The Preparation and Chemical Composition of Fractions from *Aspergillus fumigatus* Wall and Protoplasts Possessing Antigenic Activity

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A detergent-soluble fraction was prepared from the fragmented wall of *Aspergillus fumigatus* mycelium using the non-ionic detergent Triton X-100, and a wall-free extract was prepared from the same source in the form of protoplasts, released by a lytic enzyme system from *Trichoderma harzianum*. These extracts were examined by polyacrylamide gel electrophoresis and their detailed chemical composition was established. They were compared with the water-soluble fraction prepared from total mycelium, which is used routinely in this laboratory for serological tests. All fractions had immunological reactivity towards an antiserum prepared in rabbits against this water-soluble fraction of the mycelium, as shown by double diffusion. Both protein and carbohydrate moieties appear to be involved in the antigenic sites, with carbohydrate reactivity predominantly associated with the protoplast fraction. The fact that all preparations contained at least one common antigenic determinant, as judged by lines of identity to a single antiserum, is discussed in relation to antigen location.

INTRODUCTION

The importance of serological techniques in the diagnosis of all forms of human aspergillosis is well recognized (Biguet *et al.*, 1964; Longbottom *et al.*, 1964; Drouhet *et al.*, 1963). *Aspergillus fumigatus* is the fungal species most frequently implicated in the two common forms of aspergillosis, namely allergic aspergillosis and aspergilloma. The presence of precipitating antibodies in the sera of patients has generally been detected with antigens prepared from culture filtrates (Longbottom & Pepys, 1964; Dee, 1975; Coleman & Kaufman, 1972) or mycelial extracts (Tran van Ky *et al.*, 1968; Wada, 1960; Proctor, 1976) of the organism. Only in recent years has any success been reported on linking the chemical structure of these antigens with immunological activity (Azuma *et al.*, 1965, 1968, 1971; Suzuki *et al.*, 1966; Suzuki & Hayashi, 1975; Memon & Atkinson, 1977). Little is known about their localization in the organism nor has their part in eliciting an immunological response been defined. This study was undertaken to isolate antigenic components from specific fractions of the mycelium of *A. fumigatus*, namely, a fraction prepared from wall material and a fraction prepared from sub-cellular material. Our aim was to establish to what extent antigens exist in the cell-sap and whether they differ from surface antigens either in their active sites, in their overall chemical structure or in their role in triggering an immune response.

METHODS

Preparation of mycelial wall. Preparation of 3 d-old *A. fumigatus* (NCPF2109) was as described previously (Proctor, 1976). The mycelial mat was separated from the broth by filtration and stored at -20°C overnight or until required. The mycelium was then suspended in 0.05 M- NH_4HCO_3 at pH 8.0 and macerated in an MSE 'Atomix' at top speed for 1 min. The slurry was transferred as a 30% (w/v) suspension in the same medium to the precooled chamber of a 'Dynomill' cell disintegrator (Glen Creston, London) primed with Ballotini beads. Cell rupture was accomplished in 1 to 2 min at a specimen flow rate of 10 m s^{-1} . The chamber was maintained at 4°C throughout the operation. Walls were separated from cytoplasmic debris by centrifuging at 450 g and washed in hot (50 to 60°C) distilled water about 7 times until the supernatant was clear and colourless. This procedure gave wall material essentially free of attached cytoplasm, as detected by light microscopy and lactophenol cotton blue staining. It was stored at -20°C until required.

Extraction of walls with detergent (TE fraction). Routinely, 10 g (wet wt) wall pellet was suspended in 100 ml 0.05 M- NH_4HCO_3 at pH 8.0 containing 0.5% (v/v) Triton X-100 (scintillation grade from Koch-Light) and extracted with stirring for 2 h at 30°C . After centrifuging, the pellet was re-extracted under the same conditions with 50 ml bicarbonate/detergent solution and the two supernatant fractions were pooled and used as a source of wall-localized antigens. For easier handling, the Triton extracts (TE) were usually concentrated 10-fold against 10% (w/v) polyethylene glycol 6000 in 0.05 M- NH_4HCO_3 and stored at -20°C .

