

Isolation and characterization of a major allergenic component (gp55) of *Aspergillus fumigatus*

Reiko Teshima, PhD,^a Hideharu Ikebuchi, PhD,^a Jun-ichi Sawada, PhD,^a
Sumiki Miyachi, MD,^c Seiichi Kitani, MD,^d Masanori Iwama, PhD,^a
Masachika Irie, PhD,^a Masakatsu Ichinoe, PhD,^b and Tadao Terao, PhD^a
Tokyo, Japan

IgE class antibodies specific for antigens in a water-soluble extract of Aspergillus fumigatus (strain NHL-5759) were analyzed by immunoblotting with sera from patients with allergic bronchopulmonary aspergillosis. All the sera tested were reactive with a major 50 to 60 kd protein in the extract. This allergen, designated gp55, was purified by gel filtration and preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The antigen was found to be present in the water-soluble extract in the form of a complex composed of approximately eight molecules of gp55. The carbohydrate and phosphate content of the purified antigen were 23.1% and 0.46%, respectively. The molar ratio of mannose to galactose residues was 2.76:1, and the protein was glycosylated predominantly with N-linked oligosaccharides. The serologic activity of the gp55 antigen was abolished by treatment with nonspecific protease (Pronase) but not by treatment with sodium metaperiodate or endoglycosidases. Thus the major antigenic site of the glycoprotein is located within its peptide moiety. The antigen itself displayed no chymotryptic or tryptic activity. The amino acid sequence of the 20 N-terminal residues of the antigen (ATPHEPVFFSWDAGAVTSFP) is different from that of any other protein previously reported. (J ALLERGY CLIN IMMUNOL 1993;92:698-706.)

Key words: *Aspergillus fumigatus*, IgE-binding protein, glycoprotein, N-linked oligosaccharides, N-terminal sequence

Aspergillus fumigatus is a saprophyte with a worldwide distribution and is sometimes recognized as an opportunistic pathogen. It causes both allergic and nonimmunologic disorders. The allergic disorders include allergic bronchopulmonary aspergillosis (ABPA) and atopic respiratory disease (asthma).¹ The nonimmunologic disorders caused by *Aspergillus* include aspergilloma (cavi-

Abbreviations used

ABPA: Allergic bronchopulmonary aspergillosis
CBB: Coomassie brilliant blue
HRP: Horseradish peroxidase
MCA: Methylcoumarinamide
MW: Molecular weight
PAGE: Polyacrylamide gel electrophoresis
SDS: Sodium dodecyl sulfate

From ^athe Division of Biochemistry and Immunochemistry,

^bthe Division of Microbiology, National Institute of Hygienic

Sciences, ^cthe Department of Medicine, Kanto Chuo Hos-

pital, ^dthe Department of Medicine and Physical Therapy,

School of Medicine, University of Tokyo, and ^ethe Depart-

ment of Microbiology, Hoshi College of Pharmacy.

Supported in part by a research grant from the Science and Technology Agency of Japan.

Received for publication Sept. 18, 1992; revised April 7, 1993; accepted for publication April 22, 1993.

Reprint requests: Reiko Teshima, PhD, National Institute of Hygienic Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo, 158, Japan.

Copyright © 1993 by Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/1/48112

tary aspergillosis) and invasive aspergillosis (systemic aspergillosis).^{2, 3}

Serologic studies have shown high levels of IgE antibodies specific for several *A. fumigatus* antigens in ABPA and atopic respiratory disease.⁴ Therefore the detection of specific IgE antibodies against *A. fumigatus* is necessary, to make a diagnosis of *A. fumigatus*-induced allergic disorders. However, most of these antigens have not been well characterized or sufficiently purified, and

commercially available antigens are still unsatisfactory for diagnostic use.⁵

In the present study we purified an immunodominant antigen present in a water-soluble extract of *A. fumigatus* mycelia and studied its chemical properties.

METHODS

A. fumigatus mycelium extract

A. fumigatus (strain NHL-5759) was obtained from a culture collection of the Division of Microbiology, National Institute of Hygienic Sciences, Tokyo, Japan. The organism was grown as stationary cultures in completely synthetic medium (Czapek-Dox broth, Difco, Detroit, Mich.) at 37° C for 7 days.

A water-soluble extract was prepared as follows. The mycelial portion was separated from the growth medium by filtration through sterile gauze. We ground 40 gm of *A. fumigatus* mycelium with 40 gm of sea sand C (Nacalai Tesque, Inc., Kyoto, Japan) with a mortar and pestle at 2 to 4° C and then extracted with 80 ml of 0.2 mol/L phosphate buffer (pH 7.2) containing 1 mmol/L phenylmethylsulfonyl fluoride (Eastman Kodak, Rochester, N.Y.). Cell debris, unbroken cells, and sea sand C were removed by centrifugation at 45,000g for 1 hour at 4° C in a Hitachi 70-72 centrifuge. The supernatants were concentrated approximately 20 times with a PLGC cassette ultrafiltration system (Millipore Corp., Bedford, Mass.) and centrifuged for 1 hour at 105,000 g. The clear supernatant was then extensively dialyzed against distilled water and freeze-dried.

