

The influence of A-band and B-band lipopolysaccharide on the surface characteristics and adhesion of *Pseudomonas aeruginosa* to surfaces

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***Pseudomonas aeruginosa* PAO1 possesses two distinct lipopolysaccharide (LPS) O-polysaccharide species, A- and B-band LPS, the relative expression of which appears to be under environmental control. In an attempt to identify the influence these LPS types have on surface characteristics and adhesion, we examined the surface hydrophobicity and surface charge of *P. aeruginosa* PAO1 (O5 serotype) and its isogenic LPS derivatives which possessed A⁺B⁻, A⁻B⁺ and A⁻B⁻ LPS. The surface characteristics of the strains affected their ability to adhere to hydrophilic (glass) and hydrophobic (polystyrene) surfaces. Cells possessing only A-band LPS demonstrated the highest surface hydrophobicity, followed by the strain lacking both A- and B-band LPS. The presence of B-band LPS resulted in a more hydrophilic surface. Strains lacking B-band LPS (A⁺B⁻ and A⁻B⁻) had more electronegative surfaces than those possessing B-band LPS (A⁺B⁺ and A⁻B⁺), with cells lacking both A- and B-band LPS showing the highest surface electronegativity. These data suggest that the main surface-charge-determining groups reside in the core region of the LPS molecule. Cells with the lowest surface hydrophobicity and lowest surface charge (A⁺B⁺, A⁻B⁺) adhered to glass the most efficiently, implying a role for electrostatic interaction, whereas adhesion to polystyrene mirrored the relative hydrophobicities of the strains (A⁺B⁻ > A⁻B⁻ > A⁺B⁺ > A⁻B⁺). It is postulated that phenotypic variation in the relative expression of A- and B-band LPS may be a mechanism by which *P. aeruginosa* can alter its overall surface characteristics in such a way as to influence adhesion and favour survival.**

Keywords: *Pseudomonas aeruginosa*, adhesion, biofilms, surface hydrophobicity, surface charge

INTRODUCTION

Bacterial adhesion to solid substrata has been demonstrated in numerous environments and is one of the initial steps in the formation of biofilms, which occur on virtually any surface exposed to bacterial populations (Costerton *et al.*, 1987). These include such diverse settings as industrial (Christensen & Characklis, 1990) and natural ecosystems (Dazzo, 1984; Lock, 1993), pipelines, and water distribution systems (Herson *et al.*, 1987). In addition, *Pseudomonas aeruginosa* is an important opportunistic human pathogen and is often found to be

the causative organism in device-associated infections involving urinary catheters (Nickel *et al.*, 1985; Warren, 1987), contact lenses (Miller & Ahearn, 1987; Ormerod & Smith, 1986) and prosthetic joints (Brause, 1989). It has been recognized that the physiology of cells growing within established biofilms varies significantly from that of their planktonic counterparts (Davies & Geesey, 1995; Giwerzman *et al.*, 1991; Kinniment & Wimpenny, 1992). Only recently, however, has it become evident that attachment to a surface can prompt changes in the expression of certain genes (Dagostino *et al.*, 1991; Davies *et al.*, 1993), although whether these changes are prompted by attachment *per se* or by indirect surface effects (e.g. pH changes, accumulation of degradation products at the surface) remains to be investigated. Non-specific surface

Abbreviation: HIC, hydrophobic interaction chromatography.

characteristics such as hydrophobicity and surface charge can profoundly influence the initial interactions with such surfaces (Gilbert *et al.*, 1991; Gordon & Millero, 1984; Stenstrom, 1989; Van Loosdrecht *et al.*, 1987a, b), as well as with eukaryotic cells (Kihlstrom & Magnusson, 1980; Walan & Kihlstrom, 1988), phagocytes (Absolom, 1988; Svanborg-Eden *et al.*, 1987) and proteins (Absolom, 1988).

The O-polysaccharide chain of LPS has been found to be an important determinant of non-specific surface properties, and many studies have demonstrated that loss of the O-polysaccharide chain can dramatically alter the overall surface charge and hydrophobic character of the Gram-negative cell surface (Williams *et al.*, 1986; Magnusson & Johansson, 1977; Palomar *et al.*, 1995). Most *P. aeruginosa* strains possess two chemically and immunologically distinct types of LPS, termed the A- and B-bands (Lam *et al.*, 1989; Rivera & McGroarty, 1989). The high molecular mass B-band defines the serotype of the strain, with the PAO1 (O5 serotype) subunit comprising two uronic acid derivatives and one *N*-acetylfucosamine residue (Knirel *et al.*, 1988). A-band contains shorter chains composed primarily of a polymer of $\alpha 1 \rightarrow 2$ -, $\alpha 1 \rightarrow 3$ -, $\alpha 1 \rightarrow 3$ -linked D-rhamnose and low levels of 2-keto-3-deoxyoctonic acid (3-deoxy-D-manno-2-octulosonic acid) (Arsenault *et al.*, 1991). It has been demonstrated that under certain growth conditions (e.g. *in vivo* in the cystic fibrotic lung), wild-type *P. aeruginosa* LPS undergoes phenotypic alterations such that the B-band LPS becomes entirely replaced with A-band LPS (Lam *et al.*, 1989; Hancock *et al.*, 1983). At present, the conditions necessary for this change are unknown.

