Cell surface properties of *Aspergillus fumigatus* conidia: correlation between adherence, agglutination, and rearrangements of the cell wall

G. Tronchin, J.P. Bouchara, M. Ferron, G. Larcher, and D. Chabasse

Abstract: Culture conditions that lead to swelling and germination dramatically influence cell surface characteristics and properties of *Aspergillus fumigatus* conidia. Conidial adherence to polystyrene and agglutination markedly increased during swelling, in a time-dependent manner. Agglutination appeared to be sensitive to cycloheximide and calcium. Removal of cell wall polysaccharides by lyticase or sodium metaperiodate suppressed agglutination of conidia. Proteinase K weakly decreased it whereas dithiothreitol strongly dispersed the cells. These observations suggest that both cell surface carbohydrates and proteins are involved in the agglutination process. Electron microscopic observations demonstrated that the cell wall of conidia was subject to some rearrangements during swelling, involving degradation and loss of the external convoluted layer, and subsequent exposure of underlying ligands. This was confirmed using lectins labelled with gold or fluorescein isothiocyanate, which showed that some carbohydrates, particularly those acting as ligands for peanut agglutinin, are largely exposed during the process. Finally, SDS-PAGE revealed major protein changes between resting and swollen conidia. We conclude that the ability of *A. fumigatus* conidia to aggregate correlates with an increase in adherence and biochemical reorganization of the cell wall.

Key words : *Aspergillus fumigatus*, adherence, agglutination, cell wall rearrangements.

Résumé : Les conditions de culture qui conduisent au gonflement et à la germination influencent considérablement les caractéristiques de surface et les propriétés des conidies d'*Aspergillus fumigatus*. L'adhérence des conidies au polystyrène, ainsi que leur agglutination, augmentent nettement au cours du gonflement, en fonction du temps. L'agglutination apparaît sensible au cycloheximide et au calcium. L'élimination des polysaccharides pariétaux par la lyticase ou le métapériodate de sodium supprime l'agglutination des conidies. La protéinase K la réduit légèrement, tandis que le dithiotréitol disperse fortement les cellules. Ces observations suggèrent que des hydrates de carbone et des protéines de la surface cellulaire sont impliqués dans le processus d'agglutination. Les observations en microscopie électronique démontrent que la paroi des conidies subit au cours du gonflement des réarrangements impliquant la dégradation et la perte de la couche échinulée, et par conséquent la présentation de ligands sous-jacents. Ces observations sont confirmées par l'utilisation de lectines couplées à l'isothiocyanate de fluorescéine ou à des particules d'or qui montrent que certains hydrates de carbone, plus particulièrement ceux qui agissent comme ligands pour l'agglutinine d'arachide, sont largement exposés au cours du processus. Finalement, l'électrophorèse en gel de polyacrylamide révèle des changements protéiques majeurs entre les spores dormantes et les spores gonflées. En conclusion, la capacité des conidies d'*A. fumigatus* à s'agglutiner est corrélée avec un accroissement de leur capacité d'adhérence et une réorganisation biochimique de leur paroi.

Mots clés : *Aspergillus fumigatus*, adhérence, agglutination, réarrangements pariétaux.

Received December 6, 1994. Revision received April 27, 1995. Accepted April 27, 1995.

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Introduction

Aspergillus fumigatus, a ubiquitous and opportunistic fungus, can cause a wide spectrum of diseases in humans, ranging from localized (aspergilloma, bronchopulmonary aspergillosis) to invasive aspergillosis (Rinaldi 1983; Bodey and Vartivarian 1989). This respiratory pathogen mainly infects immunocompromised hosts and an increased incidence of pulmonary aspergillosis has been noted recently in patients with the acquired immunodeficiency syndrome (AIDS) (Denning et al. 1991).

The molecular mechanisms by which this fungus establishes infections are not well understood. Expression at the surface of microorganisms of specific receptors for some host ligands is a prerequisite for interaction with the host cells. This results in the adherence of microorganisms to mucosal surfaces, which is now considered to be a crucial step in the colonization of tissues. In the last few years, particular attention has been focused in our laboratory on proteins and glycoproteins that could act as ligands for *A. fumigatus* conidia at the host cell surface. Among these, fibrinogen, which plays a key role in inflammatory reactions, and laminin, the major noncollagenous basement membrane protein, have been shown to promote adherence of *A. fumigatus* in vitro (Tronchin et al. 1993*b*; Coulot et al. 1994).

Agglutination of microorganisms is a common and specialized form of cell adhesion, which can be observed among a wide range of eukaryotic and prokaryotic cells such as bacteria, filamentous fungi, algae, and yeasts (Straver et al. 1993*b*; Barki et al. 1993, 1994; Kellens et al. 1994). For example, the ability of yeasts to flocculate is of considerable interest in the brewing industry. This phenomenon is due to specific and nonspecific cell–cell interactions. Recently, several authors have pointed to a role for both cell surface hydrophobicity and a calcium-dependent lectin–sugar interaction in flocculation of yeasts (Stratford et al. 1988; Stratford and Carter 1993; Stratford 1992; Straver et al. 1993*a*). The cell surface sugar-binding proteins of one cell adhere to specific carbohydrate residues on neighbouring cell walls. In *Saccharomyces cerevisiae*, the carbohydrate ligands have been shown to be the cell wall mannan outer-chain side branches (Kihn et al. 1988).