Purification procedure

Gel filtration was carried out on a 2.5 × 70 cm Cellulofine GCL-2000m column (Seikagaku Corp., Tokyo, Japan) at 4° C. The column was calibrated with ferritin (horse spleen) (450 kd), bovine serum albumin (67 kd), cytochrome *c* (12.3 kd), and bacitracin (1.5 kd). We eluted 5 ml fractions with 50 mmol/L of Tris hydrochloride (pH 7.4) containing 0.1 mol/L potassium chloride. The protein content was determined by absorbance at 280 nm. Fractions reactive with the sera of patients with ABPA in a dot-immunobinding assay were pooled and applied to preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (continuous-elution electrophoresis, Bio-rad Model 491 Prep Cell). We electrophoresed 5 mg of antigen at 40 mA in cylindrical acrylamide gel (7.5%, 2.8 × 9 cm) containing 0.1% SDS and eluted it from the bottom of the gel at a flow rate of 1 ml/min. Fractions (1.5 ml) were collected, and their protein content was determined by absorbance at 280 nm. The samples (450 µl) from every fifth fraction were concentrated to 50 µl with Ultra-Free C3LGC (Millipore) and tested by analytic SDS-PAGE. The collected purified antigen was concentrated with Centricon 10 (W. R. Grace Co., Beverly, Mass.) and used for the chemical analyses.

Human sera

Sera were taken from 10 patients with ABPA, five with atopic respiratory disease, and two with aspergillosis. Sera from seven normal donors were also used.

Analytic SDS-PAGE

The antigen preparations (5 to 10 µg) were electrophoresed in 7.5% or 7.5% to 15% acrylamide slab gels containing 0.1% SDS according to the method of Laemmli.⁶ The gels were stained with Coomassie brilliant blue (CBB) R-250 for protein or with periodic acid-Schiff stain for carbohydrate.⁷

IgE-specific immunoblot analysis

Proteins separated by analytic SDS-PAGE were electrophoretically transferred to a nitrocellulose membrane, blocked with 0.5% casein in phosphate-buffered saline solution, and washed with 0.05% polysorbate 20 in phosphate-buffered saline solution. The membrane was treated successively with fourfold diluted patient or normal human sera, rabbit antihuman IgE antibodies (Nordic Immunology, Tilburg, The Netherlands), and horseradish peroxidase (HRP)-linked donkey antirabbit IgG antibodies (Amersham, England) and then stained with substrate solution (immunostaining HRP-kit IS-50B, Konica, Tokyo, Japan).

IgG-specific immunoblot analysis

Proteins were separated and Western blotted as described previously. The membrane was treated successively with 500-fold diluted sera from ABPA patients, rabbit antihuman IgG antibodies (Cappel Products, West Chester, Pa.) and HRP-linked donkey antirabbit IgG antibodies, and then stained with substrate solution.

Dot immunobinding assay

A diluted solution of antigen in 20 mmol/L Tris hydrochloride (pH 7.4) was dotted onto a 0.2 µm nitrocellulose membrane, and the membrane was immunostained as described in the IgE-specific immunoblot analysis.

Neutral sugar analysis

Neutral sugars were analyzed by adding 1 ml of 2N hydrochloric acid to ampules containing lyophilized samples or standards, and hydrolyzing the samples for 3 hours at 100° C. The standards included D-glucose, D-mannose, and D-galactose. All ampules contained 2-deoxy-D-glucose as the internal standard. The samples were reduced with 10 mmol/L sodium borohydride and acetylated with 250 µl of acetic anhydride and 250 µl of pyridine for 2 hours at 100° C. After being evaporated to dryness, the alditol acetate derivatives of the neutral sugars were dissolved in a small amount of chloroform and analyzed with a Shimadzu GC-9A gas-liquid chromatograph equipped with a CBP-5-25M column (SE52 equivalent, Shimadzu, Kyoto, Japan).