Preparation of dried walls. Before use in assay work, the walls were heated in a water bath at 100°C for 5 min and then dried to constant weight over P_2O_5 and stored desiccated at -20°C .

Preparation of water-soluble antigens (WS fraction). This fraction was prepared according to Proctor (1976). A number of different extracts were used which were standardized according to protein concentration.

Preparation of protoplasts (PP fraction). *Trichoderma harzianum* was a gift from Dr J. F. Peberdy of Nottingham University; the strain was originally derived from the Centraalbureau voor Schimmelcultures, Baarn (CBS 354.33). It was grown on the liquid medium recommended by Peberdy & Isaac (1976), to obtain a lytic enzyme system, but with the substitution of Rigolata's trace element solution in place of Vogel's and of 3 d-old *A. fumigatus* mycelium at 10% (wet wt/v) in the medium in place of the mixture of chitin and laminaria meal. The growth of *A. fumigatus* and the incubation with *T. harzianum* for the production of protoplasts were also carried out according to Peberdy's method. The stabilizing buffer used was 0.6 M-KCl in 0.1 M-phosphate at pH 6.0. In this system 4 to 5 g (wet wt) mycelium gave yields of about 5×10^8 protoplasts. Fresh preparations were checked by staining with 1% Tinopal 4BMT (CIBA-Geigy) in stabilizing buffer and examination by ultraviolet microscopy. No fluorescence of protoplasts was seen, indicating the absence of wall polysaccharides. Electron microscopic examination of thin sections of protoplasts confirmed that the plasmalemma was entirely free of wall material (unpublished results). Rupture of protoplasts for analysis was achieved by freezing and thawing and by dilution of the stabilizing buffer (PP fraction). After washing the protoplasts were harvested as a pellet and, if not required immediately, could be stored at -20°C without apparent loss of immunological activity.

Production of antiserum. Antiserum was obtained by the hyper-immunization of sheep or rabbit with the water-soluble fraction of 3 d-old *A. fumigatus* mycelium, prepared as described above. This fraction (at 60 mg ml^{-1}) was mixed with an equal volume of Freund's incomplete adjuvant and 1 ml was inoculated subcutaneously at weekly intervals for 2 to 3 weeks.

Double diffusion. Antigenic preparations were used at the following protein concentrations: water-soluble fraction (WS) at approximately 5 mg ml^{-1} ; Triton-soluble fraction (TE) at 1 or 2 mg ml^{-1} ; and protoplast preparation (PP) at 2.5 mg ml^{-1} (based on total protein content: what proportion of this is water-soluble was not determined). In the Ouchterlony procedure, double diffusion was done in a 1% (w/v) purified agar (Oxoid) in borate buffer at pH 8.2 containing 0.5% (w/v) EDTA, as used routinely in the Mycological Reference Laboratory. The test employed 12 ml agar in a plastic Petri dish (9 cm diam.) and a pattern of wells of 10 mm diameter with edge-to-edge distances of 4 mm (Fletcher *et al.*, 1970); $75\text{ }\mu\text{l}$ was applied to each well. When antigens were pretreated, either chemically or enzymically, thicker gels were poured to enable larger volumes to be applied in deeper wells. With the WS and TE fractions, either sheep or rabbit antiserum was used undiluted; with the PP fraction, the best results were generally obtained with a rabbit antiserum used undiluted. A 3 d development period was allowed and, after washing and fixing, the plates were stained with Coomassie Brilliant Blue R, C.I. no. 42660.

Polyacrylamide gel electrophoresis. This was done essentially according to Lund (1965). Samples were mixed 2:1 (v/v) with glycerol and layered directly into tubes containing 3.75% (w/v) stacking gel and 7.5% separating gel. The upper buffer was 0.04 M-Tris/glycine, pH 8.9, and the lower buffer was 0.12 M-Tris/HCl, pH 8.1. At the end of the separation the gels were fixed in 10% (w/v) trichloroacetic acid and stained with either Coomassie Blue for protein or with periodate-Schiff's reagent (PAS) for glycoprotein (McGuckin & McKenzie, 1958). An identical pattern was obtained with the TE fraction irrespective of whether Triton was

present or absent during electrophoresis and so the detergent was routinely omitted from the system. The protein load was 0.1 to 0.2 mg for the TE fraction, approximately 0.5 mg for the WS fraction, and 0.75 to 1 mg for the PP fraction.

