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Characterization of the Binding of *Pseudomonas aeruginosa* Alginate to Human Epithelial Cells

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The alginate produced by *Pseudomonas aeruginosa* has been reported to play a role in the adhesion of this bacterium to epithelial cell surfaces, although some controversy concerning this role exists. To clarify this controversy, we investigated the ability of alginate to bind to human buccal epithelial cells (BECs) and human tracheal epithelial cells (TECs). Alginate from *P. aeruginosa* 492c bound to both BECs and TECs. Alginate from strain 492c was found to be multivalent and thus capable of agglutinating both BECs and TECs. The multivalency of alginate complicated the determination of the number of alginate-specific receptors on the BEC and the apparent association constant (K_a). By using the analysis of Hogg and Winzor (Biochim. Biophys. Acta 843:159–163, 1985), an average valency of 2.6 BEC binding domains per alginate molecule was determined, and the maximum binding capacity per BEC was calculated to be 5.8×10^{-4} μg, with a K_a of 4.1×10^{-2} ml/μg. The binding of alginate to immobilized BECs (where only 50% of the BEC surface is exposed) yielded values of 2.52×10^{-4} μg of alginate per BEC for the maximum binding capacity per BEC and a K_a of 3.30×10^{-2} ml/μg. The alginate-specific site on the BEC surface was trypsin sensitive. Alginate from *P. aeruginosa* 492a did not bind to BECs, differing substantially from that of strain 492c. The data presented here demonstrate that alginate purified from some strains of *P. aeruginosa* may bind to TECs and BECs in a defined, specific manner, whereas alginate from other strains does not, reflecting structural diversity in *P. aeruginosa* alginates.

Mucoid strains of *Pseudomonas aeruginosa* constitute the majority of the strains isolated from cystic fibrosis (CF) patients with chronic pulmonary infections caused by this bacterium (26). The mucoid substance produced by these strains is primarily composed of an alginatelike polymer composed of D-mannuronic acid and its 5′ epimer, L-guluronic acid, polydispersed within the polymer, producing heterogeneous and homogeneous sections in the primary structure (6, 13, 16, 27). The D-mannuronic acid residues may be O acetylated, but the degree of acetylation differs from strain to strain (6, 16). Also, the percent composition of, or molar ratio of, D-mannuronic acid and L-guluronic acid in the alginic acid differs depending on the strain (6). It has also been demonstrated that alginic acid isolated from different strains is immunologically heterogeneous, and different epitopes are readily identified (22).

The initial phase of a chronic pulmonary infection in CF patients is thought to be the adhesion to and subsequent colonization of the buccal mucosa by *P. aeruginosa*, which then serves as a reservoir for a descending pulmonary infection (11, 28). Generally, nonmucoid strains are isolated early in the infection, and during the course of the infection the predominant phenotype changes from nonmucoid to mucoid (21). The mucoid phenotype also appears to be associated with the more severe cases of pulmonary infection in CF patients (21, 26). Even though *P. aeruginosa* can be eradicated from the lung, it is rarely completely cleared from the sputum or the upper respiratory tract of the CF patient (19). The persisting forms of *P. aeruginosa* may serve as a reservoir for recurrent lower respiratory tract infections.

McEachran and Irvin (17) have previously demonstrated that alginate plays a role in the adhesion of *P. aeruginosa* 492c to human buccal epithelial cells (BECs). However, McEachran and Irvin (unpublished data) have also described a mucoid strain (492a) that does not appear to use its alginate in the adhesion process. Further, Ramphal and Pier (23) have demonstrated that the mucoid exopolysaccharide binds to and enhances the binding of a number of *P. aeruginosa* strains to acid-injured mice trachea. However, neither of these investigators characterized the binding of the alginate exopolysaccharide to epithelial cells.