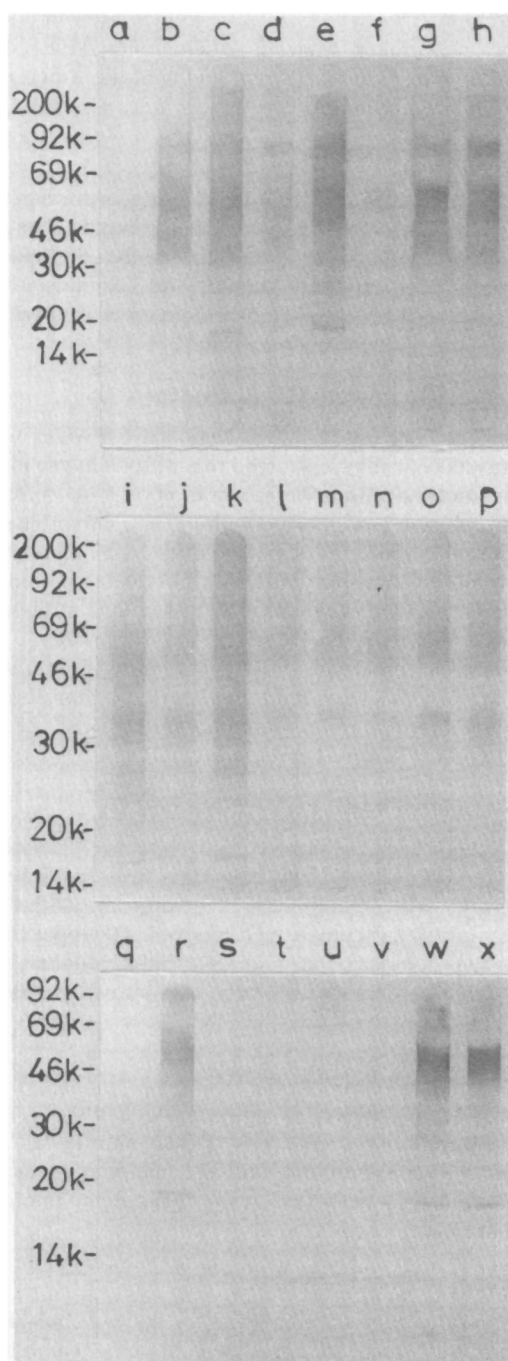


FIG. 1. IgE-specific immunoblot analysis of sera from patients with *Aspergillus*-related diseases and from normal donors. Proteins in the water-soluble extract of *A. fumigatus* were separated by SDS-PAGE in 7.5% to 15% gradient gel, transferred to nitrocellulose membrane, and immunostained with specific IgE in fourfold diluted normal sera (lanes a, f, g, s to v), and sera from patients with ABPA (lanes b to e, i to k, r, w, x), aspergilloma (lanes g, h), and atopic respiratory disease (lanes l to p), as described in Methods. MW (in kilodaltons) is indicated at left.

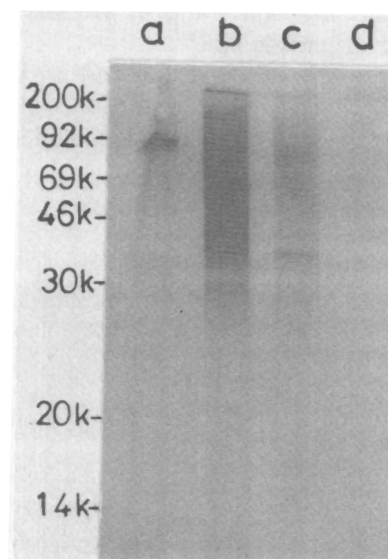


FIG. 2. IgG-specific immunoblot analysis of sera from patients with ABPA. Proteins in water-soluble extract of *A. fumigatus* were separated by SDS-PAGE in 10% to 20% gradient gel, transferred to nitrocellulose membrane, and immunostained with specific IgG in 500-fold diluted ABPA sera (lanes a to c) and normal serum (lane d), as described in Methods. MW (in kilodaltons) are indicated at left.

Amino sugar and amino acid analyses

Amino sugars were analyzed by hydrolyzing the samples with 4N HCl for 7 hours at 100° C followed by incubation with phenylisothiocyanate. Phenylthiohydantoin amino sugars were analyzed by high-performance liquid chromatography with a phenylthiohydantoin C18 column (Shiseido, Tokyo, Japan).

Amino acids were analyzed with a Hitachi 835 amino acid analyzer (Hitachi) after hydrolysis with 6N HCl for 24 hours at 110° C.

N-terminal amino acid sequencing

N-terminal sequence analysis was performed with an Applied Biosystem model 477A analyzer (Foster City, Calif.).⁸ Phenylthiohydantoin amino acids were identified with an on-line high-performance liquid chromatography system equipped with a phenylthiohydantoin C18 column (Shiseido).

Deglycosylation

Deglycosylation of gp55 was performed according to the protocol recommended by the manufacturer. Samples purified by gel filtration were used. One portion of the sample (20 µg) was incubated for 16 hours at 37° C with 40 U/ml of *N*-glycanase (Genzyme, Boston, Mass.) in 0.2 mol/L sodium phosphate (pH 8.6) containing 0.08% SDS, 10 mmol/L 10-phenanthroline hydrate, and 0.8% Nonidet P-40 nonionic detergent. Another portion (20 µg) of the purified antigen was incubated for

