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The binding of surface proteins from *Staphylococcus aureus* to human bronchial mucins

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The binding of surface proteins from Staphylococcus aureus *to human bronchial mucins. D. Trivier, N. Houdret, R.J. Courcol, G. Lamblin, P. Roussel, M. Davril. ©ERS Journals Ltd 1997.*

ABSTRACT: Colonization by *Staphylococcus aureus* is frequently observed in obstructive lung diseases, particularly in cystic fibrosis. It has been shown that the bacteria bind to mucins, the main constituent of bronchial secretions. The binding mechanism, however, remains unclear.

We have investigated the interactions of two strains of *S. aureus*, one mucoid and one nonmucoid, with human bronchial mucins. Using a solution phase assay, the binding capacity of the two strains to radiolabelled bronchial mucins was assessed. The bacterial constituents were released by lysostaphin lysis and the surface components of the nonmucoid strain were extracted with the use of a detergent (3-([3-cholamidopropyl] dimethylammonio)-1-propane sulphonate (CHAPS)). All were analysed for mucin-binding using an overlay assay.

The amount of mucins bound to the nonmucoid strain was threefold greater than that of the mucoid strain. In the lysostaphin extract from the mucoid strain, only a 57 kDa protein faintly bound ^{125}I-labelled mucins, whereas three mucin-binding proteins (52, 57 and 71 kDa) were identified from the nonmucoid strain. Two surface proteins, one major at 60 kDa and one minor at 71 kDa, bound radiolabelled bronchial mucins and their binding was almost completely inhibited by ovine submaxillary mucin.

These results indicate: 1) differences in the mucin-binding capacity from one strain of *S. aureus* to another; and 2) the presence of external and internal adhesins binding to human respiratory mucins in the nonmucoid strain.
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Staphylococcus aureus is found in the nasopharynx of 20–40% of adults at any time, and this carrier rate may increase up to 70% in the hospital environment. In spite of this frequency, overt disease is not a common event in a normal population. *S. aureus* may be responsible for lung infection in different circumstances. Acute colonization of the lower respiratory tract may occur during endotracheal intubation, and this may be due either to a temporary defect in mucociliary clearance or in local defences, or to alterations of epithelial cell surfaces. *S. aureus* can also be present in obstructive lung diseases, particularly in patients with ciliary dyskinesia syndrome, cystic fibrosis, and immunoglobulin deficiency. Many forms of chronic airway colonization are exacerbated and characterized by an inflammatory response to the bacterial infection accompanied by hypersecretion of tracheobronchial mucus, which is considered to be a barrier to bacteria in physiological conditions. However, in chronic bacterial lung infection, a mucus with abnormal rheological properties may contribute to the inefficiency of the mucociliary clearance and be responsible for the persistence of bacterial colonization.

Mucins represent the main components of mucus, and various mucins have been shown to interact with *S. aureus*. Using a ferret model, SANFORD *et al*. [1] observed the binding of this species to nasal mucus and nasal mucins. RAMPHAL [2] demonstrated the binding of *S. aureus* to bronchial mucins from patients with cystic fibrosis and chronic bronchitis. THOMAS *et al*. [3] reported the binding of staphylococci to bovine submaxillary mucin, and observed the additional role of calcium ions. More recently, SHUTER *et al*. [4] have investigated the adhesion of various strains of *S. aureus* to the human nasal mucosa, and observed several mucin-binding adhesins.

The purpose of the present study was to identify and characterize the surface proteins involved in the binding of *S. aureus* to human bronchial mucins.

Materials and methods

Bacteria

The nonmucoid *S. aureus* strain Lat is a blood culture isolate from a heart transplanted patient. It was identified by tube coagulase test and the Analytical Profile Index (API)-Staph strip (Bio Mérieux, France). The mucoid strain, SA-1, was a generous gift from R.