Several authors have used an agglutination assay to gain a better understanding of the interaction between host cells or proteins and a variety of bacteria (Vercellotti et al. 1985; Brady et al. 1993; Harty et al. 1993; Nagata et al. 1993; Tomita et al. 1994), or between plant root colonization and microbial surfaces (Buell et al. 1993; Glandorf et al. 1994). The physicochemical properties of the cell surface required for adherence and agglutination of *A. fumigatus* conidia, such as molecular composition, charge, and hydrophobicity, have not been elucidated. Morphological changes in the cell wall have been reported during germination of conidia (Campbell 1971; Ghiorse and Edwards 1973). Since differences in the expression of surface adhesins during this process may have a profound effect on the molecular interactions that promote cell attachment in vivo, we have investigated the cell surface properties involved in agglutination. Here we provide experimental evidence that culture conditions leading to spore swelling and germination dramatically influence cell surface characteristics, and that the ability of conidia to aggregate correlates with an increase in adherence and biochemical rearrangements of the cell wall.

Materials and methods

Organisms and culture conditions

Aspergillus fumigatus CBS 113.26 was grown on yeast–peptone–dextrose agar (YPDA) at 37°C as described earlier (Tronchin et al. 1993*b*). Conidia were obtained from 5-day-old cultures by flooding the agar plates with approximately 10 mL of sterile distilled water and scrapping the aerial mycelium. The suspensions were then successively filtered through 25- and 10-μm pore size nylon filters to remove the conidial heads, the hyphal fragments, and the conidial aggregates. The conidial suspensions were finally centrifuged (1200 × *g*, 3 min) and resuspended in sterile water, and the absorbance at 620 nm was adjusted to 0.6.

Adherence assay

The adherence of conidia to plastic was measured by the enzyme-linked immunosorbent assay technique (Tronchin et al. 1993*a*). To each well of microtiter plates (Nunc, Roskilde, Denmark), 200 μL of 199 medium (Biochrom, Angoulème, France) at pH 6.7 was added. Twenty microlitres of the conidial suspension (A_{620}, 0.6) was inoculated per well and incubated at 37°C from 1 to 5 h. Then nonadherent cells were removed by washing and adherent conidia were detected with a 1:100 dilution of a rabbit anti-*A. fumigatus* immune serum, followed by incubation with a 1:1000 dilution of peroxidase-labeled goat anti-rabbit IgG antibodies (Sigma Chemical Co., St Louis, Mo.). Orthophenylene diamine (Sigma) was used for color development and the absorbance at 492 nm was measured with a Titertek Multiskan. Wells without conidia were used as control.

Agglutination assay

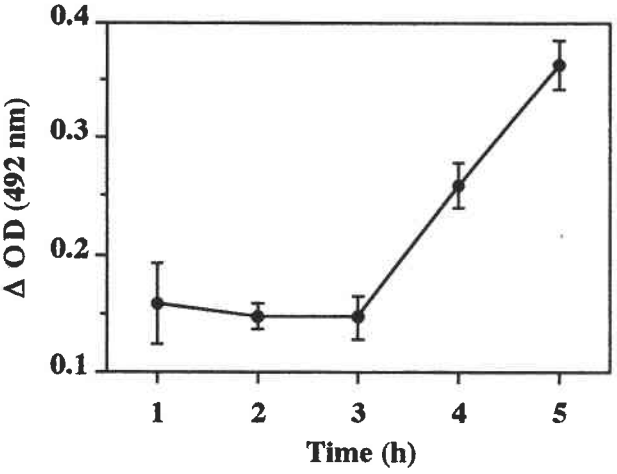
Aliquots (1.5 mL) of the conidial suspension (A_{620}, 0.6) were deposited in 94 mm diameter biologically neutral polystyrene Petri dishes (Greiner, Nürtingen, Germany) containing 15 mL of 199 medium at pH 6.7. Incubations were for 1–5 h at 37°C. Adherent conidia were removed from the plastic surface using a rubber policeman, pelleted by centrifugation, and then resuspended in phosphate-buffered saline (PBS; 0.15 M; pH 7.2) containing 0.5% sodium azide. The cell suspensions were then added to 1-mL cuvettes and diluted to a final A_{620} value of 1.5. The cuvettes were inverted three times. Agglutination of the cells was measured as the decrease in optical density every 15 min during 90 min. In each experiment, conidia stored at 4°C in PBS for 5 h and conidia incubated for 5 h in 199 medium without any treatment were used as controls.

Effect of different treatments on agglutination

After a 5-h incubation at 37°C in 199 medium, cells harvested as previously described were treated either with proteolytic (trypsin, chymotrypsin, proteinase K; Sigma) or glycolytic (lyticase; Sigma) enzymes at 200 μg/mL for 2 h at 37°C. Control cells had no enzyme treatment. Both control and enzyme-treated cells were recovered by centrifugation, washed twice in PBS, and resuspended in PBS before being assayed for agglutinability. Some cell samples were treated for 2 h at 37°C with 0.5 M dithiothreitol (DTT; Fluka Chemie AG, Buchs, Switzerland), 0.5 M 2-mercaptoethanol (2-ME; Merck, Darmstadt, Germany), 0.5% Nonidet P40 (NP 40; Merck), 0.5% sodium dodecyl sulfate (SDS; Fluka), 0.5% Tween 20 (Merck), or 0.2 M sodium metaperiodate (Sigma). After two washes, conidia were processed for agglutination.