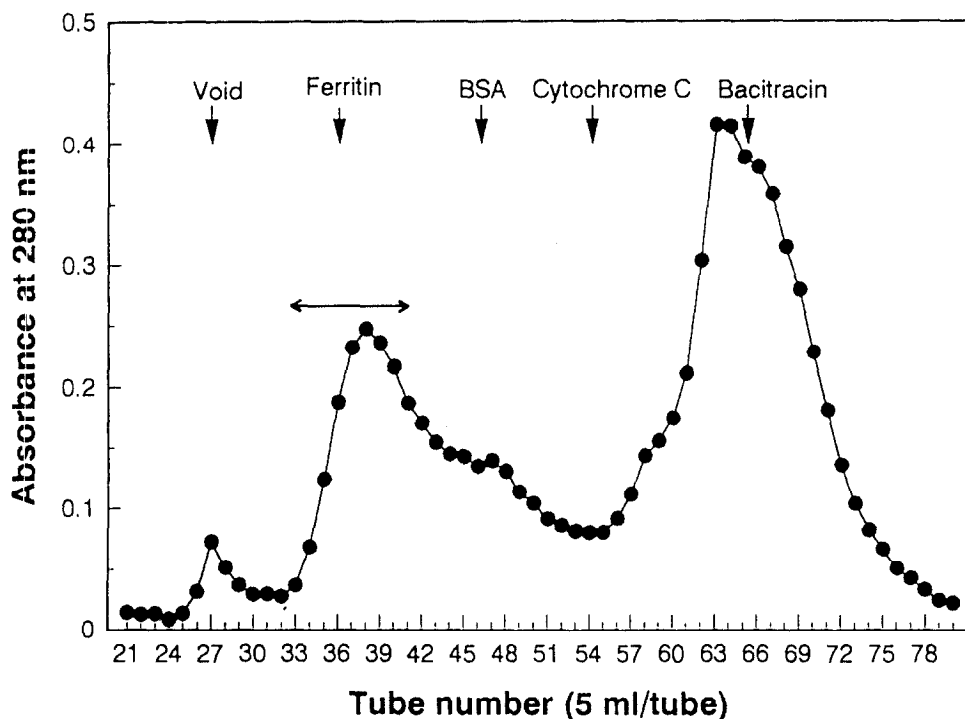


FIG. 3. Gel filtration of water-soluble extract from *A. fumigatus*. Water-soluble extract of *A. fumigatus* (20 mg proteins) was applied to column (2.5 × 70 cm) of GCL-2000m, and 5 ml fractions were eluted with 50 mmol/L Tris-HCl (pH 7.4) containing 0.1 mol/L potassium chloride. Protein concentration (absorbance at 280 nm) of each fraction is plotted. Immunoreactive fractions are indicated by horizontal arrow. Elution positions (vertical arrows) of ferritin (450 kd), bovine serum albumin (67 kd), cytochrome *c* (12.3 kd), and bacitracin (1.5 kd) are shown for reference.

16 hours at 37° C with 5.0 mU of endo- α -N-acetylglactosaminidase (Genzyme) in 20 mmol/L sodium phosphate (pH 6.5) containing 0.2% SDS and 2% Nonidet P-40 detergent.

The enzyme-treated samples were analyzed by SDS-PAGE and Western blotting with patient sera as described previously.

Sodium metaperiodate and protease treatment

Periodate oxidation of samples was carried out as follows. One hundred fifty micrograms of the antigen was incubated with 0.2 μ mol of sodium metaperiodate in 50 mmol/L acetate buffer (pH 5.0) at 24° C overnight.

Protease treatment consisted of treating 150 μ g of antigen with 500 μ g of nonspecific protease (Pronase E) (Nacalai Tesque) in 10 mmol/L Tris-HCl-buffered saline solution (pH 7.4) at 37° C for 24 hours.

The samples were analyzed by SDS-PAGE and Western blotting as described previously.

Protease assay

To measure the protease activity of gp55, a fluorogenic assay⁹ was performed in 0.7 ml of 0.05 mol/L

phosphate buffer (pH 7.4) containing 0.15 mol/L sodium chloride with a Shimadzu RF-5000 spectrofluorometer. Peptidyl-4-methylcoumarinamides (peptidyl-MCA), Boc-Val-Leu-Lys-MCA, Boc-Phe-Ser-Arg-MCA, Suc-Leu-Leu-Val-Tyr-MCA, and Suc-Ala-Pro-Ala-MCA (Peptide Institute, Inc., Osaka, Japan) dissolved in dimethyl sulfoxide were added to sample solutions in a cuvette at 37° C to yield a final concentration of 0.1 mmol/L, and the fluorescence of aminomethylcoumarin released from the substrates was measured at E_x (excitation) 380 nm and E_m (emission) 442 nm.

Estimation of phosphate

The phosphate content of the gp55 antigen was determined according to the method of Ames.¹⁰

RESULTS

IgE-specific immunoblot analysis of water-soluble antigens from *A. fumigatus* with *Aspergillus*-related patient sera

The water-soluble extract of *A. fumigatus* was analyzed by SDS-PAGE. The proteins transferred to nitrocellulose membranes were treated with

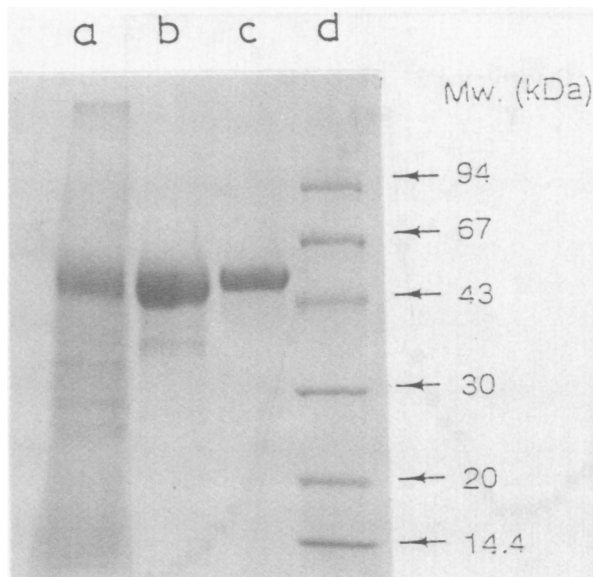


FIG. 4. Analytic SDS-PAGE of proteins in each purification step. Water-soluble extract (*a*), second peak fraction of gel filtration (*b*), and gp55 antigen purified by preparative electrophoresis (*c*) were analyzed by SDS-PAGE in 7.5% to 15% gradient gel under reducing conditions. Proteins were stained with CBB. MW standards (94 kd, 67 kd, 43 kd, 30 kd, 20 kd, 14.4 kd) were simultaneously electrophoresed (*d*).

patient sera and visualized with anti-IgE antibodies and HRP-conjugated third antibodies. Fig. 1 shows the results for the sera of 17 patients with *Aspergillus*-related conditions and of seven normal donors.