Ramphal (Gainesville, FL, USA); it is a highly encapsulated strain [5] which has a low affinity for human bronchial mucins in a microtitre plate adhesion assay [6]. Both were stored into Felix medium and cultured overnight on nutrient agar. Colonies were inoculated into 3 mL of a chemically defined medium (CDM), which contained less than 0.03 µM Fe^{3+} [7]. The inoculum, adjusted to an absorbance of 0.20 at 470 nm, was diluted to 200 mL (final volume) with CDM and incubated at 37°C, using an orbital shaking incubator. Absorbance at 470 nm and number of bacteria were measured at the initial time and the culture was stopped at the late log phase. After centrifugation at 13,000×g for 30 min at 4°C, the pellets were washed three times with 0.15 M NaCl and suspended in phosphate-buffered saline (PBS), pH 7.2. The suspensions were counted, and stored at -70°C with 15% glycerol until used.

Mucins

Human bronchial mucins were isolated from the non-purulent sputum of a patient (blood group O) suffering from chronic bronchitis. The sputum was solubilized by 1:12 dilution with deionized water and stirring overnight at 4°C. After centrifugation, the soluble components were dialysed and lyophilised, and subsequently submitted to two repeated CsBr density gradient centrifugations, as described by HOUDRET *et al*. [8]. Mucin purity was ascertained by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), carbohydrate and amino acid analyses [9].

Bovine submaxillary mucin (BSM) was obtained from Sigma (type 1-S Saint Quentin Fallavier, France) and purified on a Sepharose 4B (2×50 cm) column in 0.1 M Tris-HCl, 0.5 M NaCl, pH 8.0 buffer (data not shown).

Ovine submaxillary mucin (OSM) was isolated from submaxillary glands [10] and purified by CsBr density gradient centrifugation, as described above.

^{125}I-labelling of mucins

Bronchial mucins were radioiodinated by a modified chloramine-T method: 5 mg of mucins dissolved in 1 mL PBS were iodinated with 350 µCi of ^{125}iodine (specific activity = 17.4 mCi·µg^{-1}, Amersham, Les Ulis, France) by the addition of 2 Iodobeads (Pierce Chemicals, Interchim, Montluçon, France), and maintained for 10 min at room temperature with gentle stirring. The reaction was stopped by adding NaI to 0.5 M final concentration, and the ^{125}I-labelled mucins were separated from other reaction products by gel-filtration on Sephadex G-25 column (Pharmacia, Ozsay, France) equilibrated with PBS supplemented with 0.1% bovine serum albumin (BSA). Radioactivity was measured with an automatic γ-counter on diluted aliquots. An average specific activity of 45× 10^6 counts per minute (cpm)·mg^{-1} of mucins was obtained.

Solution phase adhesion assay

Assays were carried out in siliconized tubes to prevent ^{125}I-mucins from adhering to plastic. Tubes were filled with Sigmacote (Sigma) (2% v/v in ethanol) and left over-night for coating, then emptied and air-dried. In order to obtain radiolabelled mucin solutions of increasing concentrations, aliquots (10 µg) of ^{125}I-labelled mucins were mixed with increasing amounts of unlabelled mucins. Quantities ranging 10–1,000 µg of human bronchial mucins were mixed with 1×10^9 bacteria, in the presence of 1% (w/v) BSA in PBS. The mixtures (0.5 mL) were kept at room temperature for 2 h, and assays were terminated by adding 2 mL of ice-cold PBS containing 0.05% (w/v) Tween-20 (PBS-T). After centrifugation at 9,000×g for 20 min at 10°C, the pellets were washed twice with 2 mL of PBS, centrifuged again and measured for radioactivity. The amount of mucins bound to the different pellets was obtained by measuring the bound radioactivity and taking the specific activity of the different solutions into account. Each experiment was repeated three times with controls omitting bacteria. For measurement of calcium effect, binding assays containing a standard quantity of 40 µg of bronchial mucins and 1×10^9 bacteria were supplemented with calcium at final concentrations of 2.5, 5 and 10 mM of $CaCl_2$ and treated as described above.