To test the effect of calcium on agglutination, 1 mM $CaCl_2$ or 1 mM ethylenediaminetetraacetic acid (EDTA) in 0.15 M NaCl, 20 mM Tris buffer, pH 7.4, was added to the 199 medium at the beginning of incubation or 1 h before the agglutination assay. The effect of tunicamycin (inhibitor of *N*-glycosylation of protein; Sigma) and of cycloheximide

Fig. 1. Relationship between incubation time in 199 medium (from 1 to 5 h) and adherence of conidia to plastic. Each point represents the mean of five determinations.



(inhibitor of protein synthesis; Sigma) was tested by adding the drug (20 μg/mL) to the 199 medium at the beginning of incubation or 1, 2, or 3 h afterwards. Controls included resting or swollen conidia incubated without inhibitor. Cells were harvested after a 5-h incubation at 37°C and agglutination was tested as previously described.

Agglutination inhibition assays

For inhibition experiments, various saccharides were added to the culture medium 1 h before the agglutination assay: 0.2 M mannose, 0.2 M glucose, 0.2 M galactose, 0.2 M lactose (all from Merck), 20 mM galactosamine, 20 mM glucosamine, 20 mM *N*, *N*′-diacetylchitobiose, 20 mM *N*-acetylneuraminic acid, or 20 mM β-D-Gal-(1→3)-D-GalNAc (all from Sigma). Cells were removed after 5 h of incubation, washed twice in PBS, and resuspended in PBS before the agglutination assay.

Immunofluorescence assay for lectin binding

For immunofluorescence, washed conidia untreated or treated with *m*-periodate, DTT, or lyticase were deposited on the wells of glass slides (10 wells, diameter 8 mm, bioMérieux, France). Slides were then air dried and stored at −20°C until used. For labeling, the slides were incubated 30 min in a humid environment at 37°C with 25 μL/well of each of the following fluorescein isothiocyanate (FITC)-labeled lectins at 100 μg/mL in PBS: concanavalin A (ConA), wheat germ agglutinin (WGA), peanut agglutinin (PNA), soybean agglutinin (SBA), and *Bandeiraea simplicifolia* I (BS I). Lectins were chosen as probes on the basis of their specificity for selected sugar residues known to occur in *A. fumigatus*. For control experiments, cells were incubated with each lectin in the presence of its specific sugar: 0.2 M α-methylmannoside for ConA, *N*, *N*′-diacetylchitobiose for WGA, β-D-Gal-(1→3)-D-GalNAc for PNA, *N*-acetyl-D-galactosamine for SBA, and α-D-galactose for BS I. The slides were then washed with PBS, mounted in glycerol-PBS (9:1, v/v), and examined under a Leitz microscope equipped for epifluorescence. The intensity of fluorescence was scored from – to +++. Lectins and sugars were from Sigma.

Electron microscopy

Fungal suspensions containing different evolutionary stages from resting to swollen conidia were used. Samples were fixed in 2.5% glutaraldehyde buffered at pH 7.4 with 0.1 M sodium cacodylate for 1 h. After washing, cells were postfixed for 1 h in OsO_4 buffered with 0.1 M sodium cacodylate (pH 7.4), dehydrated in ethanol, and embedded in Epon. For labeling with PNA–gold complex (10 nm gold particles), conidia were incubated with the lectin at a 1:100 dilution, washed with PBS, and fixed as previously described. In control experiments, cells were incubated with PNA in the presence of 20 mM β-D-Gal-(1→3)-D-GalNAc. Thin sections were stained with uranyl acetate alone or with uranyl acetate and lead citrate. All observations were made on a 100 CX JEOL microscope.

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis

Resting or swollen conidia (1×10^9) were treated with 200 μL of sample buffer containing 62.5 mM Tris hydrochloride, 2% (w/v) SDS, and 10% (w/v) glycerol with or without 5% (v/v) 2-ME (pH 6.8). After being boiled for 2 min and centrifuged, 100 μL of each sample was loaded on a 1.5-mm-thick slab gel of 12.5% polyacrylamide with a 3% polyacrylamide stacking gel and electrophoresed as described by Laemmli (1970). The separated proteins were visualized by silver staining. The molecular masses of proteins were determined by using a low molecular mass electrophoresis calibration kit (Pharmacia-LKB, Uppsala, Sweden).

Results

Adherence assay

Adherence of conidia to the plastic surface was low and constant during the first 3 h of incubation in 199 medium (Fig. 1). Then it markedly and regularly increased in a time-dependent manner during the last 2 h of incubation before germination occurred.

Agglutination assay

Resting conidia appeared as single cells or small aggregates composed of two to five units (Fig. 2*a*). Agglutination was a progressive event that was low until 2 h of incubation and increased over the next 3 h, the maximum being observed at 5 h of incubation (Fig. 3). In this case, large aggregates were observed as soon as conidia were detached from the plastic surface (Fig. 2*b*).

Addition of cycloheximide at 20 μg/mL at the beginning and after 1 h of incubation completely prevented agglutination (Fig. 4). When cycloheximide was added at progressively later stages of incubation, greater degrees of agglutination were found and no effect was observed after 3 h of incubation, compared with untreated conidia. Tunicamycin did not alter the agglutination of the conidia (Fig. 5).