Hydrolysis of fractions before carbohydrate analysis. Material to be analysed for sugar content was hydrolysed using 1 M-HCl at 100 °C for 4 h at a dry weight concentration of 10 mg ml⁻¹. These conditions gave optimum release of neutral sugars and hexosamines in our system. Other hydrolysis conditions which were investigated included 4 and 6 M-HCl for various periods as well as hydrolysis with H₂SO₄ according to Bartnicki-Garcia (1966). The HCl was either removed by evaporation to dryness *in vacuo* at room temperature or neutralized by the addition of the appropriate amount of sodium hydroxide.

Paper chromatography. Individual monosaccharides were characterized in acid hydrolysates by descending paper chromatography at room temperature on Whatman no. 1 paper in the 1-butanol/pyridine/water (6:4:3, by vol.) solvent system. The spots were visualized with the alkaline silver nitrate reagent of Trevelyan *et al.* (1950).

Gas-liquid chromatography. The results of paper chromatography were confirmed by analytical gas-liquid chromatography, kindly performed by Mr R. A. Faulkes (National Institute for Medical Research, Mill Hill, London) according to the method of Clamp (1974). Cleavage of the polymeric material was achieved by methanolysis, followed by conversion of the methyl glycosides to *O*-trimethylsilyl ethers.

Chemical analysis. Total neutral reducing sugars were estimated according to Dubois *et al.* (1956). Glucose was measured either by the glucose oxidase/peroxidase system or by the hexokinase/glucose-6-phosphate dehydrogenase system (both Sigma kits), while galactose was estimated specifically using the galactose dehydrogenase method (Boehringer kit). Standards were subjected to the same chemical procedure for comparative purposes. Mannose, the only other neutral sugar present, was calculated by difference. The procedure of Lee & Montgomery (1961) was followed for the analysis of hexosamines as neutral sugars after deamination. Total reducing sugars were determined by the method of Somogyi (1952). 2-Keto-3-deoxyoctonic acid and dideoxy sugars were analysed according to Aminoff (1961). Protein was measured with the Folin-Ciocalteu reagent with bovine serum albumin as a standard (Lowry *et al.*, 1951). *N*-Acetylhexosamines were determined according to Reissig *et al.* (1955). Total phosphorus was estimated following digestion for 1 h at 200 °C by the method of Bartlett (1959). Total lipid was analysed colorimetrically by the sulphophosphovanillin reaction of Zöllner & Kirsch (1962) using the Boehringer test-combination kit. Detergent interferes with this reaction and therefore the lipid content of the TE fraction was determined by measuring the dry weight of the chloroform/methanol extract (after prior removal of Triton). Nucleic acid in the WS fraction was determined using the A_{230}/A_{260} ratio; otherwise it was extracted with perchloric acid according to Marriott (1975) and ribose was measured with the phloroglucinol reagent (Dische & Borenfreund, 1957), while deoxyribose was estimated with the diphenylamine reagent (Burton, 1955). Ash was determined by combustion at 900 to 1000 °C in an electrically heated muffle furnace for 3 h. Dry weight was estimated by heating overnight at 110 °C and cooling to constant weight over P₂O₅.

Concanavalin A treatment. Concanavalin A (Con A; Calbiochem) was incubated at 0.75 to 2.5 mg ml⁻¹ with antigenic fractions containing different amounts of carbohydrate for 18 h at 20 °C in the presence of phosphate buffer (pH 7.2), according to So & Goldstein (1967). The effect of Con A on the precipitin activity of the various fractions was tested in plates similar to those used for routine double diffusion. Con A controls and antigenic preparations incubated in the absence of lectin were included.