The use of chemical mutagenesis and LPS-specific phages has generated isogenic *P. aeruginosa* strains in which the A⁺B⁺ phenotype has been altered to A⁺B⁻, A⁻B⁺ and A⁻B⁻ attributes (Lightfoot & Lam, 1991). In the present study, we have utilized these mutants to investigate the contribution of A- and B-band LPS to the overall surface character of the cell, and to determine the influence these characteristics have on adhesion to both hydrophilic and hydrophobic surfaces.

METHODS

Bacterial strains and maintenance. The *P. aeruginosa* strains used were PAO1 (H103) (wild-type: O5 B-band, A-band LPS; Hancock & Crey, 1979), AK1401 (A⁺B⁻; Berry & Kropinski, 1986), dps89 (A⁻B⁺; Kadurugamuwa *et al.*, 1993) and rd7513 (A⁻B⁻; Lightfoot & Lam, 1991). A chemically defined medium (CDM) was used for routine culture and consisted of 50 mM MOPS, 1.2 mM K₂HPO₄, 3.2 mM MgSO₄·7H₂O, 12 mM (NH₄)₂SO₄, 3 mM NaCl, 3 mM KCl, 3.2 mM FeSO₄·7H₂O and 10 mM glucose. The medium was supplemented with 1% (w/v) Casamino acids (vitamin-free), which were filter-sterilized and added to the autoclaved CDM. All bacterial strains were grown at 37 °C with vigorous shaking (200 r.p.m.) and harvested at mid-exponential phase (OD₄₇₀ = 0.6). All experiments were performed at room temperature (approximately 20 °C).

Determination of relative surface hydrophobicity. Relative surface hydrophobicity was determined using hydrophobic interaction chromatography (HIC) as first described by Smyth *et*

al. (1978). Octyl Sepharose CL-4B was used as the hydrophobic substrate with Sepharose CL-4B as control. Small columns (1 ml bed vol.) were constructed in the barrels of small transfer pipettes and held in place using a small circle (6 mm diameter) of Whatman no. 4 filter paper. The columns were rinsed with 3 × 2 ml sterile nanopure water followed by 5 × 2 ml of the appropriate saline solution (i.e. 0, 1, 2, 3 or 4 M NaCl buffered with 10 mM phosphate buffer, pH 7.4). All solutions had been previously passed through a 0.45 µm membrane filter. Mid-exponential-phase cells (50 ml; OD₄₇₀ = 0.6, approximately 4 × 10⁸ c.f.u. ml⁻¹) were harvested (20000 g, 10 min), washed in 0.1 M phosphate buffer (K₂HPO₄:KH₂PO₄, pH 7.4), re-centrifuged and resuspended in 2 ml of the appropriate saline solution buffered with 10 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of the cell suspension were added to the top of the columns and allowed to equilibrate for 15 min. The columns were then rinsed with 5 × 2 ml of the appropriate saline solution. The turbidities (OD₄₇₀) of the eluates from the hydrophobic and control columns were determined and hydrophobicities were expressed as the percentage retention to the Octyl Sepharose column relative to the control column (Sepharose CL-4B). It should be noted that no significant binding of cells to the control columns was found for any of the strains studied (data not presented).

Determination of relative surface charge. Relative surface charge was assayed using DEAE-Sepharose CL-6B, an anion-exchange resin, with Sepharose CL-6B as control. Previous studies have utilized anion-exchange resins to determine relative surface charge (Pendersen, 1981); however these methods did not utilize a control and did not, therefore, correct for non-specific trapping within the assay column. In the present study, binding to the positively charged beads, relative to identical beads lacking the charged ligand, was determined and thus gave a reliable measure of relative surface charge.

Before construction of the columns the DEAE-Sepharose beads were mixed with control beads in a ratio of 2:3 (DEAE beads to control beads) as previous experiments had shown that this led to increased sensitivity (data not presented). Columns were constructed and prepared as for HIC columns, as were cell suspensions. The eluting medium used was phosphate buffer (pH 7.4, 0–0.2 M). Aliquots (0.1 ml) of the cell suspensions were added to the columns and allowed to equilibrate for 15 min. The columns were rinsed with 5 × 2 ml of the appropriate phosphate buffer and the turbidity (OD₄₇₀) of the eluates was determined. The retention to the positively charged DEAE resin relative to the Sepharose CL-6B column was calculated and expressed as percentage of cells retained. Again, no significant retention of cells to the control columns was found (data not presented).

Light microscopy of cells bound to DEAE-Sepharose beads.

DEAE-Sepharose columns with cells attached were disassembled and the beads rinsed with 10 mM phosphate buffer (pH 7.4). Cells were fixed in 2.5% (v/v) glutaraldehyde (buffered with 10 mM phosphate buffer, pH 7.4) for 30 min at room temperature, rinsed twice with phosphate buffer, and post-fixed with 1% (w/v) osmium tetroxide (buffered with 10 mM phosphate buffer, pH 7.4) for 30 min at room temperature. The beads (with bound cells) were enrobed in Noble agar, dehydrated in a graded ethanol series, and embedded in LR White resin (Marivac). Ultrathin sections were prepared using a Sorvall MT1 microtome and stained with 1% (w/v) toluidine blue. Sections were examined and photographed with a Zeiss light microscope.