We kinetically analyzed the binding of alginate purified from *P. aeruginosa* 492c to human BECs by using a Langmuir adsorption isotherm and examined the agglutination of BECs and human tracheal epithelial cells (TECs) by using alginate. The results indicated that alginate from this strain binds to untrypsinized BECs and TECs in a specific and defined kinetic manner but not to trypsinized BECs. We also examined the binding of alginate derived from *P. aeruginosa* 492a to BECs and found that alginate from strain 492a did not bind to BECs, confirming the observations of McEachran and Irvin (17) and establishing a functional diversity in alginates.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *P. aeruginosa* 492c and 492a have been previously described (10) and were originally isolated from the sputum of a CF patient (10). Strain 492c is a stable, mucoid, antibiotic-hypersusceptible strain, and strain 492a is a stable mucoid strain that is highly resistant to antibiotics (10). Strains were maintained on brain heart infusion agar (Difco Laboratories) slants at −70°C and were routinely cultured on brain heart infusion agar. A single colony was used to inoculate 10 ml of M-9 medium (1), which was incubated at 37°C for 8 h with shaking at 150 rpm in a New Brunswick Scientific Co. Gyrotory shaker. This culture was the source of a 2% (vol/vol) inoculum for 500 ml of M-9

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which was cultured, as described above, for 17 h. In order to label the alginate, the culture was grown in M-9 medium for 14 h, supplemented with either 0.1 μCi of L-[^{35}S]methionine per ml (typical specific activity, 1,100 mCi/mmol) or 0.5 μCi of [^{14}C]sodium acetate per ml (typical specific activity, 56 mCi/mmol) (New England Nuclear Corp.)–0.2 μg of unlabeled sodium acetate per ml (Sigma Chemical Co.), and then incubated for an additional 3 h. The labeled *P. aeruginosa* cells were then harvested by centrifugation (6,000 × *g* for 20 min at 4°C), and the exopolysaccharide was purified from the supernatant.

Purification of alginate. The supernatant from a 500-ml culture of *P. aeruginosa* was brought to pH 1.7 with 4 N HCl and incubated at 37°C for 1 h. The previous step assured that the alginate was fully protonated. Disassociated and denatured material was then removed from the supernatant by centrifugation (12,000 × *g* for 20 min at 4°C). The supernatant was then brought to pH 8.0 with 4 N NaOH, and the alginate was precipitated by the addition of an equal volume of cold redistilled acetone. This mixture was kept at −20°C for 30 min, and the precipitate, which contained the crude alginate, was collected by centrifugation (12,000 × *g* for 20 min at −20°C). The crude alginate was suspended in a minimum volume of double distilled water. Sodium dodecyl sulfate (SDS) (Bio-Rad Laboratories) was added to a final concentration of 0.1% (wt/vol), and the crude alginate was then heated for 30 min at 80°C in a water bath. After the alginate was cooled to room temperature, proteinase K (Sigma) was added to a final concentration of 50 μg/ml and incubated at 37°C for 2 h. The solution was then adjusted to pH 1.7 with 4 N HCl and incubated for 1 h at 37°C. Disassociated and denatured material was then removed by centrifugation (12,000 × *g* for 20 min at 4°C). The alginate was exhaustively dialyzed against warm tap water, dialyzed against distilled water to remove any remaining SDS and low-molecular-weight impurities, and finally lyophilized.

BECs. Human BECs were collected with wooden applicator sticks from healthy, nonsmoking, male volunteers (n = 10). BECs were removed from the applicator sticks by agitation in 0.01 M sodium phosphate-buffered saline (PBS), pH 7.2. The BECs were washed three times (2,000 × *g* for 10 min at 4°C) with PBS, pH 7.2. BECs were suspended in two portions, cells used "as is" in the binding assays and cells that were first trypsinized. BECs were trypsinized by the addition of trypsin (Sigma) to a final concentration of 50 μg/ml and incubated for 30 min at 37°C. Trypsinized BECs were then washed three times with PBS, pH 7.2. The cell concentrations for both untrypsinized and trypsinized BECs were determined with a hemacytometer, and the BEC concentrations were adjusted to 2.0 × 10^5 cells per ml.