Sodium periodate treatment. Two volumes of the test fraction, at various carbohydrate concentrations, were held with one volume of 0.15 M-NaIO₄ for 24 h at room temperature (approx. 20 °C) in the dark. Controls, in which saline was substituted for periodate, were treated similarly.

Treatment with enzymes. A range of enzymes were tested for their effect on the precipitin activity (as measured by double diffusion) of the various *A. fumigatus* antigenic fractions. These included α -amylase, β -amylase, cellulase, pronase and snail gut juice ex *Helix pomatia*, all from Koch-Light; β -galactosidase, maltase, *Streptomyces griseus* protease and papain, all from Sigma; lytic enzyme L₁ from Cytophaga and trypsin, both from BDH. Amylo-1,4- α -1,6- α -glucosidase was purchased as a 1% (w/v) suspension from Boehringer. An enzyme with α -mannosidase activity, capable of hydrolysing yeast mannan, was purified from jack bean meal according to the method of Li (1966). Crude culture filtrate from *Trichoderma harzianum* [prepared using chitin (Koch-Light) and laminaria stipe (Alginate Industries, Ayrshire) instead of *A. fumigatus* mycelium in the growth medium], which is capable of hydrolysing a wide range of polysaccharides in addition to protease activity, was also tested.

Most of the commercial enzymes were used at a final concentration of 3% (w/v) with WS, TE and PP antigen preparations; the exception was amyloglucosidase which was used at 0.5% (w/v). In each case the appropriate buffer and temperature were selected for the incubation mixture which contained either 2.5 mg WS protein or 2.5 mg PP protein or 0.5 mg TE protein. Antibiotics (chloramphenicol, 12.5 μ g, and cycloheximide, 25 μ g) were included and hydrolysis was allowed to proceed for 18 to 30 h. Carbohydrases were

Fig. 1

Fig. 2

Fig. 1. Polyacrylamide gel electrophoresis of fractions from *A. fumigatus* mycelium stained for protein and carbohydrate. A, WS fraction from fragmented total mycelium, 0.65 mg protein; B, PP fraction from protoplasts, 0.90 mg protein; C, TE fraction from mycelial walls, 0.325 mg protein. 1, Gels stained with Coomassie Blue; 2, gels stained with periodate-Schiff's reagent.

Fig. 2. Polyacrylamide gel electrophoresis of fractions from *A. fumigatus* mycelium, at higher concentrations than in Fig. 1. A, WS fraction from fragmented total mycelium, 0.82 mg protein; B, PP fraction from protoplasts, 1.6 mg protein; C, TE fraction from mycelial walls, 0.65 mg protein. All gels stained with Coomassie Blue.

added in a single aliquot at zero time, proteases were added in three aliquots during a 30 h period. The α -mannosidase from jack bean meal was tested using similar substrate concentrations at a final enzyme protein concentration of 0.5 % (w/v). Crude *T. harzianum* was used in 0.01 M-citrate, pH 4.5, at a final protein concentration of 0.1 % (w/v). Control samples were held under the same conditions in the absence of either substrate or enzyme. Reactions were stopped by freezing the test mixtures. In addition to monitoring the immunological activity of the enzyme-treated antigenic preparations, chemical analysis of the products was carried out to determine release of reducing sugars, specifically glucose. The enzymes used in this experiment included β -amylase, lytic enzyme from *Cytophaga*, crude *T. harzianum* culture filtrate and an ammonium sulphate fraction prepared from the culture filtrate of *Streptomyces violaceus* (Chattaway *et al.*, 1976), rich in α -mannosidase activity and used at a final protein concentration of 0.25 % (w/v).