Among the different chemical or enzyme treatments used to dissociate agglutinates, only *m*-periodate, DTT, and lyticase had a notable effect (Figs. 6 and 7). Proteinase K weakly decreased agglutination, whereas *m*-periodate, DTT, and lyticase strongly dispersed the cells. *m*-Periodate at 0.2 M and DTT at 0.5 M were the most effective treatments. The action of *m*-periodate is illustrated in Fig. 2*c*. Trypsin and chymotrypsin did not alter the agglutination of the conidia. Addition

Fig. 2. Suspension in PBS of resting conidia obtained from a 5-day-old culture on yeast–peptone–dextrose agar (*a*), and large aggregates of swollen conidia after a 5-h incubation of resting conidia in 199 medium (*b*). Sodium metaperiodate treatment (0.2 M) of swollen conidia completely inhibited agglutination (*c*). ×800. Scale bars = 10 μm.

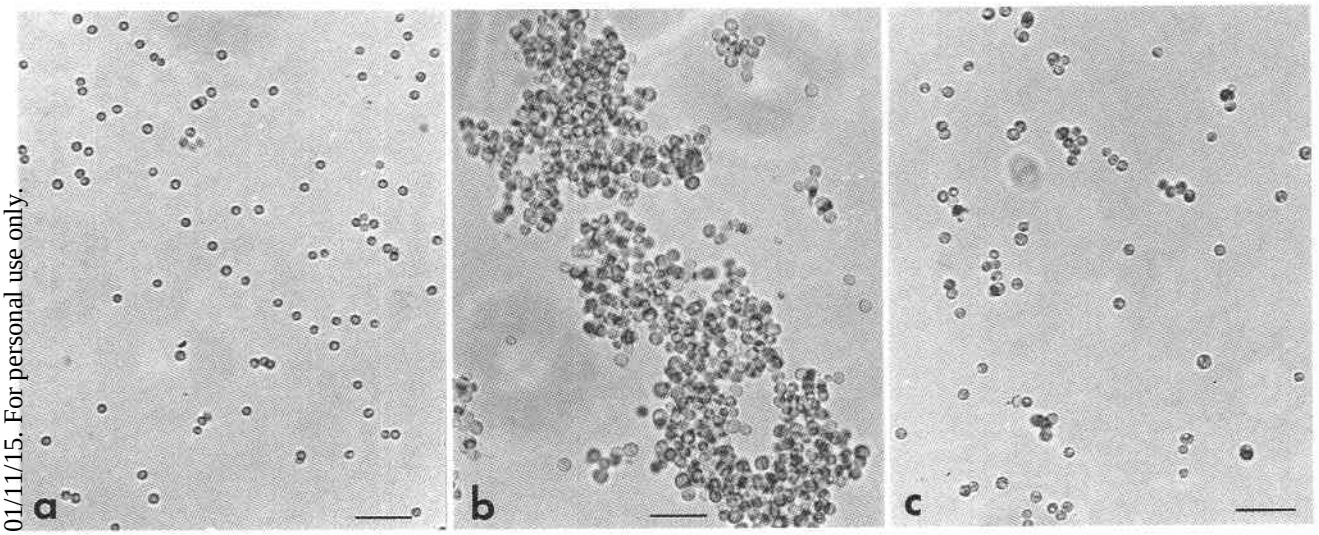


Fig. 3. Effect of increasing incubation time in 199 medium (from 2 to 5 h) on agglutination of conidia (■, PBS; ▲, 2 h; △, 3 h; ●, 4 h; □, 5 h). This is a typical example from an experiment repeated three times.

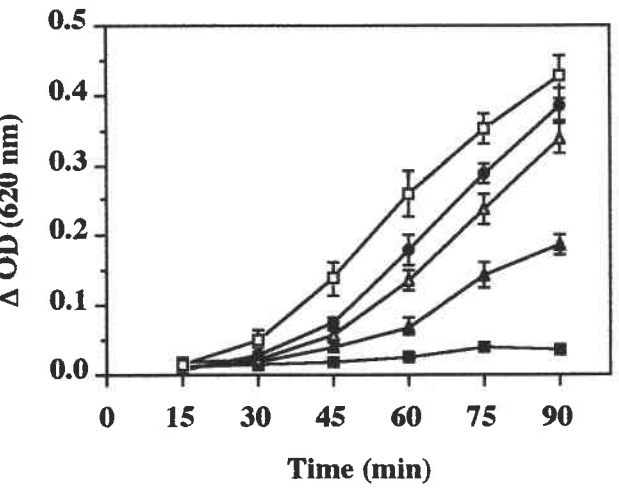
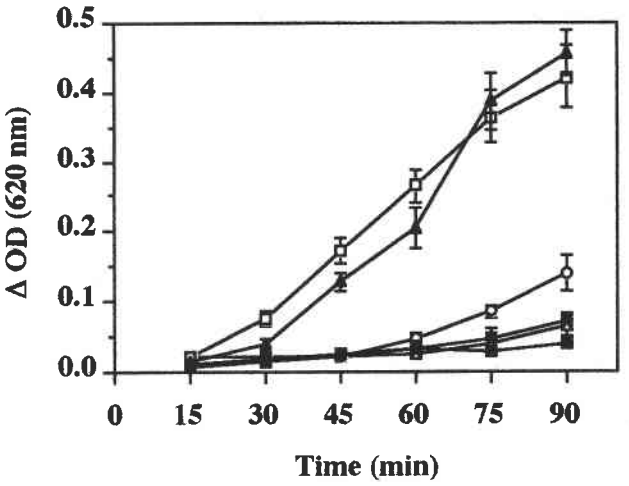