Human TECs. Human TECs were obtained by bronchoscopic brushing of the tracheal bronchial mucosa. Bronchoscopy was conducted in two distinct groups of patients, with subtle differences in technique between these groups. All procedures were approved by the Toronto General Hospital Ethics Committee, and informed consent was obtained from relatives or from the patients themselves.

TECs were obtained from intubated-ventilated patients in the Intensive Care Unit. There were no patients with tracheostomies. Because most of these patients were conscious, the procedure was preceded by intravenous injection of diazepam (5 to 10 mg) and the injection of 5 ml of 2% xylocaine down the airway. Bronchoscopy was performed with a flexible Olympus Type 2 BF bronchoscope inserted through an endotracheal tube. A cytology brush was passed through the suction channel and was used to abrade the tracheal-bronchial mucosa. The bronchoscope and brush were removed after each brushing to avoid loss of epithelial cells by withdrawing the brush through the suction channel itself. The cells were eluted from the bronchial brush by agitation in high-glucose Dulbecco modified Eagle medium containing 1% sodium citrate and were kept at 4°C. A total of 10 brushings were performed on each patient.

A group of volunteers served as a control group. After informed consent was obtained, 2% xylocaine spray was administered to a single nares, posterior pharynx, and larynx. No sedative was administered. The Olympus Type 2 BF flexible bronchoscope was passed through the nose and directed through the larynx. An additional 5 ml of 2% xylocaine was administered through the bronchoscope into the trachea. Brushing of the tracheal mucosa was undertaken for all 10 brushings as noted above, with the exception that the brush was withdrawn each time through the suction channel to avoid subjecting the volunteer to the reintroduction of the bronchoscope.

TECs were isolated from contaminating blood cells and mucus as previously described (6a). Epithelial cell suspensions were briefly vortexed (half speed). The cell suspensions were then passed through a 70-μm-pore-size mesh, followed by passage through a 30-μm-pore-size mesh to remove cell clumps and mucus. The cells were then washed twice with PBS, pH 7.2 (500 × *g* for 15 min at 4°C), and resuspended in 1 ml of PBS, pH 7.2. The cell suspension was then placed on a PBS (pH 7.2)-preformed (48,000 × *g* for 40 min at 4°C) 65% (vol/vol) percoll gradient and centrifuged at 500 × *g* for 30 min at 4°C. The cell band just above the percoll-aqueous portion interface was collected and placed on a second 65% (vol/vol) percoll gradient and centrifuged as described above. The cell band just above and the percoll-aqueous portion interface was collected and washed twice with PBS, pH 7.2. Cell numbers were determined with a hemacytometer.

Binding of alginate from strain 492c to free BECs. BECs (1.0 ml at 2.0 × 10^5 cells per ml) or TECs (0.5 ml at 10^5 cells per ml) were mixed with an equal volume of ^{14}C-labeled alginate suspended in PBS (pH 7.2) in a 15-ml polystyrene test tube and incubated for 1 h. The amount of alginate added to each tube varied from 10 to 100 μg/ml. With TECs, a fixed concentration of alginate was added (50 μg/ml) due to the limited number of cells available. BECs and TECs with bound alginate were then collected by filtration on 5.0-μm-pore-size polycarbonate filters and on 12.0-μm-pore-size filters (Nuclepore), respectively, washed with 15 ml of PBS (pH 7.2), and then dried in scintillation vials. Omnifluor (5 ml) (New England Nuclear Corp.) was added to each vial, and the amount of radioactivity was determined by scintillation counting by using a Beckman LS-150 scintillation counter or by planchet counting. All binding assays were performed in triplicate. Binding of alginate to BECs was corrected for nonspecific binding of the alginate to the 5.0-μm-pore-size filter. BEC concentration was determined at the end of the assay to correct for BECs lost during incubation. In order to reduce nonspecific binding of alginate, the filters were pretreated with 0.5 ml of 1% (wt/vol) bovine serum albumin (BSA) in PBS (pH 7.2) for 10 min, washed with 15 ml of PBS (pH 7.2), and then incubated with 1.75 ml of kelp alginate (100 μg/ml) (Sigma) in double distilled water for 15 min. The reaction mixture was applied directly to the filter with the vacuum operating. Typically, the nonspecific binding values were <15% of the experimental values.