RESULTS

Polyacrylamide gel electrophoresis

The components of the water-soluble (WS) fraction of total mycelial extract, the Triton-soluble (TE) fraction prepared from isolated mycelial wall and the water-soluble (PP) fraction from protoplasts (released by freezing and thawing) were each separated by gel electrophoresis (Fig. 1). With the WS fraction approximately eight bands were seen on staining with Coomassie Blue. When the same extract was stained with PAS, the bands visualized showed similar electrophoretic mobilities to those stained with Coomassie Blue. Some minor bands were not seen with PAS, probably because this reagent is less sensitive than Coomassie Blue. Results with the PP extract indicated that those components which stained with Coomassie Blue also stained with PAS; evidence of identity of these components

Fig. 3. Precipitin patterns obtained on double diffusion with fractions from *A. fumigatus*.

(a) Immunological identity of components prepared from wall and cytoplasmic sources. The centre well contained sheep antiserum, undiluted. Other wells contained: A, WS fraction (batch 18.2), 0.6 mg protein; B, PP fraction (batch 1), 0.2 mg protein; C, TE fraction, 0.06 mg protein; D, duplicate of C; E, duplicate of A; F, PP fraction, 0.05 mg protein.

(b) Additional component visible in the TE fraction at higher concentration and its immunological identity with a water-soluble component. The centre well contained sheep antiserum, undiluted. Other wells contained: A, TE fraction, 0.15 mg protein; B, duplicate of A; C, WS fraction (batch 4.11), 0.6 mg protein; D, TE fraction, 0.06 mg protein; E, duplicate of D; F, TE fraction, 0.15 mg protein.

was based on identical R_F values. The gel obtained with the TE fraction had a somewhat similar appearance to that of the PP fraction; the fast-moving components which stained with both Coomassie Blue and PAS had identical R_F values. However, the slow-moving bands had different mobilities.

With higher protein loads a somewhat different pattern was seen (Fig. 2). As many as 12 components were apparent in the WS and PP fractions, two components of the latter being too large to penetrate the separating gel. Approximately five bands were seen in a TE preparation.

Demonstration of precipitins by double diffusion

The WS, PP and TE preparations all showed one or more arcs of precipitation when allowed to diffuse against an antiserum raised to the WS fraction (Fig. 3a, b). The results indicate the presence of a line(s) of identity between the three antigenic preparations and this antiserum.

Qualitative sugar analysis

Neutral and amino sugars present in the different extracts were identified by paper and gas-liquid chromatography. Mannose, galactose, glucose, galactosamine and glucosamine were found in all samples tested; in addition, the protoplast preparation contained ribose (as RNA) and unextracted wall material contained trace amounts of dideoxy sugars. Mannose was the dominant neutral sugar in all fractions except unextracted wall where galactose was the major component. The highest levels of glucose were found in the PP fraction. The ratio of neutral sugar to hexosamine varied from fraction to fraction being highest in the PP and WS preparations and lowest in the wall fractions. Although glucosamine and galactosamine were both present in all fractions, glucosamine was found preferentially in the TE preparation, and galactosamine was the major hexosamine released from unextracted mycelial wall under the conditions used for hydrolysis.

Table 1. *Percentage composition of Aspergillus fumigatus fractions*

Component	Soluble fractions*		Insoluble fractions*	
	WS	TE	PP	Total wall
Hexosamine	4.4 ± 3	13.1 ± 5	2.7 ± 1	22.0 ± 6
Neutral sugar	22.5 ± 8	24.5 ± 6	10.9 ± 5	58.0 ± 10
Glucose	2.1 ± 0.5	3.8 ± 2	3.5 ± 1	17.0 ± 3
Galactose	8.6 ± 4	8.4 ± 3	1.8 ± 0.1	30.0 ± 5
Mannose	11.8	12.3	4.0	11.0
Ribose	ND	ND	1.6 ± 1	U
Protein	51.2 ± 15	37.3 ± 12	26.9 ± 10	10.0 ± 3
Total lipid	1.4 ± 0.5	8.0 ± 2	5.2 ± 2	7.2 ± 0.5
Total P	1.5 ± 0.4	2.8 ± 0.3	0.6 ± 0.1	0.4 ± 0.1
DNA†			0.6 ± 0.3	
RNA†	6.4 ± 0	ND	9.0 ± 3	U
Ash	8.8 ± 2	U	29.5 ± 8	U

ND, Not done; U, undetected.