In inhibition studies, lysostaphin lysate or 3-([3-cholamidopropyl] dimethylammonio)-1-propane sulphonate (CHAPS) extract (protein amounts equivalent to 1×10^9 bacteria) was preincubated for 2 h at room temperature with radiolabelled mucins (40 µg) before addition of cells.

^{125}I-surface labelling of bacteria

Surface proteins were labelled as above. Briefly, 1×10^{11} bacteria in 2 mL of PBS were mixed with 2 Iodobeads and 500 µCi of 125iodine for 5 min, with gentle shaking, at room temperature. The reaction was stopped and the ^{125}I-labelled bacteria were separated from other reaction products by centrifugation at 3,200×g, for 10 min at 10°C. The pellets were washed twice with PBS and radioactivity was measured on diluted aliquots.

Lysostaphin digestion of S. aureus

Bacteria (1×10^{10}) were suspended in 0.5 mL of 50 mM Tris-HCl, 10 mM $MgCl_2$ buffer pH 7.5, and incubated with 100 units of lysostaphin, 2 mM phenylmethyl sulphonyl fluoride (PMSF) and 2 mM Nα-p-tosyl-L-lysine chloromethyl ketone (TLCK), for 2 h at 37°C, with gentle shaking; 10 µg of deoxyribonuclease (DNase) I and 10 µg of ribonuclease (RNase) were then added. After 30 min of incubation at room temperature, the mixture was centrifuged at 1,000×g for 10 min at 4°C. The supernatant was designated as lysostaphin lysate.

Extraction of S. aureus *cell surface proteins with CHAPS*

Aliquots (2×10^{10} cells) of the two strains were suspended in 2 mL of 50 mM Tris-HCl buffer (pH 7.5) containing 150 mM NaCl supplemented with 1% (w/v)

CHAPS. The suspensions were gently stirred at room temperature for 5 h in the presence of 2 mM PMSF and 2 mM TLCK. The supernatants were recovered by centrifugation at 3,200×g for 10 min at 4°C, and concentrated on Centricon 10 tubes (Amicon, Grace, Epernon, France). The material retained was exhaustively washed with the Tris-HCl buffer above to remove most of the detergent. The viability of the treated bacteria was checked by cell count on blood agar.

Protein A removal from the CHAPS extract

Four milligrams of CHAPS extract were loaded on immunoglobulin G (IgG) Sepharose 6 Fast Flow (Pharmacia) column (1×5 cm), according to the manufacturer's instructions. The unretained fraction containing the adhesins was eluted with the equilibration buffer, 50 mM Tris, 150 mM NaCl and 0.05 % Tween 20, adjusted to pH 7.6. Protein A could be obtained by further elution with 0.5 M acetic acid adjusted to pH 3.4 with ammonium acetate.

Protein determination

Protein was determined with the bicinchoninic acid (BCA) protein assay reagent (Pierce Chemicals) and bovine serum albumin as standard, in microtitre plates.

Electrophoresis of S. aureus *extracts*

Samples treated with β-mercaptoethanol were subjected to SDS-PAGE according to LAEMMLI [11] on 0.75 mm thick, 10% acrylamide gels with a Mini Protean II equipment (BioRad, Ivry-sur-Seine, France). After staining with Coomassie blue R-250 and drying, gels containing radiolabelled proteins were exposed to Hyperfilm MP (Amersham) at -70°C. The molecular masses of ^{125}I-labelled proteins were estimated by comparison with ^{14}C-methylated protein standards. On Western blots, the protein standards used were from Pharmacia.

Western blotting and detection of mucin-binding adhesins

After electrophoresis of *S. aureus* extracts, electrotransfer was performed on nitrocellulose membranes (Hybond-C extra, 0.45 µm, Amersham) in 25 mM Tris, 192 mM glycine buffer pH 8.3 containing 20% (v/v) methanol, using the Mini Transblot System (BioRad). The blots were first maintained under gentle shaking, for 120 min at room temperature, with 2% (w/v) BSA in PBS, and then incubated with ^{125}I-labelled bronchial mucins (100 µg·mL^{-1} in PBS-BSA 1%) for 120 min. They were then washed thoroughly four times with PBS and four times with PBST, for 15 min each, dried and exposed at -70°C to X-ray film.