Adhesion assays

Adhesion to glass. Cell suspensions were prepared as for HIC and resuspended in a final volume of 2 ml 0.1 M phosphate buffer (pH 7.4). Sufficient suspension was added to 100 ml 0.1 M

phosphate buffer (pH 7.4, previously passed through a 0.45 µm filter membrane) such that the final cell suspension had an OD₄₇₀ of 0.75 (approximately 5×10^8 c.f.u. ml⁻¹).

Circular glass coverslips (10 mm diameter, Chance Proper) were soaked in concentrated nitric acid to remove any adsorbed contaminants and then rinsed five times in sterile nanopure water. The coverslips were carefully placed onto a stage made from an embedding mould with small incisions made in it, such that the coverslips were held vertically in a radial arrangement with little decrease in the surface available for bacterial adhesion. Eight coverslips were used for each strain in each experiment. The adhesion stage, with coverslips, was immersed in the cell suspension and the whole system was incubated at 37 °C and gently shaken (30 r.p.m.). At regular intervals, the glass coverslips were carefully removed using fine sterile forceps and placed directly into a vial containing 5 ml sterile 0.1 M phosphate buffer (pH 7.4) and 1 g previously washed and sterilized glass beads (diameter < 106 µm). The coverslips were vortexed at top speed on a Vortex Genie II mixer (Fisher Scientific) for 1 min to remove attached cells. Vortexing with glass beads for this time had previously been shown to remove all attached cells, but had no effect on cell viability (data not presented). Viable counts were then conducted (in quadruplicate) to determine the number of cells initially adhering.

Adhesion to hydrophobic polystyrene. Cell suspensions were prepared as for the glass adhesion assay. Whenever possible, adhesion to glass and to polystyrene was determined using cells derived from the same culture. To 50 ml cell suspension (approximately 5×10^8 c.f.u. ml⁻¹) was added 0.5 g hydrophobic polystyrene SM-2 Biobeads (Bio-Rad, mean size 75–150 µm) which had been previously washed and sterilized. An identical suspension, without Biobeads, served as a control. At regular time intervals, 1 ml aliquots of the suspensions were removed and passed through a small circle (6 mm diameter) of Whatman no. 4 filter paper held in a 3 ml syringe barrel to remove the Biobeads. Viable counts were conducted on the cell suspensions at various time intervals. From the drop in the cell number, relative to the control, it was possible to calculate the number of cells adhering to the beads.

Preparation of LPS. LPS was prepared by the proteinase K method described by Hitchcock & Brown (1983).

SDS-PAGE analysis and staining. The various LPS samples were analysed by SDS-PAGE using 12% (w/v) acrylamide gels (Lugtenberg *et al.*, 1975). Silver staining of the separated LPS bands was according to the method of Tsai & Frasch (1982).

Western immunoblotting. LPS samples were transferred from SDS-polyacrylamide gels to nitrocellulose and reacted with monoclonal antibody (mAb) MF 15-4 (specific for B-band serotype O5 LPS) or mAb N1F10 (specific for A-band polysaccharide). Bands were visualized using goat anti-mouse antibodies conjugated to horseradish peroxidase which was developed using 4-chloro-1-naphthol.

RESULTS

SDS-PAGE banding patterns and immunoreactivities of LPS samples

LPS samples from the *P. aeruginosa* strains were separated by SDS-PAGE and silver-stained to visualize the banding patterns (Fig. 1a). A characteristic ladder pattern can be seen for wild-type PAO1, with irregularities in the spacings and intensities of the bands. Immunoblotting of the PAO1 (A⁺B⁺) LPS with both A-band-specific and B-band-specific mAbs showed co-expression of the A-band

and B-band LPS (Fig. 1b, c). The banding pattern of the B-band LPS can be clearly seen at lower molecular masses; however at higher molecular mass values the bands are poorly resolved and reacted more as a smear. A-band units can be seen in a different position in the gels, with the spacing and intensity of the ladder pattern being much more regular than that of B-band LPS, confirming the results of earlier studies (Lightfoot & Lam, 1991; Rivera *et al.*, 1988). The silver-stained LPS profile for strain AK1401 (A⁺B⁻) also showed the ladder-like bands but these were not as extensive as those for PAO1 (A⁺B⁺), indicating that the bands were of lower molecular mass than B-band LPS.

Immunoblots of LPS from strain dps89 (A⁻B⁺) indicated that the high molecular mass material was B-band LPS and confirmed that A-band was not present. Conversely, the immunoblot of LPS derived from AK1401 confirmed the presence of A-band LPS only, without the expression of B-band LPS. Silver-stained LPS from rd7513 (A⁻B⁻) lacked the ladder pattern and did not react with either A-band- or B-band-specific mAbs, confirming the absence of A- and B-band LPS.