Fig. 4. Effect of cycloheximide (20 μg/mL) added 0 (●), 1; (△), 2 (○), or 3 h (▲) after the beginning of incubation, on agglutination of conidia (□, untreated swollen conidia; ■, resting conidia). This is a typical example from an experiment repeated three times.



of EDTA at the beginning of incubation or 4 h afterwards, prevented agglutination (Fig. 5), whereas calcium did not modify it.

None of the sugars tested significantly inhibited agglutination. On the contrary, *N*-acetylneuraminic acid increased agglutination (data not shown).

Protein patterns of cell wall extracts

The cell surface protein patterns revealed major protein changes between resting and swollen conidia, including an increase in the number and intensity of bands (Fig. 8). In resting conidia, only a few polypeptides with molecular masses larger than 60 kDa were present. A swollen conidial extract run on a 12.5% polyacrylamide slab gel had, after silver staining, a large number of polypeptide chains with molecular masses ranging from 20 to >150 kDa. In conidia incubated for 1–5 h in 199 medium, all of these bands were detected from the 4th h of incubation (data not shown).

Binding of lectins

To study the dynamic changes in the cell wall polysaccharides during incubation of conidia in 199 medium, cells were treated with FITC-labeled lectins. Results are shown in Table 1. No labeling was observed with SBA (specific for *N*-acetyl-D-galactosamine) and BS I (specific for α-D-galactosaminyl residues). ConA (specific for α-D-mannose and α-D-glucose) showed similar labeling in both resting or swollen conidia, and

Fig. 5. Effect of 20 μg/mL tunicamycin (●), 1 mM EDTA (○), and 1 mM $CaCl_2$ (▲), added at the beginning of incubation, on agglutination of conidia (□, untreated swollen conidia; ■, resting conidia).This is a typical example from an experiment repeated three times.

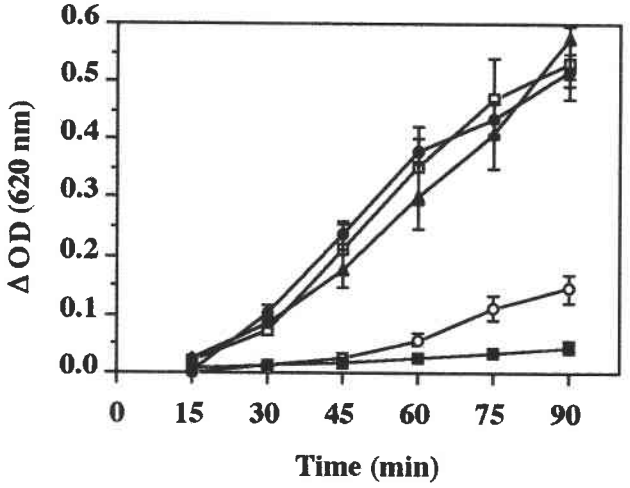
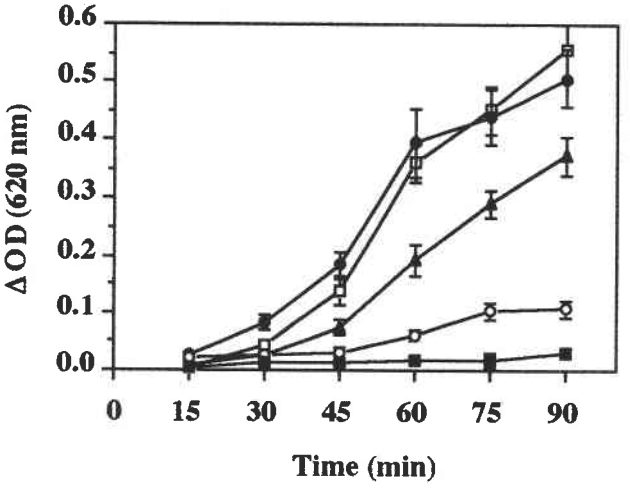


Fig. 6. Effect of treatment of conidia incubated for 5 h in 199 medium by 200 μg/mL proteinase K (▲), trypsin (●) and lyticase (○), on agglutination (□, untreated swollen conidia; ■, resting conidia). This is a typical example from an experiment repeated three times.



its binding was only very slightly affected by lyticase, *m*-periodate, or DTT treatment. An intense fluorescence was observed in resting conidia treated with WGA (specific for D-GlcNAc and NeuNAc) whereas the labeling was weak in swollen conidia. Conversely, PNA (specific for β-D-Gal-(1→3)-D-GalNAc) weakly labeled the resting conidia, but the labeling increased markedly during swelling. *m*-Periodate and DTT treatment of the swollen conidia restored the binding of WGA and inhibited the binding of PNA.