As shown in Fig. 1 (*lanes b to e, i to k, r and w and x*), IgE antibodies from ABPA patients were reactive with several proteins (92 kd, 55 kd (50 to 60 kd), 35 kd, and 18 kd). Although the staining patterns of these proteins differed slightly, the 55 kd protein seemed to be a major and common antigen. IgE antibodies from patients with aspergilloma (Fig. 1, *lanes g and h*) and from those with atopic respiratory disease were also reactive with the 55 kd protein. In contrast, IgE antibodies from normal donors (Fig. 1, *lanes a, f, g, and s to v*) did not react with the 55 kd protein. Thus we attempted to purify this 55 kd antigen with one of the ABPA patient sera (*lane b*) for immunoblotting and dot immunobinding assay.

IgG-specific immunoblot analysis of water-soluble antigens from *A. fumigatus* with ABPA sera

To determine whether both IgG and IgE antibodies are produced against the 55 kd antigen, we

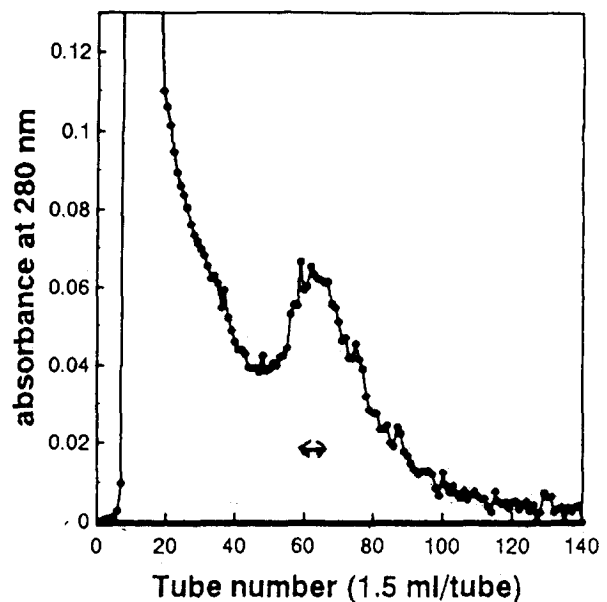


FIG. 5. Elution profile in preparative SDS-PAGE. Immuno-reactive fraction obtained by gel filtration of water-soluble extract (5 mg as protein) was applied to continuous-elution SDS-PAGE as described in Methods. Protein concentration (absorbance at 280 nm) of each fraction is plotted. Fractions collected (fractions 61 to 65) are indicated by horizontal arrow.

carried out IgG-specific immunoblot analysis. The protein present in water-soluble extract was separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was treated with ABPA sera and visualized with anti-IgG antibodies and HRP-conjugated third antibodies. Fig. 2 shows typical results for the sera of three patients with ABPA and one normal donor. As shown in Fig. 2 (*lanes a to c*), the major antigen recognized by the IgG antibodies of ABPA patient sera was a 92 kd protein, not a 55 kd protein.

Purification of the 55 kd antigen

Three main peaks (fraction number 25 to 30, 33 to 41, and 46 to 51) were obtained when the water-soluble extract of *A. fumigatus* was separated by gel filtration (Fig. 3). The second peak (fractions 33 to 41) was found to be positive in the IgE-specific dot immunobinding assay with ABPA serum.

The relative molecular weight (MW) of the second peak was estimated to be 450 kd. When the 450 kd proteins were analyzed by SDS-PAGE, a major band of 55 kd was stained by CBB (Fig. 4, *lane b*) and by immunoblotting. No appreciable differences were found in the migration patterns

TABLE I. Amino acid and sugar composition of gp55 antigen

	Weight %* (mol %)
Amino acid	
Asx	8.7 (8.6)
Thr	6.0 (6.7)
Ser	6.2 (7.8)
Glx	6.9 (6.1)
Gly	7.2 (8.1)
Ala	6.6 (9.8)
Val	4.3 (4.8)
Met	0.5 (0.4)
Ile	3.4 (3.4)
Leu	4.2 (4.2)
Tyr	1.8 (1.3)
Phe	4.0 (3.1)
Lys	1.7 (1.5)
His	0.2 (0.2)
Arg	4.5 (3.4)
Pro	10.7 (12.2)
Total sugar†	23.1 (18.4)
Mannose	13.9 (10.9)
Galactose	5.1 (4.0)
Glucosamine	3.6 (3.0)
Galactosamine	0.6 (0.5)

*Total protein content was measured by the method of Lowry et al.²⁵ Cysteine and tryptophan levels were not determined.