Relative surface hydrophobicity of *P. aeruginosa* and its LPS derivatives

The hydrophobicity of the cell surface of *P. aeruginosa* strains was investigated using HIC, at a range of ionic strengths, and the results are presented in Fig. 2. A similar pattern was seen at all salt concentrations above 1 M NaCl, with the strain possessing only A-band LPS (AK1401) having the most hydrophobic surface (effectively 100% retention), followed by the 'rough' mutant (rd7513, A⁻B⁻). Both strains possessing B-band LPS (PAO1, A⁺B⁺; dps89, A⁻B⁺) had hydrophilic surfaces. At all ionic strengths ≥ 1 M NaCl, the strain possessing just B-band LPS (dps89) was the most hydrophilic. The fact that the wild-type organism (PAO1, A⁺B⁺) had a mean surface hydrophobicity between AK1401 (A⁺B⁻) and dps89 (A⁻B⁺) would suggest that the overall hydrophobicity is a balance between the strongly hydrophobic nature of A-band LPS and the hydrophilic nature of B-band LPS. When a 'salting out' agent [2 M (NH₄)₂SO₄] was used as the eluting medium, the percentage retention to the Octyl Sepharose was accordingly higher, but the previous order was maintained (i.e. A⁺B⁻ > A⁻B⁻ > A⁺B⁺ > A⁻B⁺, data not presented).

When HIC is employed to study surface hydrophobicity of relatively hydrophilic organisms, high salt concentrations are used in an attempt to 'cloak' charged groups on the cell surface and maximize hydrophobic interactions. Thus, the increased binding of cells to the hydrophobic column when 10 mM phosphate buffer alone was used was unexpected.

Surface charge characteristics of *P. aeruginosa* and its LPS derivatives

The relative surface charge of *P. aeruginosa* and its LPS derivatives was investigated using a modification of

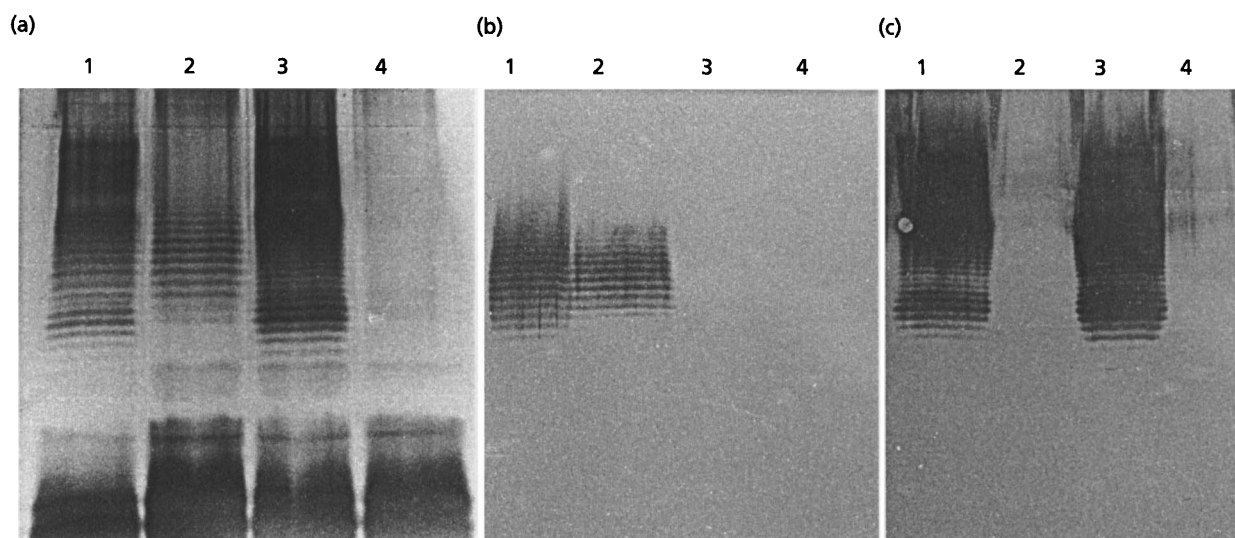


Fig. 1. (a) Silver-stained SDS-polyacrylamide gel of LPS from *P. aeruginosa* PAO1 (A^+B^+) (lane 1), AK1401 (A^+B^-) (lane 2), dps89 (A^-B^+) (lane 3) and rd7513 (A^-B^-) (lane 4). Western immunoblots of these LPS profiles were reacted with A-band-specific mAb (b) and B-band-specific mAb (c).

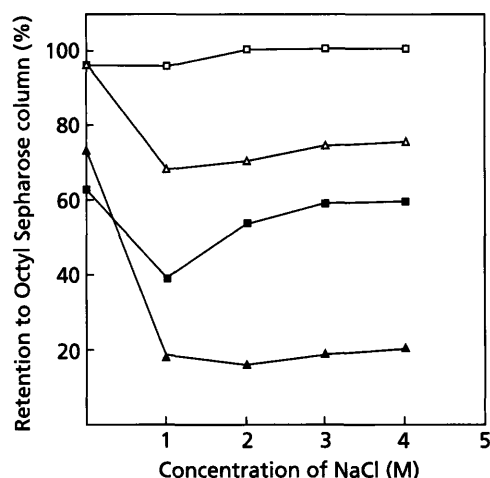


Fig. 2. Relative cell surface hydrophobicities of *P. aeruginosa* PAO1 (A^+B^+) (■) and its LPS derivatives AK1401 (A^+B^-) (□), dps89 (A^-B^+) (▲) and rd7513 (A^-B^-) (△) as determined by HIC. Relative hydrophobicities are expressed as the percentage of cells retained on the hydrophobic Octyl Sepharose column relative to a control Sepharose CL-4B column. The experiment was performed three times and a representative result is shown.