Fig. 7. Effect of treatment of conidia incubated 5 h in 199 medium, by 0.5% SDS (●), 0.2 M sodium metaperiodate (○), and 0.5 M DTT (▲), on agglutination (□, untreated swollen conidia; ■, resting conidia). This is a typical example from an experiment repeated three times.

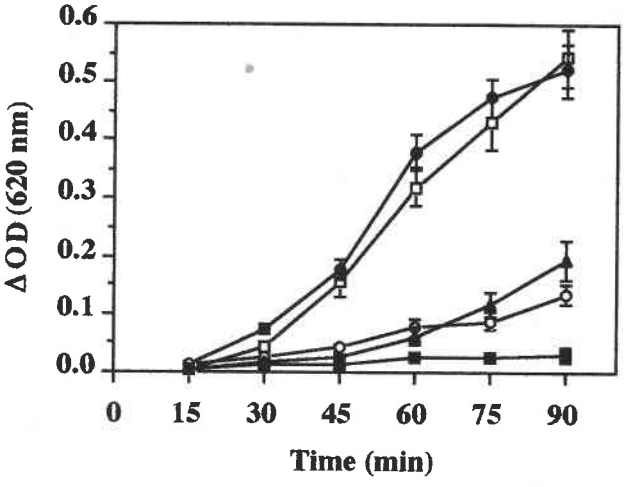


Fig. 8. Silver-stained SDS-polyacrylamide gels of resting (A) or swollen conidia (B), treated by sample buffer, without (lane 1) or with (lane 2) 2-ME treatment. A total of 5×10^8 conidia were loaded per lane. Molecular masses of the standard proteins (in kDa) are indicated on the left.

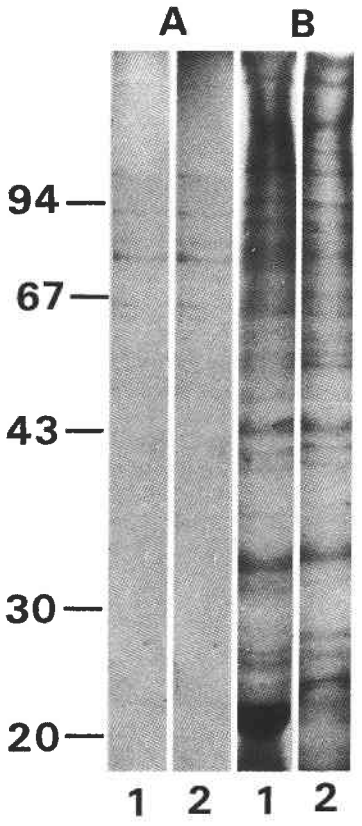
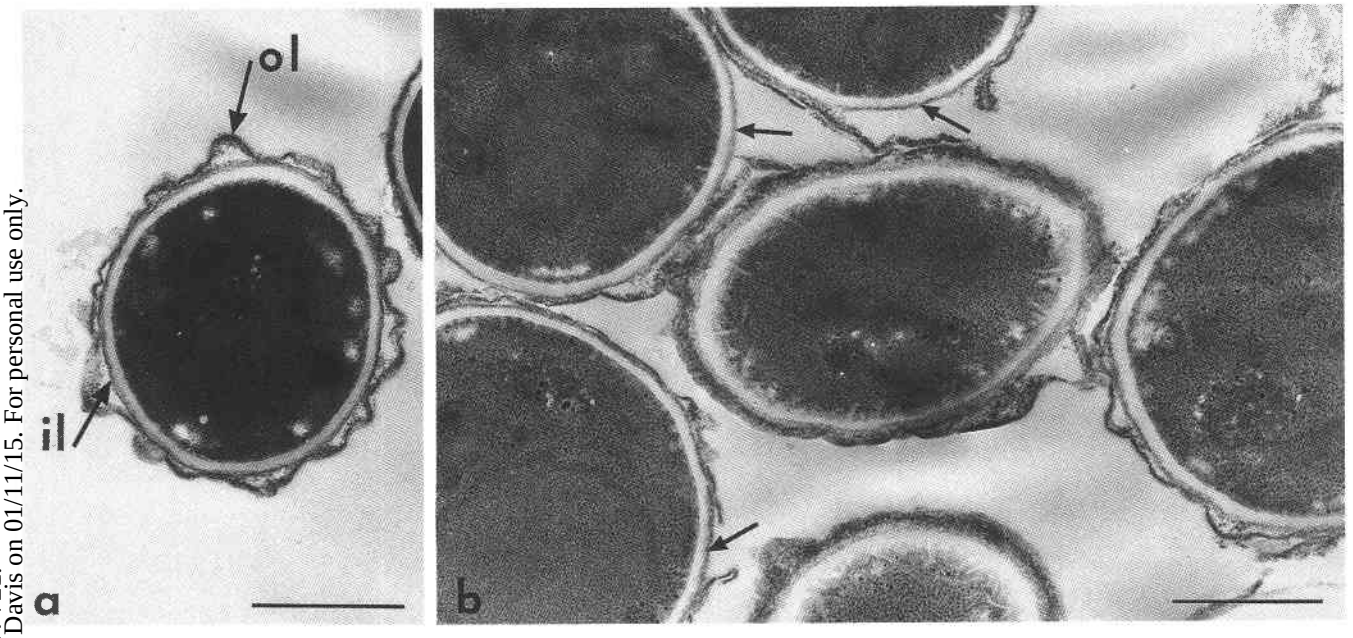


Fig. 9. Electron micrographs of resting conidia. (*a*) The characteristic cell wall with its convoluted outer layer (*ol*) and the inner cell wall layer (*il*). × 20 000. (*b*) After a 2-h incubation in 199 medium, the outer layer appeared to be disrupted, unmasking the inner layer (arrows). × 20 000.