Inhibition assays were performed with glycopeptides obtained by pronase digestion of human bronchial mucins [12], and with BSM and OSM. Blots were first incubated with the various inhibitors, at concentrations ranging 1–8 mg·mL^{-1} in PBS-BSA 1% for 120 min, and then with the radiolabelled bronchial mucins, and processed as described above.

Results

Differential binding of human bronchial mucins to two strains of S. aureus

Two strains of *S. aureus* were investigated, one nonmucoid (Lat) and one mucoid (SA-1), which had previously been shown to be poorly adhering in a microtitre plate adhesion assay [2].

Fixed numbers of bacteria (1×10^9) were incubated in a solution phase assay with increasing amounts of airway mucins. Figure 1 shows that the binding was mucin concentration-dependent for both strains. The binding increased almost linearly and, at the final concentration of 2 mg·mL^{-1} mucin added (1,000 µg in the assay), strain Lat fixed three times more mucins than strain SA-1. Although no saturation could be obtained with either strain, a tendency towards a plateau from 1 mg·mL^{-1} mucin concentration was visible for SA-1, whereas the slope still increased for strain Lat. When performing assays using strain Lat (1×10^9 cells) with a fixed concentration of mucin (40 µg) and varying quantities of $CaCl_2$, there was a twofold increase of the binding in the presence of ≥5 mM $CaCl_2$ (data not shown).

Extraction of surface proteins with lysostaphin and CHAPS

The two strains Lat and SA-1 were treated with CHAPS or lysostaphin, and the amounts of soluble proteins recovered from 1×10^{10} bacteria were compared. For strain Lat, a mean (±SD) of 0.16 (±0.04) mg of protein was solubilized by CHAPS, and 1.01 (±0.5) mg by lysostaphin (three determinations). For strain SA-1, the recovery of soluble proteins was 0.15 mg with CHAPS, and 0.75 mg with lysostaphin, (one experiment). The effect of the detergent was milder than that of lysostaphin, and cell counts indicated that almost all bacteria remained alive following CHAPS treatment. Most of them, when examined under a transmission electron microscope (not

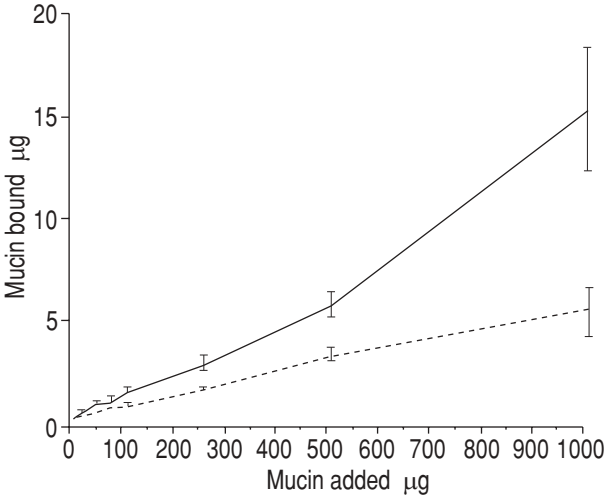

Fig. 1. – Binding of ^{125}I-labelled bronchial mucins to nonmucoid *Staphylococcus aureus* strain Lat (——) and mucoid strain SA-1 (- - -), in a solution phase assay, performed as described in "Materials and methods". Values are presented as mean±SD of triplicate determinations.