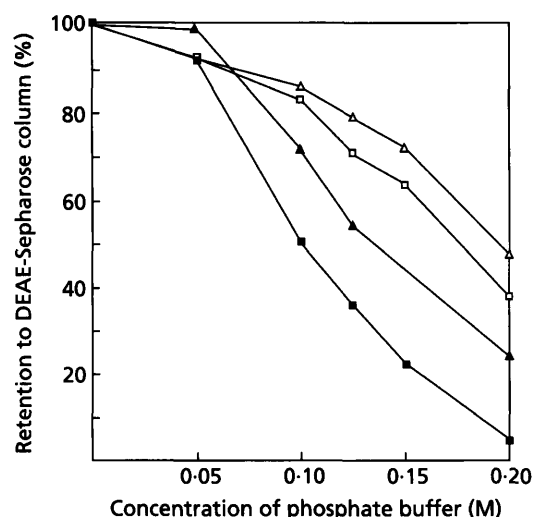


Fig. 3. Relative cell surface charge of *P. aeruginosa* PAO1 (A^+B^+) (■) and its LPS derivatives AK1401 (A^+B^-) (□), dps89 (A^-B^+) (▲) and rd7513 (A^-B^-) (△) as determined by electrostatic interaction chromatography. Relative surface charges are expressed as the percentage of cells retained on a positively charged DEAE-Sepharose column relative to a control Sepharose CL-6B column, at a range of ionic strengths. The higher the retention values, the higher the cell surface electronegativity. The experiment was performed three times and a representative result is shown.

electrostatic interaction chromatography. The binding of cells to a positively charged ligand (DEAE-Sepharose CL-6B) relative to Sepharose CL-6B, at a range of ionic strengths, is presented in Fig. 3.

At low ionic strengths (< 50 mM phosphate buffer) there was effectively 100% retention of all strains to the charged columns. As the ionic strength of the eluting medium increased, however, there was a gradual decrease in the percentage of cells retained, due to the competition

between anion groups on the cell surface and those present in the eluting medium (phosphate). The decrease was, however, dependent upon the LPS composition. PAO1 cells (A^+B^+) were eluted most readily from the charged column, followed by the strain possessing only B-band LPS (dps89, A^-B^+). Both strains lacking B-band LPS (rd7513, A^-B^- ; AK1401, A^+B^-) were retained to the

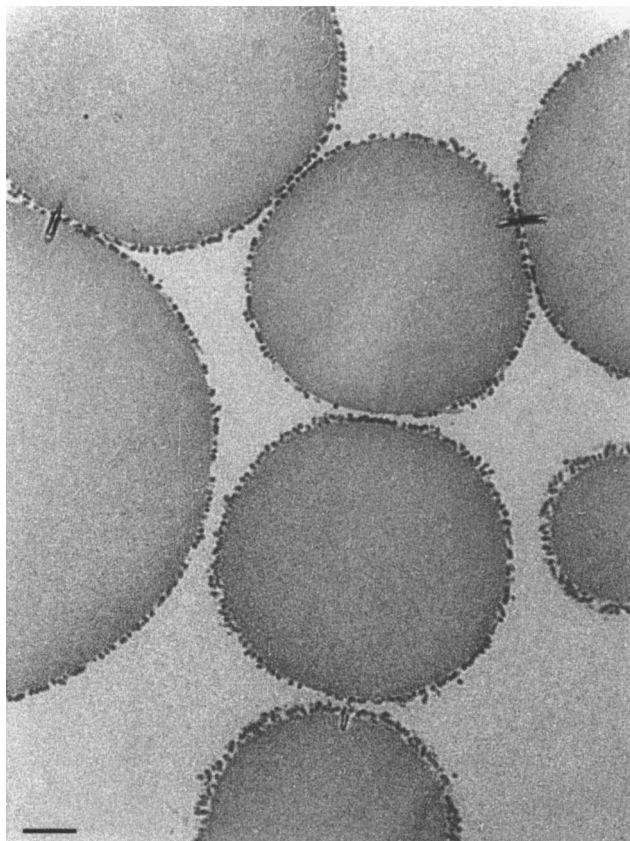


Fig. 4. *P. aeruginosa* rd7513 (A^-B^-) attached to the surface of positively charged DEAE-Sepharose beads. Beads with attached cells were fixed, dehydrated, embedded in resin and sectioned. Sections were stained with toluidine blue and examined using light microscopy. Cells can be seen to form uniform layers on the surface of the beads. Bar, 10 μ m.

greatest extent. The fact that there is competition for the charged groups on the beads would suggest that the number of anionogenic groups exposed per unit surface area is greatest for the strain lacking both A- and B-band LPS (rd7513, A^-B^-), and lowest for the wild-type strain (PAO1, A^+B^+). At a phosphate buffer concentration of 0.2 M, only 4.2% of PAO1 cells were retained by the column, compared to 46.8% of the 'rough' mutant (rd7513, A^-B^-).

