. H. Plummer, Jr., and F. Maley.** 1974. The release of intact oligosaccharides from specific glycoproteins by endo-beta-N-acetylglucosaminidase H. J. Biol. Chem. **249:**818–824.
17. **Weis, J. J., and D. T. Fearon.** 1985. The identification of N-linked oligosaccharide on the human CR2/Epstein-Barr virus receptor and their function in receptor metabolism, plasma membrane expression and ligand binding. J. Biol. Chem. **260:** 13824–13830.
18. **Weis, J. J., S. A. Richards, J. A. Smith, and D. T. Fearon.** 1986. Purification of the B lymphocyte receptor for the C3d fragment of complement and the Epstein-Barr virus by monoclonal antibody affinity chromatography and assessment of its functional capacities. J. Immunol. Methods **92:**79–87.