* All results are based on at least three experiments; standard deviations are given.

† The nucleic acid of the WS fraction was determined using the A_{280}/A_{260} ratio; in the PP fraction, DNA and RNA were determined individually, as described in Methods.

Chemical composition

The detailed chemical composition of the different fractions is shown in Table 1. In the wall, polysaccharides constituted the major components, while in the TE fraction, the protein to carbohydrate ratio was 1:1. For the WS and PP preparations, the ratio of protein to carbohydrate was 2:1. The wall fractions contained a high proportion of hexosamine, relative to the total sugar present, whereas the hexosamine content in the WS and PP preparations was only about 20% of the total carbohydrate.

Significant amounts of lipid were found in the unextracted wall as well as in the TE and PP samples, but it was not further characterized. The DNA content of the PP antigen preparation was very low but an appreciable quantity of RNA was present. Unextracted wall showed no evidence of nucleic acid, and this was taken as a criterion of purity of the preparation. The high ash content of the PP fraction was due to the presence of concentrated salts from the osmotically stabilizing buffer.

Effect of Con A on immunological activity

Addition of Con A at concentrations as low as 0.75 mg ml⁻¹ to the WS fraction at a carbohydrate content of 2.6 mg ml⁻¹ gave appreciable precipitation on standing at 20 °C. On subsequent analysis by double diffusion against sheep antiserum, the number of precipitin arcs was decreased from three in the control antigen without Con A to one in the test sample (Fig. 4a). Similarly when Con A was incubated with the TE fraction of mycelial wall (at a carbohydrate concentration of 1.3 mg ml⁻¹) visible precipitation in the test tube was accompanied by loss of the single precipitin band seen in the control on double diffusion (Fig. 4b). When PP antigens of comparable carbohydrate content were incubated with Con A at up to 2.5 mg ml⁻¹, neither precipitation nor loss of immunological activity was detected.

Effect of periodate on immunological activity

Sodium periodate, when used under the conditions stated in Methods, had no effect on the immunological activity of the WS and TE fractions, as judged by double diffusion against a sheep antiserum. The extracts were used at a carbohydrate content of about 200 to 300 µg. However, when a PP preparation containing approximately 500 µg carbohydrate was treated in the same way, one of the two precipitin arcs disappeared (Fig. 4c).

Fig. 4. Effect of Con A on double diffusion patterns obtained with *A. fumigatus* fractions and the effect of NaIO_4 on the PP fraction.

(a) Abolition of precipitin activity of at least two components of the WS fraction on treatment with Con A. The centre well contained sheep antiserum, undiluted. Other wells contained: A, WS fraction (batch 18.2), control; B, WS fraction incubated with Con A; C, WS fraction, control; D, Con A only; two empty wells.

(b) Comparison of the TE and WS fractions on treatment with Con A. The centre well contained sheep antiserum, undiluted. Other wells contained: A, WS fraction (batch 5.10), control; B, WS fraction incubated with Con A; C, Triton-soluble fraction from total mycelium, control; D, Triton-soluble fraction from total mycelium incubated with Con A; E, TE fraction from mycelial wall, control; F, TE fraction from mycelial wall incubated with Con A.

(c) Effect of incubation with either Con A or NaIO_4 on the immunological activity of the PP fraction. The centre well contained rabbit antiserum, undiluted. Other wells contained: A, PP fraction (batch 2) incubated with 0.15 M- NaIO_4 ; B, PP fraction incubated with 0.03 M- NaIO_4 ; C, PP fraction stored at -20°C , control; D, PP fraction incubated with 100 μg Con A; E, PP fraction incubated with 500 μg Con A; F, PP fraction, control, incubated without NaIO_4 —retention of the PP fraction at 20°C for 18 h caused considerable loss of immunological activity.

Fig. 5. Effect of hydrolytic enzymes on the antigenic fractions of *A. fumigatus*. Each antigenic mixture was incubated with each enzyme before determining the number of precipitin arcs formed in double diffusion tests. Details of antigen and enzyme concentrations are given in Methods. The control contained no enzyme.