Since the most hydrophobic strains (rd7513, A^-B^- ; AK1401, A^+B^-) bound to the charged column the most effectively, co-operative binding could be responsible (i.e. multiple layers of cells binding due to hydrophobic cell-cell interactions). To investigate this hypothesis, beads with cells attached were fixed, dehydrated, embedded in resin and sectioned. The sections were stained with toluidine blue and examined under the light microscope. Fig. 4 shows beads which have been 'saturated' with cells by loading approximately 10 times the number of cells routinely loaded, and is representative of all four strains. Binding of cells was primarily as a monolayer with almost complete coverage of the bead surface. No co-

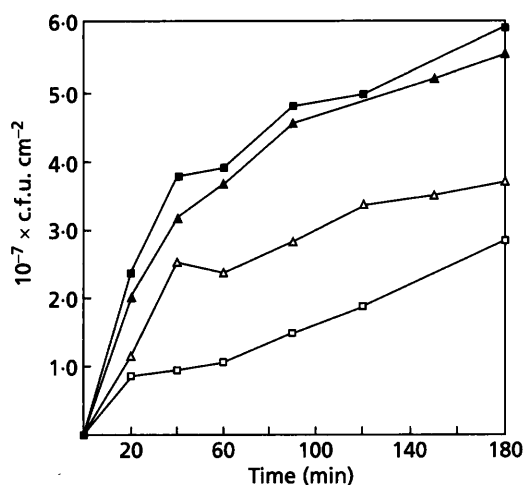


Fig. 5. Adhesion to glass of *P. aeruginosa* PAO1 (A^+B^+) (■) and its LPS derivatives AK1401 (A^+B^-) (□), dps89 (A^-B^+) (▲) and rd7513 (A^-B^-) (△). Cleaned glass coverslips were placed in washed cell suspensions (approximately 5×10^8 c.f.u. ml $^{-1}$) and incubated at 37 °C. At regular intervals, coverslips were removed and the number of cells adhering was determined by vortexing with glass beads and viable counting. The experiment was performed three times and a representative result is shown.

operative binding was seen, even for the most hydrophobic strains. These results indicate that the extent of binding to the positively charged beads is mediated primarily by charge-charge interactions.

Adhesion to glass

The adhesion of PAO1 and its LPS derivatives to glass was determined using nitric-acid-cleaned glass coverslips as a substratum. The number of cells adhering was determined by removal of attached cells and viable counting. Results are presented in Fig. 5. Adhesion to glass was rapid for all strains (up to 2.4×10^7 c.f.u. cm $^{-2}$ in 20 min). The extent of adhesion was, however, dependent upon the LPS composition of the cell surface. Strains possessing B-band LPS (PAO1, A^+B^+ ; dps89, A^-B^+) adhered to the greatest extent; after 180 min, in strains possessing B-band LPS, the numbers adhering were approximately double when compared to those lacking B-band LPS (AK1401 and rd7513). The presence or absence of A-band LPS seemed to have little influence on the tendency to adhere. The fact that there was no significant difference between PAO1 (A^+B^+) and dps89 (A^-B^+) would again suggest that the presence of B-band LPS was the main mediator of adhesion to glass.

Adhesion to polystyrene

The adhesion of cells to hydrophobic polystyrene occurred extremely rapidly (Fig. 6). The number of cells attached to the Biobeads rose sharply in the first 10 min and reached a plateau value within 20 min. The extent of adhesion was, again, highly dependent upon the LPS

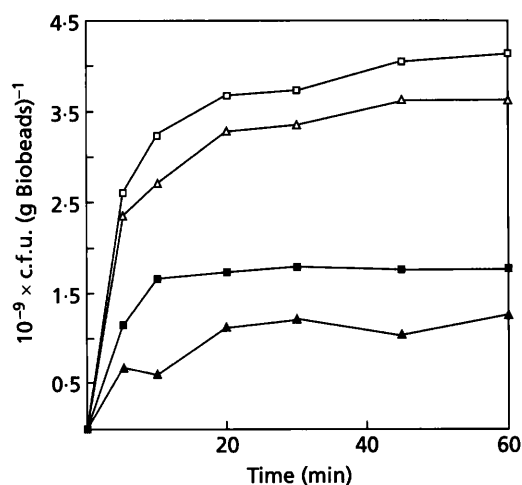


Fig. 6. Adhesion to polystyrene of *P. aeruginosa* PAO1 (A⁺B⁺) (■) and its LPS derivatives AK1401 (A⁺B⁻) (□), dps89 (A⁻B⁺) (▲) and rd7513 (A⁻B⁻) (△). Washed cell suspensions (approximately 5×10^8 c.f.u. ml⁻¹) were incubated (37 °C) with hydrophobic SM2 Biobeads. At regular intervals, 1 ml aliquots of the suspension were taken and the Biobeads were removed by filtration. The number of cells attaching was calculated from viable counting of suspended cells, relative to a suspension without Biobeads. The experiment was performed three times and a representative result is shown.

composition of the cell surface; the greatest adhesion was seen for the strain possessing A-band LPS only (AK1401) followed by the 'rough' mutant lacking both A- and B-band LPS. Strains possessing B-band LPS (PAO1, A⁺B⁺; dps89, A⁻B⁺) adhered less well. It is interesting to note that phenotypic removal of B-band LPS from wild-type cells resulted in adhesion to polystyrene increasing by almost 240%.