†Total neutral sugar and amino sugar content was measured by the phenol-sulfuric acid method²⁶ and the Elson-Morgan method,²⁷ respectively.

of the proteins when analyzed by SDS-PAGE under reducing and nonreducing conditions (data not shown). Thus the antigen in the second peak seemed to be composed of about eight molecules of 55 kD protein with no disulfide bonding.

To remove a lower MW (35 kD) protein present in the second peak fraction (approximately 5%), the proteins in this fraction were applied to preparative electrophoresis in the presence of SDS. The elution profile is shown in Fig. 5. The tracking dye (bromophenol blue) was eluted in the first peak (fractions 10 to 20). The eluted fractions were checked by analytic SDS-PAGE. The 55 kD antigen was found in the second peak (fractions 55 to 70), and fraction 61 to 65 were collected as the purified gp55. As shown in Fig. 3 (*lane c*), the purified antigen produced a single band visualized at 55 kD by CBB staining.

Characterization of the 55 kD antigen

On the basis of the broad band observed on SDS-PAGE, it was anticipated that the 55 kD antigen was a glycosylated protein. In fact, this

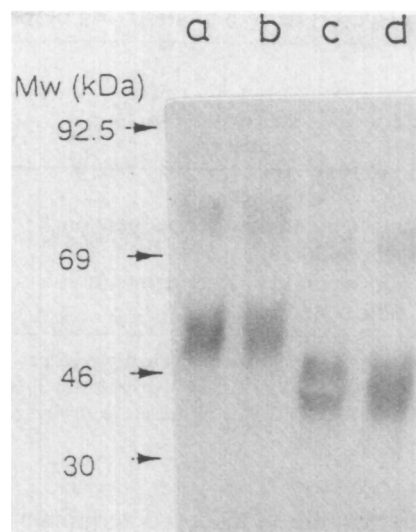


FIG. 6. Endoglycosidase treatment of gp55 antigen. The gp55 antigen was digested with endoglycosidases, separated by SDS-PAGE in 7.5% gels, transferred to nitrocellulose, treated with ABPA serum, and visualized with anti-IgE antibodies and HRP-conjugated third antibodies. *Lane a*, Untreated antigen; *lane b*, treated with *O*-glycanase; *lane c*, treated with *N*-glycanase followed by *O*-glycanase, *lane d*, treated with *N*-glycanase. MW (in kilodaltons) are indicated at left.

protein was stained with periodic acid-Schiff stain and had the ability to bind concanavalin A (data not shown). These findings suggest that this 55 kD protein is glycosylated, and we therefore decided to designate it antigen gp55.

Enzymatic deglycosylation of gp55 was undertaken to confirm the presence of carbohydrates. The gp55 antigen was treated with peptide *N*-glycosidase F (*N*-glycanase) to cleave *N*-linked oligosaccharides between *N*-acetylglucosamine and asparagine residues or endo- α -*N*-acetylglactosaminidase (*O*-glycanase) to hydrolyze *O*-glycosidic bonds between *N*-acetylglactosamine and serine residues. After the enzyme treatment the protein was separated by SDS-PAGE, transferred to nitrocellulose membranes, and stained with the patient serum. As shown in Fig. 6 (*lane d*), *N*-glycanase treatment transformed the 55 kD protein to a 43 kD protein, whereas *O*-glycanase treatment did not reduce the molecular weight of gp55 (Fig. 6, *lane b*). No additive effect of *O*-glycanase to *N*-glycanase treatment was found (Fig. 6, *lane c*). As shown in Fig. 6 (*lanes a and d*), the 55 and 43 kD bands were stained with ABPA serum to a similar extent. These data indicate that the gp55 antigen contains *N*-linked carbohydrate

TABLE II. Hydrolysis of peptide MCAs by proteases present in *A. fumigatus* mycelium extract

Enzyme source	Protease activity (nmol/mg/15 min)*		
	Substrate 1	Substrate 2	Substrate 3
Water-soluble extract	0.052	0.072	1.226
Fraction separated by gel permeation (fractions 36-39)	<0.001	<0.001	<0.001
Fraction separated by gel permeation (fractions 48-51)	0.330	0.500	11.500

*Values are expressed as nanomoles hydrolyzed per 15 minutes per milligram of protein. Peptide MCAs used were as follows: Substrate 1, Boc-Val-Leu-Lys-MCA; substrate 2, Boc-Phe-Ser-Arg-MCA; substrate 3, Suc-Leu-Leu-Val-Tyr-MCA. Hydrolysis of Suc-Ala-Pro-Ala-MCA by proteases in *A. fumigatus* mycelium extract was not detected.

chain but that the antigenic determinant is not located in the N-linked oligosaccharide moiety.

Moreover, sodium metaperiodate treatment of the gp55 antigen did not affect its antigenicity. Protease treatment, on the other hand, completely abolished it. These data clearly indicate that the major epitopes for the IgE antibodies in the patient serum that we used are present in the peptide moiety.

Chemical analysis and N-terminal amino acid sequencing of gp55 antigen

The amino acid and sugar composition of the electrophoretically purified gp55 antigen is shown in Table I. The carbohydrate content was 23.1%. The neutral sugars mannose and galactose were found in a molar ratio of 2.76:1. The amino sugars glucosamine and galactosamine were detected in a molar ratio of 5.68:1.