Alginate-BEC equilibrium assay. A time course study to

determine when maximum binding of alginate from strain 492c to BECs occurred was performed. Equal volumes (1.0 ml) of BECs (10^5 BECs per ml) and ^{35}S-labeled alginate (100 μg/ml) from strain 492c were mixed and incubated at 37°C with shaking at 300 rpm. Starting at time zero (at mixing), and every 15 min after for a period of 2 h, a tube was removed, and the amount of alginate bound per BEC was assayed as described above.

In order to determine the reversibility of alginate binding, equal volumes (1.0 ml) of BECs (10^5 BECs per ml) and ^{35}S-labeled alginate (100 μg/ml) from strain 492c were mixed and incubated as described above for 1 h. The BECs were then collected by centrifugation (2,000 × *g* for 10 min at 4°C), washed once with PBS (pH 7.2), and resuspended in 1 ml of PBS (pH 7.2). To the BECs with bound, ^{35}S-labeled alginate, 1 ml of unlabeled alginate from strain 492c in PBS (pH 7.2) (concentration range, 0 to 100 μg/ml) was added, these mixtures were incubated as described above for 1 h, and the amount of alginate bound per BEC was determined as described above.

Agglutination assay. BECs (0.5 ml of trypsinized or untrypsinized cells at 2.0×10^5 cells per ml) or TECs (0.5 ml at 10^5 TECs per ml) were mixed with an equal volume of alginate suspended in PBS (pH 7.2) in a 15-ml polystyrene test tube and incubated with an equal volume of alginate in PBS (pH 7.2) for 1 h at 37°C with shaking at 300 rpm. The amount of alginate added to each tube varied from 0 to 100 μg/ml. A sample of the reaction mixture was then placed on a slide, the percentage of BECs that had formed clumps per 10 fields per slide (five slides were examined) was determined compared with the control, and the level of significance was determined by the Kruskal-Wallis nonparametric test (12).

Solid phase assay for the binding of alginate to BECs. The solid phase assay of McEachran and Irvin (18) was modified for determining the binding of alginate to BECs. Tissue culture plates (24-well) were washed with double distilled water and then were coated with poly-L-lysine (1.75 ml of poly-L-lysine [400 μg/ml] [molecular weight, 525,000; Sigma] in double distilled water) by drying the poly-L-lysine onto the wells overnight at 60°C. The wells were then washed three times with PBS (pH 6.9). To each well, 0.5 ml of 20% (vol/vol) aqueous glutaraldehyde (grade II; Sigma) was added, and the plate was incubated at 37°C for 1 h. Each well was incubated twice at room temperature with 1.0 ml of PBS (pH 6.9), followed by four washes with PBS (pH 6.9) to remove remaining glutaraldehyde. BECs (0.5 ml of cells at 2.0×10^5 cells per ml) were added to each well and incubated at 37°C for 2 h at 75 rpm in a Gyrotory shaker. Unattached BECs were removed, and the wells were washed three times with PBS (pH 6.9). In order to block remaining free aldehyde groups, 1.0 ml of 3% (wt/vol) BSA in PBS (pH 6.9) was added to each well and incubated at 37°C for 1 h with shaking at 75 rpm. The wells were then washed once with 1% (wt/vol) BSA in PBS at pH 6.9 and twice with 1% (wt/vol) BSA in PBS at pH 7.2. Labeled alginate (0.5 ml at 0 to 100 μg/ml) was added to the wells and incubated at 37°C for 1 h with shaking at 75 rpm. Nonspecific binding was assessed by adding the labeled alginate to wells to which PBS (pH 6.9) was added instead of BECs. Wells were washed four times with PBS (pH 7.2). To remove bound alginate from the BECs, 0.5 ml of 10% SDS was added, and the plate was incubated for 1 h at 60°C. The SDS was then removed from the wells and placed in scintillation vials with 5 ml of Aquasol (New England Nuclear Corp.), and radioactivity was determined in a Beckman LS-150 liquid scintillation counter. All assays were performed in triplicate. The total number of BECs bound per well was calculated from the average number of BECs observed per 10 fields with an Olympus CK inverted microscope by using a calibrated ocular micrometer to determine field size.