The adhesion to polystyrene exactly mirrored the hydrophobicity data (i.e. A⁺B⁻ > A⁻B⁻ > A⁺B⁺ > A⁻B⁺) and strongly suggested that the cell surface hydrophobicity *per se* determines the extent of adhesion to a hydrophobic substratum.

DISCUSSION

In the present study, major differences were found between the physicochemical characteristics of *P. aeruginosa* A- and B-band LPS. The parent PAO1 strain (A⁺B⁺) was hydrophilic when compared to the 'rough' mutant lacking both A- and B-band LPS (rd7513). This is presumably due to the exposure of hydrophobic substituents of outer-membrane components such as phospholipids and proteins, and is supported by previous studies which have shown that variations in the O-antigen chain length, and complete loss of the O-antigen, can profoundly affect the cell surface hydrophobicity of Gram-negative bacteria (Hermansson *et al.*, 1982; Palomar *et al.*, 1995; Williams *et al.*, 1986) (it should be noted that no significant differences were found between the outer-membrane protein profiles of the strains used; data not presented). The finding that possession of A-band LPS

alone resulted in a more hydrophobic surface than cells lacking any O-polysaccharide chains would appear to be in contrast to previous studies. In *P. aeruginosa* PAO1, however, A-band LPS has been found to consist of short chains of predominantly neutral polysaccharides (D-rhamnose, and lesser amounts of 3-O-methyl-rhamnose, ribose, glucose and mannose; Arsenault *et al.*, 1991). Thus, a relatively hydrophobic LPS O-polysaccharide chain may shield charged groups closer to the outer membrane (2-keto-3-deoxyoctonic acid, phosphate and sulphate) and result in a higher overall hydrophobicity.

The presence of B-band LPS was found to result in a low surface hydrophobicity. B-band LPS has been found to contain an unusually high number of phosphate groups (approximately 12 per molecule) and amino sugars (Wilkinson, 1983), in contrast to A-band LPS which is effectively lacking in phosphate groups (but does contain lesser amounts of sulphate; Rivera & McGroarty, 1989). Indeed, results from our laboratory have demonstrated that the outer membrane of strains with only A-band LPS do not easily stain with electron-dense uranyl ions (UO₂²⁺) during the preparation of specimens for electron microscopy, whereas strains that possess B-band LPS stain darkly because of their abundant electronegative sites (Lam *et al.*, 1992). Although the high phosphate content of B-band LPS would explain why this LPS was highly hydrophilic in nature, it would seem to conflict with the data obtained using electrostatic interaction chromatography in which both strains possessing B-band LPS were retained least by the positively charged resin. Cells lacking B-band LPS demonstrated the highest surface electronegativity, with the rough mutant (rd7513, A⁻B⁻) possessing the most highly electronegative surface. [It should be noted that, although the combination of a high surface potential and a hydrophobic surface (i.e. rd7513) would seem to be contradictory, the charged groups occupy only a minor fraction of the total surface area of the exterior outer-membrane face (approximately 8%, Van Loosdrecht *et al.*, 1987b).]

One explanation for the observed charge characteristics is that the main surface-charge-determining substituents reside in the core/lipid A region of the LPS, which is especially high in phosphate (Peterson *et al.*, 1985). The O-side-chain of B-band LPS has been found to be significantly longer than that for A-band LPS (Rivera & McGroarty, 1989) and electron microscopical examination of freeze-substituted cells would support the hypothesis that the LPS polysaccharide chains extend perpendicularly from the surface of the cell (Lam *et al.*, 1992). Thus, the charge originating from phosphate groups in the core/lipid A may be 'shielded' by O-polysaccharide chains, which would be stabilized with divalent cations in the case of B-band LPS (Peterson *et al.*, 1985) and the charge at the environmental interface of the bacterium lowered (estimated to extend 31–36 nm from the outer-membrane bilayer in *P. aeruginosa*; Lam *et al.*, 1992). If this were the case, the decrease in the charge originating from the core/lipid A in cells possessing only A-band LPS would be less than that for cells possessing only B-band LPS, due to the shorter length of A-band

LPS. This was found to be the case, and the fact that complete removal of A- and B-band O-side-chains resulted in maximal binding to the positively charged groups would support the hypothesis that the major charge-determining species reside in the LPS core/lipid A. These findings are supported by a previous study in which the retention of rough and smooth *Salmonella typhimurium* on an anion-exchange resin was examined (Hermansson *et al.*, 1982). These authors found that loss of the O-side-chain significantly increased the binding to the charged column.