The gp55 antigen contained covalently bound phosphate groups (0.46%). The phosphate content in the 55 kd antigen was estimated to be 8 mol phosphate per mole of 55 kd antigen.

The N-terminus of the purified gp55 antigen was sequenced. The sequence obtained was ATPHEPVFFSWDAGAVTSFP. No proteins with a homologous N-terminal sequence were found when the SWISS-PROT protein data base (Release 19) was searched. This sequence was different from that of another *A. fumigatus* allergen (*Asp Af1*) reported by Arruda et al.¹¹

Protease activity determination of gp55 antigen

To determine whether gp55 antigen has any protease activity, we tested it with fluorogenic peptide substrates. As shown in Table II, the water-soluble fraction showed strong protease ac-

tivity, whereas the gp55 antigen, which was separated by gel filtration and collected into fractions 36 to 39 as shown in Fig. 3, failed to hydrolyze any fluorogenic peptide substrates. Moreover, almost all protease activity was separated and collected into fractions 48 to 51 by gel filtration. The MW of the protein in this fraction was estimated to be 20 to 30 kd.

These findings suggest that the gp55 antigen was easily separated from the protease activity by gel filtration.

DISCUSSION

The allergic responses to *A. fumigatus* have been shown to be highly diverse with respect to their antigen specificity. The variability of the allergens may be due to differences in the source of the extracts (i.e., culture filtrate,¹²⁻¹⁴ intracellular extracts,^{11, 15-19} or cell walls²⁰⁻²²), the age of the cultures, and the method of extraction. Water-soluble materials would be the most desirable source for antigen preparation to diagnose atopic allergy, if the water-soluble antigens were reactive with sera from most patients with ABPA or other *Aspergillus*-related conditions.

We analyzed the allergens present in the water-soluble extract of *A. fumigatus* with an IgE-specific immunoblotting technique. All ABPA sera were found to be reactive with the gp55 antigen (Fig. 1), whereas the major antigen recognized by the IgG antibodies of ABPA sera by immunoblotting was a 92 kd protein (Fig. 2). The gp55 protein was not only immunodominant (Fig. 1, lane b) but also abundant in the mycelial extracts, comprising approximately 20% of the CBB-stained proteins (Fig. 4, lane b).

Fratamico and Buckley²³ reported a 58 kd protein of *A. fumigatus* recognized by the IgG of

patients with invasive aspergillosis. The serologic activity of their antigen was totally destroyed by treatment with sodium metaperiodate and partially destroyed by treatment with protease. Treatment of gp55 antigen with endoglycosidases (Fig. 6, lanes b to d) and periodate did not eliminate its serologic activity. The gp55 antigen has *N*-glycosidically linked oligosaccharides (Fig. 6, lane d). They speculated that their 58 kd antigen had mannose and galactose residues linked to serine or threonine. Both our own findings and the differences in amino acid composition indicate that the properties of our gp55 antigen are different from those of the 58 kd protein reported by Fratafico and Buckley. However, the two proteins have similar properties (MW and the binding ability to concanavalin A), and thus it is possible that their 58 kd antigen and our gp55 antigen are homologous proteins with strain-to-strain differences.

The gp55 antigen also contained phosphate groups as part of its structure. Lloyd²⁴ reported that the peptide-phosphogalactomannan complex of *Cladosporium werneckii* contains phosphodiester linkages in the main chain of the phosphogalactomannan polymer and between the polymer and the peptide. Therefore the broad band (MW 40 to 46 kd) shown after the *N*-glycanase treatment (Fig. 5, lane b) may be explained by differences in the amount of phosphodiester linkages. Further studies are needed to determine the position and the mode of linkage of phosphate groups in the gp55 antigen.

The N-terminal sequence is different from that of another 18 kd *A. fumigatus* allergen (*asp Af1*) reported by Arruda et al.¹¹ As shown in Fig. 1 (lanes c, e, i, k, r, w, and x), some ABPA sera reacted with an 18 kd antigen. The MW of this protein was similar to that of *Asp Af1* allergen; however, our gp55 is clearly different from the *Asp af1* allergen. The different staining patterns for allergens in our experiment and theirs may be attributable to differences in the fungal strains and/or the sera used.

IgE antibodies to the gp55 antigen were detectable in 100% of ABPA sera (n = 6), atopic respiratory disease sera (n = 5) and aspergilloma sera (n = 2), as shown in Fig. 1. Therefore the gp55 antigen should be useful in developing a diagnostic allergosorbent assay for *A. fumigatus*-induced allergic disorders.

An allergenic protein from *A. fumigatus* was shown to possess protease activity.^{18, 22} However, gp55 did not hydrolyze any fluorogenic peptide

substrates of proteases. At present neither the biologic nature nor the function of the gp55 antigen or other antigens is known. Further studies are needed to clarify whether this antigen has any biologic activity.