Electron microscopy

Electron micrographs showed that the thick electron dense cuticle that covered the resting conidia was composed of two layers, an outer convoluted layer more or less intimately associated with an inner regular thin layer (Fig. 9*a*). During incubation in 199 medium, the outer cell wall layer stretched and disintegrated. As early as 2 h after inoculation of conidia in 199 medium, the outer layer was appreciably thinner and had become fragmented (Fig. 9*b*). After 4 h of incubation, swollen conidia had almost completely shed the outer layer and some appeared to be surrounded by the inner layer (Fig. 10*b*).

After incubation with PNA–gold, no gold particles were observed on the surface of resting conidia. Labeling appeared after 2 h of incubation in 199 medium, in areas where the outer cell wall layer was loosely arranged or detached (Fig. 10*a*). After 4 h incubation, this layer was completely shed, and an intense labeling regularly distributed over the cell wall surface was observed (Fig. 10*b*). Controls incubated with β-D-Gal-(1→3)-D-GalNAc were free of label.

Discussion

Agglutination has been reported for numerous microorganisms, more particularly yeasts (Stratford 1992) and bacteria (Savage and Fletcher 1985), as well as for higher organisms (Edelman 1985). In *S. cerevisiae*, biochemical and molecular studies led to the hypothesis that both cell surface hydrophobicity and a specific lectin–carbohydrate interaction are involved in this process (Straver et al. 1993*b*). In the present study we report that, when *A. fumigatus* conidia are incubated under conditions that promote swelling and germination, cell wall modifications occur, strongly correlated with adherence to inert surfaces and with agglutination of cells. Electron microscopy suggests that the cell wall of conidia is subject to some rearrangements, which probably involve enzymatic degradation of the external layer and subsequent exposure of underlying ligands. For example, FITC-labeled lectins revealed that carbohydrates acting as ligands for WGA are lost or masked during swelling, whereas the carbohydrate ligands of PNA are synthesized or unmasked. As observed by electron microscopy with PNA–gold, these components could represent preexisting molecules that have become exposed, rather than a de novo synthesis.

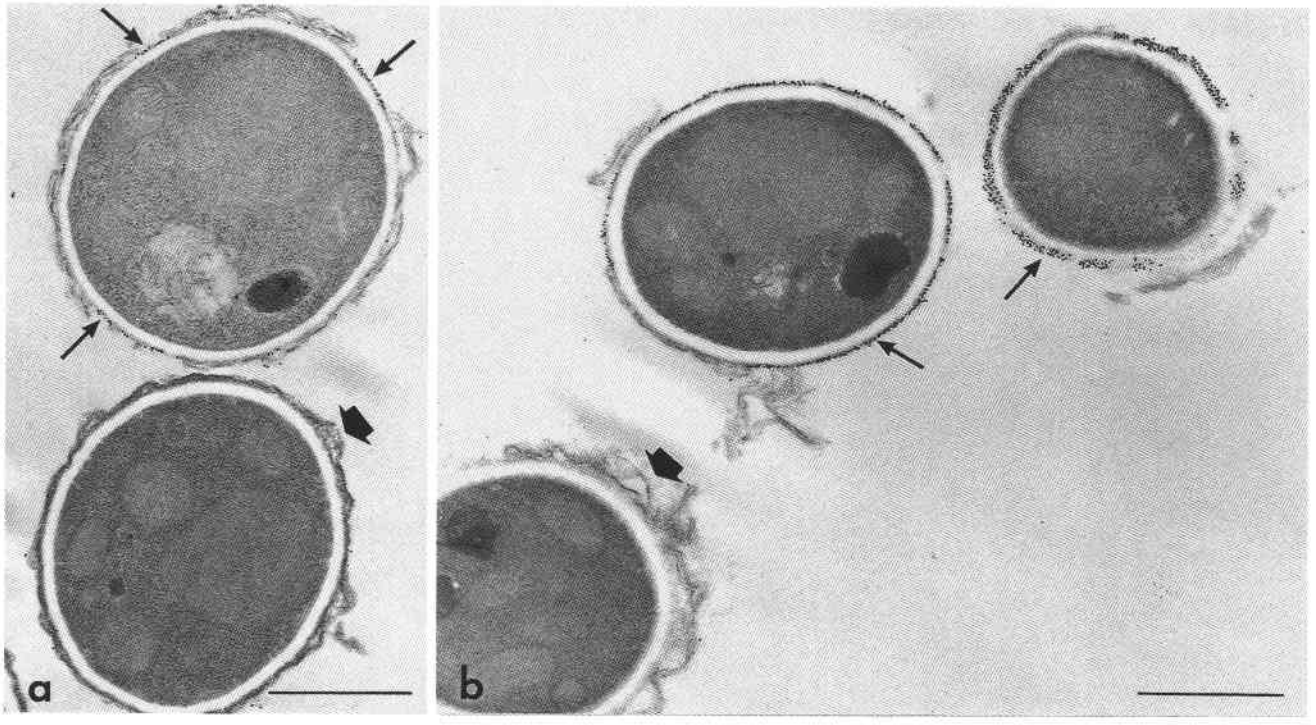
Hydrophobic interactions play a crucial role in various microbial adhesion phenomena. Such interactions facilitate nonspecific adhesion of the opportunistic fungal pathogen *Candida albicans* to inert surfaces (Klotz et al. 1985; Rotrosen et al. 1986), and also enhance its specific adhesion to epithelial cells (Hazen 1989; Hazen et al. 1991). Likewise, hydrophobic interactions enhance adhesion of bacteria to plants cells (Smit and Stacey 1990). Cell surface hydrophobicity was also found