Many authors have attempted to apply colloid models such as the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory to explain the adhesion of bacteria to surfaces (Busscher *et al.*, 1984; Gilbert *et al.*, 1993; Marshall *et al.*, 1971; Rutter & Vincent, 1980; Van Loosdrecht *et al.*, 1990). In this model, if contact pressure and temperature are constant and the molecular composition of the surface remains constant, then it is valid to assume that there is an inverse correlation between bacterial surface free energy and hydrophobicity (Busscher *et al.*, 1984). In addition, as the bacterial cell surface and most substrata in natural environments possess net negative charges, one of the most obvious parameters influencing adhesion would seem to be surface electronegativity (Rutter & Vincent, 1980). In recent years, it has thus become evident that correlations exist between adhesion to surfaces and non-specific surface characteristics such as surface charge and hydrophobicity (Gilbert *et al.*, 1991; Gordon & Millero, 1984; Hermansson *et al.*, 1982; James *et al.*, 1985; Rainey, 1991; Van Loosdrecht *et al.*, 1987a, b).

In the present study, the surface characteristics primarily responsible for adhesion were dependent upon the physicochemical characteristics of the substratum. When glass was used as a hydrophilic, negatively charged surface, the greatest adhesion was seen for the strains possessing B-band LPS (PAO1 and dps89). These strains were the most hydrophilic and had the lowest cell surface charge. Strains AK1401 and rd7513 (A^+B^- and A^-B^- , respectively), which were more hydrophobic and had higher negative surface charges, adhered to glass less well. These data would imply that the surface charge was the main mediator of adhesion to a hydrophilic surface, the decreased surface electronegativity accounting for the increased adhesion to the negatively charged surface. For hydrophobic polystyrene, however, higher adhesion was seen for the more hydrophobic strains (AK1401, A^+B^- ; rd7513, A^-B^-) and the results obtained exactly mirrored the HIC data ($A^+B^- > A^-B^- > A^+B^+ > A^-B^+$).

These findings are supported by Van Loosdrecht *et al.* (1987b), who determined the hydrophobicities, electrophoretic mobilities and adhesion characteristics of 23 bacterial strains and concluded that for bacteria with hydrophobic cell surfaces, hydrophobicity was the primary mediator of adhesion to a negatively charged, hydrophobic surface, whereas for cells possessing more hydrophilic surfaces, surface charge played an increasingly important role. Gilbert *et al.* (1991) also concluded that for *Escherichia coli* there was a good correlation between

adhesion to glass and electronegativity, but not hydrophobicity.

Paul & Jeffrey (1985) found evidence for separate adhesion mechanisms for hydrophilic and hydrophobic surfaces in *Vibrio proteolytica* and proposed that proteins were involved in the latter. They found that various proteolytic enzymes and surfactants decreased cell surface hydrophobicity and inhibited (> 97%) the attachment of *V. proteolytica* to hydrophobic polystyrene, but had no effect on adhesion to hydrophilic glass. Conversely, Van Haeke *et al.* (1990) quantified the surface hydrophobicities of 15 *P. aeruginosa* strains and examined the adhesion to electropolished stainless steel. In contrast to our present study, they found a correlation only between hydrophobicity (as determined by contact angle measurement and bacterial adhesion to hydrocarbons) and adhesion, although the authors failed to investigate the LPS characteristics of the strains used. No correlation was found between surface charge and adhesion. Miller & Ahearn (1987) also found greater adhesion of *P. aeruginosa* to hydrophobic contact lenses when compared to hydrophilic contact lenses, although no attempt was made to characterize the bacterial cell surface hydrophobicity or charge.

In *P. aeruginosa*, the relative amounts of A- and B-band LPS produced on the cell surface appear to be under environmental control. In the present study we have shown that both surface charge and cell surface hydrophobicity are profoundly influenced by the expression of these two LPS types which ultimately help control their adhesion to hydrophobic and hydrophilic surfaces. In the wild-type organism, the hydrophobic A-band LPS would presumably be shielded by the longer, and more hydrophilic, B-band LPS. Conformational changes in the B-band structure (i.e. dynamic folding), however, could expose the more hydrophobic A-band LPS. If these conformational changes were prompted by attachment to a surface, it may aid in the firm attachment of the bacterium. In addition, the distribution of A-band and B-band LPS upon the cell surface is, at present, unclear and it is not known if the LPS types are randomly distributed or if distinct domains, enriched in A-band or B-band LPS, exist. If such domains were to exist, they would offer the possibility of distinct areas on the cell surface which would favour attachment to either hydrophilic or hydrophobic surfaces. Conversely, if the distribution of the different LPS types was random and disperse, it may be that attachment to a surface would prompt migration of a particular LPS type to the point of attachment in such a way as to strengthen the attachment. Attachment to surfaces with subsequent colonization and growth as a biofilm offers distinct survival advantages when compared to planktonic growth. These advantages may be due to accumulation of nutrients at the hydrodynamic boundary layer (Fletcher, 1992), modulation of the physicochemical microenvironment (Davies *et al.*, 1993; Davies & Geesey, 1995) and increased resistance to antimicrobial agents (Anwar *et al.*, 1990; Gilbert *et al.*, 1990; Nickel *et al.*, 1985). We propose, therefore, that changes in surface characteristics which favour adhesion to surfaces,

mediated by alterations in the LPS composition and/or configuration of the outer membrane, may constitute an important adaptation and survival strategy for *P. aeruginosa* and, possibly, other Gram-negative bacteria.

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