Effect of enzymes on immunological activity

Under the conditions of the experiment, none of the carbohydrases abolished the immunological activity of any of the antigenic fractions, as evidenced by similar precipitin patterns with test and control samples. Crude enzyme preparations, i.e. snail gut juice, Cytophaga lytic enzyme and *T. harzianum* culture filtrate, likewise had no apparent effect. Of the

Table 2. *Release of sugars from antigenic preparations of Aspergillus fumigatus by hydrolytic enzymes*

Conditions of incubation were as described in Methods. Percentage yields are based on an initial sugar content of 2.2 mg for the WS and PP preparations and 1.0 mg for the TE preparation. Figures in parentheses show glucose levels as a percentage of the total sugar released.

Fraction	<i>T. harzianum</i> culture filtrate	α -Mannosidase ex <i>S. violaceus</i>	Lytic enzyme L ₁ ex <i>Cytophaga</i>	β -Amylase
WS	20.0 (8.0)	2.0 (0.2)	5.7	10.0 (11.0)
PP	13.4 (10.3)	6.2 (ND)	9.8 (10.0)	10.9 (11.3)
TE	U	12.6 (ND)	39.5	U

ND, Not done; U, undetected.

proteases, trypsin appeared to be ineffective while papain destroyed at least one antigenic component. The proteases which proved most effective were *S. griseus* protease and pronase, which, at the concentrations used, appeared to abolish all immunological activity in the WS and TE fractions. With the PP fraction, one component was unaffected by either pronase or protease. A diagrammatic representation of these results is shown in Fig. 5.

Enzymic release of sugars

Although immunological activity was unaffected by the action of carbohydrases, they released considerable quantities of reducing sugars from the WS, PP and TE preparations (Table 2).

DISCUSSION

Chemical analysis showed that the same five major sugar constituents were present in different fractions of the hyphal mycelium of *A. fumigatus*, though the relative proportions of these sugars varied. The high levels of galactose and mannose, especially in the soluble fractions, are indicative of the presence of galactomannans; these are reported to be a common constituent of many fungi, including the aspergilli. A high proportion of the carbohydrate appears to exist as glycoprotein, as judged by selective staining on polyacrylamide gel electrophoresis, which also revealed the presence of macromolecules which differed in number and mobilities in the various extracts.

All extracts appeared to contain at least one common precipitating antigen, as evidenced by a line of apparent identity on double diffusion against an antiserum prepared from macerated mycelium. This could be accounted for by a soluble antigen in the wall which is also present in protoplasts.

Differences among precipitating antigens prepared from different sources were established after chemical and enzymic treatment. The precipitin reactivity of the WS and TE fractions was abolished by proteolytic enzymes, whereas periodate oxidation and polysaccharase treatment, though removing considerable quantities of reducing sugars, were ineffective. Concanavalin A caused aggregation of these immunologically active substances with resultant loss of precipitin arcs on double diffusion. It is therefore concluded that some of these antigens are glycoprotein in nature with protein moieties as their active sites. Only in the WS fraction was one component still visible on double diffusion after Con A treatment; this may be a protein antigen (cf. Azuma *et al.*, 1965; Memon & Atkinson, 1977).

With the PP fraction, one of two antigenic components was lost by periodate oxidation; this suggests that at least one antigen has carbohydrate at its active site. The nature of the sugar residues involved is uncertain, but it is of interest that Con A, which binds to α -linked sugar residues such as D-glucose and D-mannose, has no detectable effect in this system. The activity of the other component of the PP fraction was destroyed by proteolytic hydrolysis and so it may be protein rather than glycoprotein in nature.

The results indicate that there are common antigenic components which exist both in the

wall and the sub-cellular material. In addition, extracts from the wall and protoplasts contain antigens which differ both in immunological reactivity and in chemical structure. These differences may perhaps be linked to cellular location and reflect incomplete structures in the process of biosynthesis and transfer to wall sites where they become immobilized. It is also possible that cell fractionation has achieved the separation of different antigens whose immunological role remains to be determined.

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