Solid phase assay for the binding of BECs to alginate. Tissue culture wells coated with poly-L-lysine as described in the previous paragraph were used for the solid phase assay for the binding of BECs to alginate. The plates were washed three times with double distilled water, and various amounts (0 to 2.5 ml) of a 100 μg/ml solution of alginate in double-distilled water were added to each well. The alginate was then dried onto the well overnight at 60°C. Before use, the wells were washed three times with PBS (pH 7.2). To reduce nonspecific binding of BECs to the coated wells, 1.0 ml of 3% (wt/vol) BSA in PBS (pH 7.2) was added, and the plate was incubated at 37°C for 1 h with shaking at 75 rpm. The wells were washed three times with 1% (wt/vol) BSA in PBS (pH 7.2). BECs (0.5 ml of cells at 2.0×10^5 cells per ml) were added to each well and incubated at 37°C for 2 h with shaking at 75 rpm. Unattached BECs were removed, and the wells were washed four times with PBS (pH 7.2). Attached BECs were stained with crystal violet, and the average number of BECs per field was determined (10 fields per well, three wells per assay) as described above. Significant differences were determined with a Kruskal-Wallis test (12).

In order to test the efficiency of binding of alginate from strains 492c and 492a to the poly-L-lysine-coated wells, radioactively labeled alginate was added to the wells in place of cold alginate, and the binding assay was performed as described above. To remove the alginate from the wells, 0.5 ml of Aquasol was added to each well for 5 min. The solubilized alginate was then placed in a scintillation vial, 5.0 ml of additional Aquasol was added, and radioactivity was determined as described above.

Paper chromatography. Alginate was hydrolyzed as described by Haug and Larsen (7), and the neutralized hydrolysate was analyzed by descending paper chromatography. Paper chromatography was performed by using a solvent system of ethyl acetate-acetic acid-pyridine-water (5:1:5:3, vol/vol) and by staining by the method of Linker and Jones (13). Kelp alginate (Sigma) was used as an authentic alginate standard.

Biochemical assays. Uronic acid was determined by the colorimetric carbazole assay (3), and carbohydrate was determined by the phenol-sulfuric acid assay (5) by using D-glucuronic acid (Sigma) as a standard. Protein was determined by ninhydrin assay and by the method of Lowry et al. (14) by using BSA as a standard. Lipopolysaccharide was determined as 2-keto-3-deoxyoctonate by the method of Osborn (20) by using 2-keto-3-deoxyoctonate as a standard (Sigma). Percent acetylation of the alginate was determined by the method of Hestrin (8) by using acetylcholine as a standard and unacetylated alginate as a blank.

RESULTS

Purification of alginate from strain 492c. Purification of alginate from 500 ml of culture supernatant typically yielded 10 mg of an off-white powder. Analysis of 100 μg/ml solutions of the purified alginate revealed a trace of 2-keto-3-deoxyoctonate, less than 1.7% protein by the method of Lowry et al. (17), no detectable protein by using the ninhydrin assay, 88.7% uronic acid, and 89.3% carbohydrate. The alginate was found to be 11 ± 0.55% (mean ± standard deviation) acetylated. Further, no absorbance was noted at