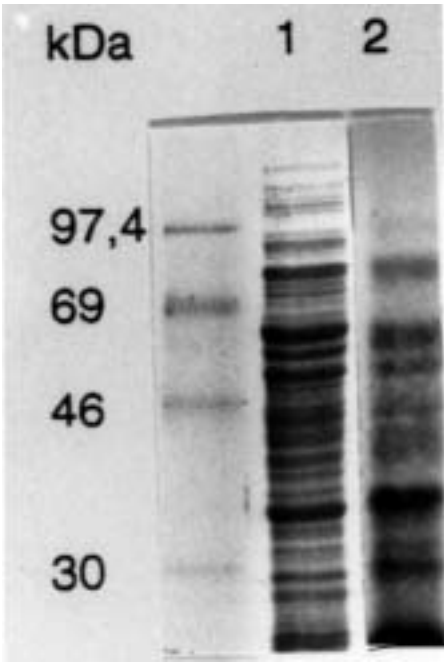

Fig. 2. – Protein profiles of the CHAPS extract of *Staphylococcus aureus* strain Lat on SDS-PAGE. Proteins (10 µg per lane) were stained with Coomassie blue (lane 1). Labelled surface proteins were revealed by autoradiography (lane 2). Molecular mass standards are shown on the left. CHAPS: 3-([3-cholamidopropyl] dimethylammonio)-1-propane sulphonate; SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis.

shown), still maintained their integrity, indicating the mild action of CHAPS on the surface.

Comparison of profiles after staining (fig. 2, lane 1) and after autoradiography (fig. 2, lane 2) showed that the stained bands having a molecular weight above 40 kDa were also radiolabelled, indicating that they are surface components. Moreover, there are differences from one band to another in the relative intensity of Coomassie blue staining and radiolabelling. These differences probably correspond to amino groups and tyrosine contents of each band.

Differential binding of human bronchial mucins to CHAPS and lysostaphin extracts

Amounts of CHAPS and lysostaphin extracts corresponding to 1×10^9 nonmucoid bacteria were preincubated with radiolabelled mucins (40 µg) for 2 h, before addition of a corresponding number (1×10^9) of living bacteria. Results are presented in table 1. Preincubation of radiolabelled mucins with the lysostaphin extract decreased the binding capacity of human respiratory mucins to the nonmucoid strain (residual binding of 45%), whereas preincubation with the CHAPS extract led to a higher residual binding (66%). These data indicate that each extract contains proteins having an affinity for mucins; these proteins might be different from one procedure of extraction to another.

Characterization of S. aureus *proteins binding to human bronchial mucins*

The proteins solubilized by lysostaphin treatment of the two strains (Lat and SA-1) were submitted to Western blotting onto nitrocellulose (fig. 3). The efficiency of the transfer was assessed by staining replicas with amido black (lanes 1 and 2). The adhesins having an affinity for bronchial mucins were identified by overlay of other replicas of the lysostaphin extracts with ^{125}I-mucins and autoradiography. Three bands were observed from strain Lat, one major at 52 kDa and two minor at 57 and 71 kDa (fig. 3, lane 4). A faint band at 57 kDa was also present and barely visible in the extract from strain SA-1 (fig. 3, lane 3).

When the CHAPS extract from strain Lat was analysed with ^{125}I-mucins in the same conditions, one major adhesin was observed at 60 kDa and one minor band

Table 1. – Inhibition of the binding of human bronchial mucins to *Staphylococcus aureus* by lysostaphin and CHAPS extracts

Pretreatment	Mucin bound µg	% of control
None	1.73±0.09	100
Lysostaphin extract	0.77±0.03	45±2
CHAPS extract	1.15±0.04	66±2

Values are presented as mean±SD of two experiments performed in triplicate *i.e.* six determinations. Lysostaphin extract containing 97 µg of proteins, CHAPS extract containing 38 µg of proteins, or PBS ("None") were preincubated for 2 h at room temperature with 40 µg of ^{125}I-bronchial mucins, before addition of 1×10^9 cfu of live *S. aureus* (strain Lat). The binding assay was then processed as described under "Materials and methods". Quantities of lysostaphin and CHAPS extracts correspond to those recovered from 1×10^9 bacteria. CHAPS: 3-([3-cholamidopropyl] dimethylammonio)-1-propane sulphonate; cfu: colony-forming units.