REFERENCES

- Anderson CJ, Craig S, Bardana EJ Jr. Allergic bronchopulmonary aspergillosis and bilateral fungal balls terminating in disseminated aspergillosis. *J ALLERGY CLIN IMMUNOL* 1980;65:140-4.
- Rinaldi MG. Invasive aspergillosis. *Rev Infect Dis* 1985; 5:1061-77.
- Bardana EJ Jr. The clinical spectrum of aspergillosis. Part 2. Classification and description of saprophytic, allergic, and invasive variants of human disease. *CRC Crit Rev Clin Lab Sci* 1980;13:85-159.
- Reijula KE, Kurup VP, Fink JN. Ultrastructural demonstration of specific IgG and IgE antibodies binding to *Aspergillus fumigatus* from patients with aspergillosis. *J ALLERGY CLIN IMMUNOL* 1991;87:683-8.
- Mackenzie DWR, Hearn VM. Serodiagnosis of aspergillosis. *Jpn J Med Mycol* 1991;32:95-101.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680-5.
- Zacharius RM, Zell TE, Morrison JM, Woodlock JJ. Glycoprotein staining following electrophoresis on acrylamide gels. *Anal Biochem* 1969;30:148-52.
- Hewick RM, Hunkapiler MW, Hood LE, Dreyer WJ. A gas-liquid solid phase peptide and protein sequenator. *J Biol Chem* 1981;256:7990-7.
- Kato H, Adachi N, Ohno Y, Iwanaga S, Takada K, Sakakibara S. New fluorogenic peptide substrates for plasmin. *J Biochem* 1980;88:183-90.
- Ames BN. Assay of inorganic phosphate, total phosphate and phosphatases. *Methods Enzymol* 1966;8:115-7.
- Arruda LK, Platts-Mills TAE, Fox JW, Chapman MD. *Aspergillus fumigatus* allergen I, a major IgE-binding protein, is a member of the mitogillin family of cytotoxins. *J Exp Med* 1990;172:1529-32.
- Calvanico NJ, Du Pont BL, Huang CJ, Patterson R, Fink JN, Kurup VP. Antigens of *Aspergillus fumigatus* I. Purification of a cytoplasmic antigen reactive with sera of patients with *Aspergillus*-related disease. *Clin Exp Immunol* 1981;45:662-71.
- Kurup VP, Ting EY, Fink JN. Immunochemical characterization of *Aspergillus fumigatus* antigens. *Infect Immun* 1983;41:698-701.
- Kurup VP, Resnick MS, Scribner GH, Gunasekaran M, Fink JN. Enzyme profile and immunochemical characterization of *Aspergillus fumigatus* antigens. *J ALLERGY CLIN IMMUNOL* 1986;78:1166-73.
- Kim SJ, Chaparas SD, Brown M, Anderson MC. Characterization of antigens from *Aspergillus fumigatus* II. Fractionation and electrophoretic, immunologic, and biologic activity. *Am Rev Respir Dis* 1978;118:553-60.
- Kim SJ, Chaparas SD. Characterization of antigens from *Aspergillus fumigatus*. I. Preparation of antigens from organisms grown in completely synthetic medium. *Am Rev Respir Dis* 1978;118:547-51.
- Harvey C, Longbottom JL. Characterization of a major

- antigenic component of *Aspergillus fumigatus*. Clin Exp Immunol 1986;65:206-14.
18. Harvey C, Longbottom JL. Characterization of a second major antigen Ag13 (antigen C) of *Aspergillus fumigatus* and investigation of its immunological reactivity. Clin Exp Immunol 1987;70:247-54.
 19. Schønheyder H, Storgaard L, Anderson P. Variation of a 470,000 dalton antigen complex and catalase antigen in clinical isolates of *Aspergillus fumigatus*. Sabouraudia 1985; 23:339-48.
 20. Hearn VM, Mackenzie DWR. The preparation and chemical composition of fractions from *Aspergillus fumigatus* wall and protoplasts possessing antigenic activity. J Gen Microbiol 1979;112:35-44.
 21. Hearn VM, Proctor AG, Mackenzie DWR. The preparation and partial characterization of antigenic fractions obtained from the mycelial walls of several *Aspergillus* species. J Gen Microbiol 1980;119:41-9.
 22. Piechura JE, Kurup VP, Daft LJ. Isolation and immunochemical characterization of fractions from membranes of *Aspergillus fumigatus* with protease activity. Can J Microbiol 1990;36:33-41.
 23. Fratamico PM, Buckley HR. Identification and characterization of an immunodominant 58-kilodalton antigen of *Aspergillus fumigatus* recognized by sera of patients with invasive aspergillosis. Infect Immun 1991;59:309-15.
 24. Lloyd KO. Molecular organization of a covalent peptide-phospho-polysaccharide complex from the yeast form of *Cladosporium wernneckii*. Biochemistry 1972;21:3884-90.
 25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
 26. Dubois M, Gilles KA, Hamilton JK, Rebers DA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956;28:350-6.
 27. Elson LA, Morgan WTL. Colorimetric method for the determination of glucosamine and chondrosamine. Biochem J 1933;27:1824-8.

Bound volumes available to subscribers

Bound volumes of THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY are available to subscribers (only) for the 1993 issues from the Publisher, at a cost of \$60.00 for domestic, \$82.20 for Canadian, and \$78.00 for international subscribers for Vol. 91 (January-June) and Vol. 92 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby, Subscription Services, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318; phone 1 (800) 453-4351 or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.