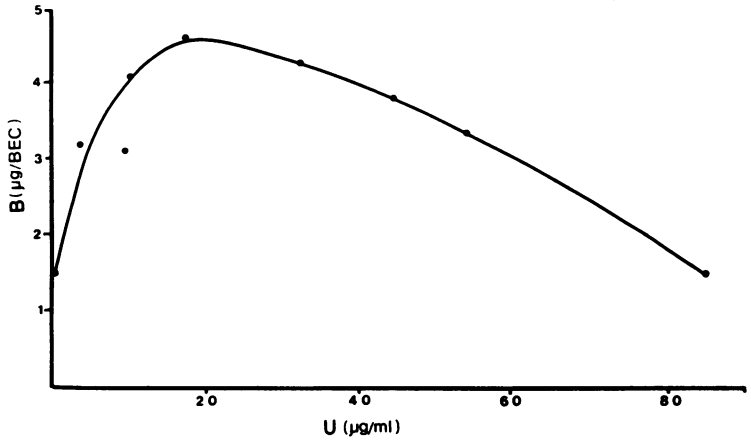

FIG. 1. Binding isotherm for binding of alginate from *P. aeruginosa* 492c to free BECs. Abbreviations: *B*, bound alginate (10^4 μg per BEC); *U*, unbound alginate at equilibrium.

240 nm, indicating a lack of nucleic acids in the purified alginate.

Paper chromatography of the acid-hydrolyzed alginate revealed spots with relative mobilities (R_ms) corresponding to D-mannuronic acid, L-guluronic acid, and their lactones (the lactones are formed during hydrolysis [13]). The paper chromatogram of alginate from strain 492c was almost identical to that of kelp alginate. Infrared spectra of purified alginate were typical of acetylated alginate and provided no indication of any amide bonds (data not shown).

Alginate binding time course and reversibility. Binding of alginate from strain 492c increased with time, stabilized after about 45 min, and remained constant through 90 min. Therefore, all subsequent assays were run for 1 h. Addition of increasingly greater amounts of unlabeled alginate to BECs with attached, labeled alginate decreased the amount of labeled alginate bound per BEC in a concentration-dependent manner. This indicated that binding of alginate to BECs was in reversible equilibrium at the time of the assay.

Binding of alginate from strain 492c to free BECs. The concentration of ^{14}C-labeled alginate from strain 492c binding to BECs increased until a concentration of about 15 μg of alginate per ml was obtained, and then it decreased significantly (Fig. 1). By replotting these data as a Langmuir adsorption isotherm [$U/B = U/N + 1/(K_aN)$, where U is the concentration of unbound alginate, B is the amount of alginate bound per BEC, N is the maximum amount of alginate that can bind per BEC, and K_a is the apparent association constant (4)], an exponential curve was obtained which indicated that alginate from strain 492c was probably multivalent. This curve was transformed by using the counterpart of the Langmuir equation for multivalent ligands described by Hogg and Winzor (9). Solving this equation for different datum points allowed for the calculation of the average valency, f (f = 2.6). Plotting $U^{1/f}/R$ versus $T^{(f-1)/f}U^{1/f}$, where U is unbound alginate at equilibrium, f is the valency, R is the binding function defined by Hogg and Winzor (9), and T is the total alginate added, a straight line was obtained (correlation coefficient = 0.94) with a slope of f/N and an intercept of $1/(K_aN)$ (Fig. 2). This allowed the calculation of the maximum amount of alginate that could bind per BEC ($N = 5.82 \times 10^{-4}$ μg per BEC) and the determination of the apparent association constant ($K_a = 4.1 \times 10^{-2}$ ml/μg).