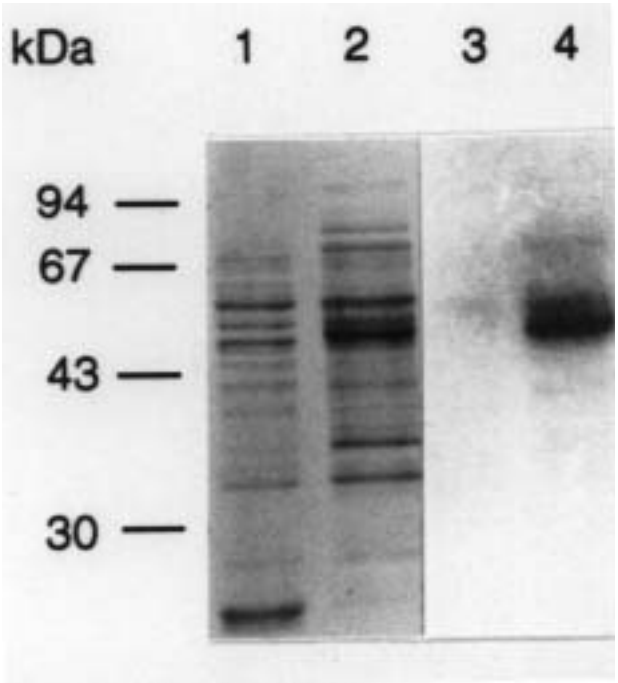

Fig. 3. – Western blotting analysis of *Staphylococcus aureus* proteins solubilized by lysostaphin and characterization of mucin adhesins, Lysates of strain SA-1 (lanes 1 and 3) and strain Lat (lanes 2 and 4) were stained with amido black (lanes 1 and 2) and assessed for adhesins with ^{125}I-mucins (lanes 3 and 4). The positions of the protein standards (Pharmacia) are indicated by bars.

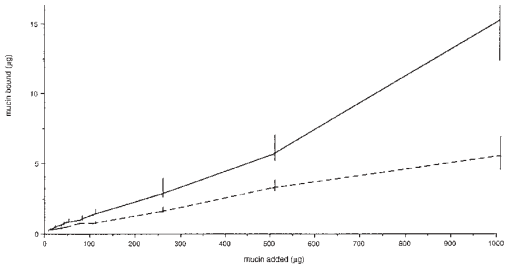

Fig. 4. – Western blotting analysis of *Staphylococcus aureus* strain Lat proteins. Lysostaphin lysate (lane 1) is shown for comparison. Proteins solubilized by CHAPS (lanes 2–4) were assessed for adhesins with ^{125}I-mucins (lanes 3 and 4). Inhibition of binding to ^{125}I-bronchial mucins by preincubation with OSM (8 mg·mL^{-1}) corresponds to lane 4. The positions of the protein standards (Pharmacia) are indicated by bars. Proteins were stained with amido black (lanes 1 and 2). OSM: ovine submaxillary mucin; CHAPS: 3([3-cholamidopropyl] dimethylammonio-1-propanesulphonate.

was less visible at 71 kDa (fig. 4, lane 3). In order to eliminate the possibility of interference with protein A, the CHAPS extract was submitted to an affinity chromatography on IgG-Sepharose, which retained protein A. Profiles on SDS-PAGE of the CHAPS extract, before and after chromatography on IgG-Sepharose, were identical (data not shown) to the profile shown in figure 4. No adhesin could be observed in the CHAPS extract from strain SA-1 (data not shown).

Specificity of the binding

In order to assess the specificity of the binding, inhibition experiments were performed by preincubating replicas of CHAPS extracts with OSM, or BSM, or human bronchial mucin glycopeptides, before incubation with radiolabelled bronchial mucins. Inhibition was obtained only with OSM. The inhibition was already visible when using OSM at 1 mg·mL^{-1} and was almost complete at 8 mg·mL^{-1} (fig. 4, lane 4). Preincubation of the replicas with BSM or human bronchial glycopeptides had no effect.