Table 1. Binding of FITC-labeled lectins to resting conidia (T0) and to conidia incubated for 2 h (T2) or 4 h (T4) in 199 medium before labeling.

	T0	T2	T4	Lyticase	*m*-Periodate	DTT
ConA	+	+	±	+	+	+
WGA	++	++/+++	+	+++	++/+++	+/++
PNA	±	±	++	++	−	±
SBA	−	−	−	−	−	−
BSI	−	−	−	−	−	−

Note: The effect of cell wall treatment by lyticase, *m*-periodate, and DTT on labeling of conidia incubated for 4 h in 199 medium was measured. Labeling was scored from − to +++ according to its intensity.

Fig. 10. Ultrathin section of cells labeled with PNA–gold after 2 h (*a*) and 4 h (*b*) of incubation in 199 medium. Note that the gold particles were associated with the inner cell wall layer in areas where the outer layer was loosely arranged or lost (small arrows). No labeling was observed at the surface of conidia showing an intact cell wall (large arrows). × 20 000.



to be a major determinant of flocculation of *S. cerevisiae* (Straver et al. 1993*b*). Here we demonstrated that adherence of conidia to polystyrene, which reflects an increase of hydrophobicity (Mozes and Rouxhet 1987), correlates with an increase in agglutination. This could indicate that disintegration of the cell wall and unmasking of the inner cell wall layer lead to exposure of hydrophobic macromolecules at the cell surface, thus enhancing cell surface hydrophobic binding abilities. This would be an essential process to promote adherence of conidia.

Agglutination also appeared to be correlated with rearrangements of the cell wall surface. It was low in the early stages of swelling (from 1 to 2 h of incubation in 199 medium), compared with later stages prior to germination (from 3 to 5 h of incubation). Removal of cell wall polysaccharides by lyticase or sodium metaperiodate suppressed agglutination. This clearly demonstrated the involvement of cell surface carbohydrates in the agglutination process. Moreover, calcium appeared to be essential in conidial agglutination, since EDTA prevented it. It has been postulated that calcium could act as a salt bridge between saccharide and a proteinaceous receptor (Kihn et al. 1988). The fact that in our study proteinase K slightly affected agglutination and that DTT completely suppressed it could indicate that proteins of the cell wall surface, more or less deeply inserted into the cell wall, are involved in the agglutination process. This agrees with the result obtained with cycloheximide, an inhibitor of protein synthesis that has been previously shown to stop synthesis of yeast cell wall mannoproteins (Elorza and Sentandreu 1969) and to prevent development of flocculation of yeast (Stratford and Carter 1993). Although cycloheximide did not alter swelling and growth when it was added at a concentration of 20 μg/mL at the beginning of incubation, the agglutination appeared to be cycloheximide sensitive, thus indicating inhibition, during swelling, of the synthesis of cell wall proteins involved in the agglutination process.

The cell wall proteins that may interact to cause cell aggregation were therefore examined by SDS-PAGE. Major protein changes were revealed between resting and swollen conidia. It remains to be determined whether these additional bands reflect the synthesis of new molecules or result from newly exposed proteins in swollen conidia. In resting conidia, proteins were difficult to extract because they were embedded in a thick polysaccharide matrix. On the other hand, since the cell wall is thinner in swollen conidia, cytoplasmic, periplasmic, or membrane components may have been extracted. Therefore, it is likely that the protein patterns observed do not exactly reflect changes in cell wall proteins during swelling. However, because extraction is more successful in swollen conidia it may provide an opportunity to study the cell wall receptors (adhesins) involved in adherence and agglutination.

Concomitant with morphological rearrangements of the cell wall, these results suggest that changes in culture conditions influence cell surface characteristics. We have previously demonstrated a similar sequence of events in the course of germination of the pathogenic yeast *C. albicans* (Tronchin et al. 1989). Here, by examining the temporal relationship between adhesion, agglutination, and cell wall morphology, we have provided evidence that adhesins are present at the cell surface during swelling. Further work is needed to identify the cell wall components involved in the agglutination process of

A. fumigatus, and to determine its implications in vivo. It is possible that agglutination may allow the organism to elude phagocytic host defenses or to establish high concentrations of conidia at sites of infection, allowing conidia to interact with host protein. Indeed, differences in the distribution of fibrinogen (Annaix et al. 1992) and laminin receptors (Tronchin et al. 1993*b*) have been reported during swelling. Because it is both simple and easily quantifiable, the agglutination assay described here is useful to assess interactions of cell surface molecules with host proteins.

Acknowledgments

This work was supported by a grant from Pfizer Laboratories and from Fondation pour la recherche médicale (France).

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