Discussion

In the present study, proteins binding to human bronchial mucins have been characterized among the surface proteins of a nonmucoid strain of *S. aureus*. Previously, *S. aureus* has been shown to have the potential to bind to a number of host components [13–21], including several types of mucins, nasal from human and ferret [1, 3], bovine submaxillary and human respiratory [4].

A solution phase adhesion assay was used in the present study. The binding of a nonmucoid strain of *S. aureus* was threefold higher than the binding of a mucoid strain (SA-1) that was poorly adherent in a microtitre plate adhesion assay [2]. This mucoid strain is known to elaborate a capsular polysaccharide [5], which probably prevents the binding of these cells to mucins.

Solution phase adhesion assays allow the investigation of whether or not the binding of mucins to *S. aureus* is saturable. In the range of mucin concentrations that were used, the binding of human bronchial mucins to the nonmucoid strain was concentration-dependent. The usual concentration of mucins in mucus from patients suffering from chronic bronchitis is in the order of 1 mg·mL^{-1} [9] and, even at a twofold higher mucin concentration, a plateau was not reached (fig. 1). Similar results were obtained by DEVARAJ *et al*. [22] for interactions of human respiratory mucins with *P. aeruginosa*. One possible explanation for such observations is based on the physical properties of mucins. Mucins are a polydisperse family of very high molecular weight glycoproteins. On electron microscopy, they appear as filaments with sizes ranging 0.5–5 µm, sometimes longer than the width of a single coccus. Moreover, at high concentrations, as in mucus, they have a tendency to form aggregates [9], especially in the presence of calcium [23]. Therefore, Scatchard analysis [24] is not applicable to such bacterial/mucin interactions and, at physiological concentrations of mucins, one may observe an apparent increase of *S. aureus*/mucin interactions due to the self-aggregating properties of mucins.

Similarly, the binding of airway mucins to *S. aureus* was increased by using 5 mM calcium. Analogous data have previously been obtained by THOMAS *et al*. [3] for BSMs. However, one may question whether this corresponds, at least to some extent, to the aggregating property of calcium on mucins [23] and, moreover, one may question the physiological or pathological relevance of this observation, since this calcium concentration corresponds to more than twice the normal blood concentration.

In order to characterize the *S. aureus* surface proteins which interact with human respiratory mucins, two procedures were used, which solubilized proteins able to inhibit the binding of mucins to *S. aureus* (table 1).

Lysostaphin, an enzyme which rapidly lyses *S. aureus* cell walls, leads to a mixture of cytoplasmic and membrane-bound proteins among other components. An attempt was also made to solubilize the surface proteins from strain Lat with CHAPS, a nondenaturing zwitterionic detergent, which has been used for the solubilization of rat liver microsomal membranes [25]. To our knowledge, this detergent has never been used to solubilize surface proteins from bacteria. This reagent is easy to remove from solutions, and the integrity of CHAPS-treated cells was checked on electron microscopy and by cell count. It should be mentioned that the amount of protein extracted from the mucoid strain was lower than that from the other strain; this might be related to the thickness of the capsular polysaccharide [5].

In order to determine whether all proteins solubilized with CHAPS derived from the cell surface, radiolabelling of the bacterial surface of strain Lat was performed under mild conditions. The majority of the proteins above

40 kDa, extracted with CHAPS and stained with Coomassie blue (fig. 2, lane 1) were also visible on the autoradiograph (fig. 2, lane 2), indicating that they were bacterial surface proteins. Some proteins were not radiolabelled, suggesting that, as the cell wall integrity was preserved, their tyrosine residues, if any, were nonreactive to iodination.

Among the proteins released by lysostaphin treatment of strain SA-1, only one adhesin was identified with a very faint binding to mucin (fig. 3, lane 3). This result suggests that, for *S. aureus* SA-1, surface constituents other than proteins, such as capsular polysaccharide, may be responsible for the weak binding of this strain observed in the solution phase assay (fig. 1).

Conversely, among proteins obtained from strain Lat, one main adhesin could be identified at 52 kDa and two minor adhesins at 57 and 71 kDa when using lysostaphin (fig. 3, lane 4). The major adhesin, obtained by CHAPS treatment, has a higher molecular weight (60 kDa). This adhesin is still present when protein A is removed from the CHAPS extract, indicating that these two proteins are not related. The molecular masses of the main adhesins extracted by CHAPS or by lysostaphin are slightly different, and there are two possible explanations for this. Firstly, the lysostaphin extract contains both internal and surface proteins, therefore the surface adhesins may be less visible in this extract because of their minor amount. Secondly, the major band at 52 kDa observed after lysostaphin lysis may be a degradation product of the band at 60 kDa observed after CHAPS extraction. Although the lysostaphin treatment was performed in the presence of PMSF and TLCK, it might have released some bacterial proteases responsible for the cleavage of some peptide bonds in the surface proteins.

Tetramethylurea, used at a 0.5 M concentration, has been claimed to disrupt hydrophobic interactions [26]. In the present case, it had no effect on the binding (data not shown), suggesting that the interactions between mucins and those bands were not simply hydrophobic.

Adhesins binding to various mucins have been described previously in several strains of *S. aureus*. Using ^{125}I-BSM type-IS and a *S. aureus* mutant strain, THOMAS *et al.* [3] characterized three adhesins with molecular masses lower than those in the present study (40, 35 and 29 kDa). Such differences emphasize the variations observed using mucins of various origins on the one hand, and an isogenic mutant of *S. aureus* (strain DU 5723) on the other [3].

Bands at 127 and 138 kDa with an affinity for nasal mucins have recently been reported in a lysostaphin-solubilized *S. aureus* strain by SHUTER *et al.* [4]. Until now, there has been no information about the similarities between nasal and bronchial human respiratory mucins. The sites which are recognized on the nasal and bronchial human mucins are probably not the same, since the binding to nasal mucins is inhibited by BSM [4] in contrast to the binding to human respiratory mucins.

Mucins are a very large family of polydisperse glycoproteins. They may vary from species to species, within a species, from individual to individual, and within an individual, from one mucosa to another. Such variations may occur at the peptide as well as at the carbohydrate level. Therefore, the adhesins reported by THOMAS *et al.* [3], SHUTER *et al.* [4] and in the present study may correspond to surface proteins with different affinities for mucins of various origins, as well as to a strain-specific expression of surface proteins.

Human bronchial mucins contain highly glycosylated domains resistant to proteolysis and so-called naked regions, which are more or less devoid of carbohydrate and susceptible to proteolysis. In the present study, bronchial mucin glycopeptides corresponding to highly glycosylated domains, which were obtained by proteolysis of mucins, did not inhibit the binding of whole mucin to the putative adhesins, suggesting that, if carbohydrate chains are involved in the interactions, they are not located on the highly glycosylated domains, but on the naked regions. At a concentration of 10 mg·mL^{-1} (*i.e.* no more than 1–2 mg of naked region), which represents the upper limit of solubility of whole bronchial mucins, we found no inhibition of adhesion (data not shown). However, OSMs, which contain very short carbohydrate chains [10], partially inhibited the binding of human respiratory mucins to the adhesins. Therefore, it is possible that the receptor sites located on the naked or poorly glycosylated regions correspond to peptide or carbohydrate/peptide epitopes sharing some similarities with OSMs. SHUTER *et al.* [4] consider that there are specific protein-carbohydrate interactions between *S. aureus* and nasal mucin, whereas SANFORD *et al.* [1] and THOMAS *et al.* [3] suggest that part of the binding requires the presence of calcium.

In conclusion, the present data report variations in the binding of human bronchial mucins to two strains of *Staphylococcus aureus* and, more importantly, they demonstrate the expression of surface adhesins binding to these bronchial mucins.

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