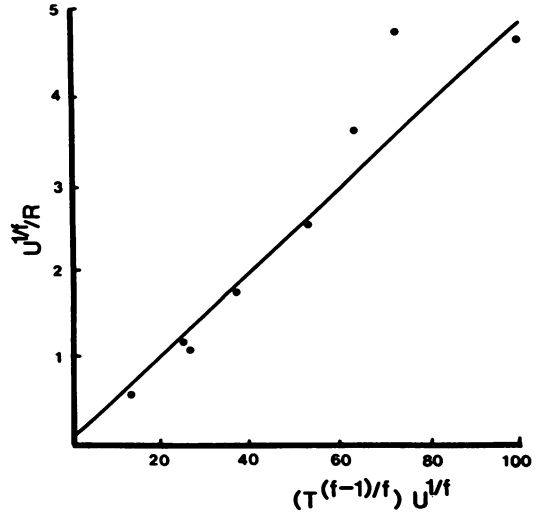

FIG. 2. Langmuir adsorption isotherm counterpart for multivalent ligands for binding of alginate from *P. aeruginosa* 492c to free BECs. For the definitions of *U*, R, f, and T, see the text.

Agglutination of BECs with alginate from strain 492c. Alginate agglutinated BECs in a concentration-dependent manner. The addition of 10 μg of alginate per ml resulted in 67.2% agglutination of all BECs. All values were significantly different ($P < 0.001$) from the control by using the Kruskal-Wallis nonparametric test (12). Alginate caused approximatelyy 20% of the total trypsinized BECs to agglutinate ($P < 0.001$), and the agglutination appeared to be independent of the alginate concentration (test values were not significantly different from each other; $P > 0.1$).

TEC agglutination and binding of alginate from strain 492c. TECs agglutinated in the presence of alginate. Addition of 50 μg of alginate per ml resulted in 59% agglutination of TECs. Often, agglutination of the TECs appeared to occur by cilia-cilia contact between TECs. All values were significantly different ($P < 0.005$) from the control by using the Kruskal-Wallis nonparametric test (12). Further, at a concentration of 50 μg/ml, 8.55×10^{-5} μg of alginate bound per TEC.

Binding of alginate to immobilized BECs. The binding of alginate to immobilized BECs was investigated. By varying the concentration of ^{14}C-labeled alginate added, a binding isotherm was generated. However, the low specific activity of the ^{14}C-labeled alginate (specific activity 200 cpm/μg)

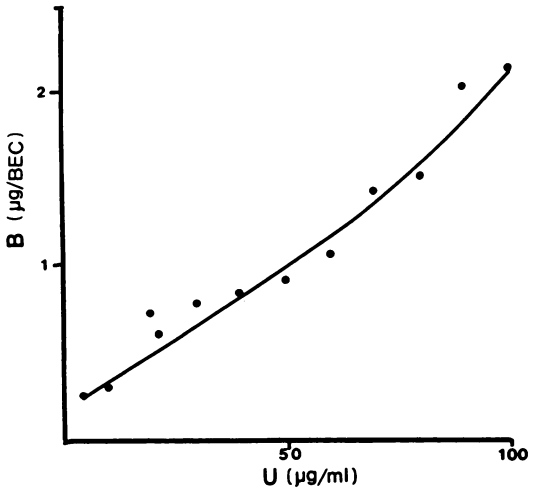

FIG. 3. Binding isotherm for binding of alginate from *P. aeruginosa* 492c to immobilized BECs. For the definitions of *B* and *U*, see the legend to Fig. 1.

prevented resolution at the lower range of the isotherm (10 to 50 μg/ml). To overcome this problem, ^{35}S-labeled alginate was used. The ^{35}S label is intimately associated with the alginate such that it remains associated with the alginate even under denaturing conditions (data not shown). Further, the binding of ^{35}S-labeled alginate to BECs was kinetically identical to the binding of ^{14}C-labeled alginate in identical experiments (data not shown).

The binding of ^{35}S-labeled alginate to BECs did not saturate over the range of concentrations examined (Fig. 3). The Langmuir adsorption isotherm for these data produced a straight line (Fig. 4). By using the Langmuir equation, the maximum amount of alginate bound per BEC and the association constant were calculated to be 2.52×10^{-4} μg per BEC and 3.30×10^{-2} ml/μg, respectively.

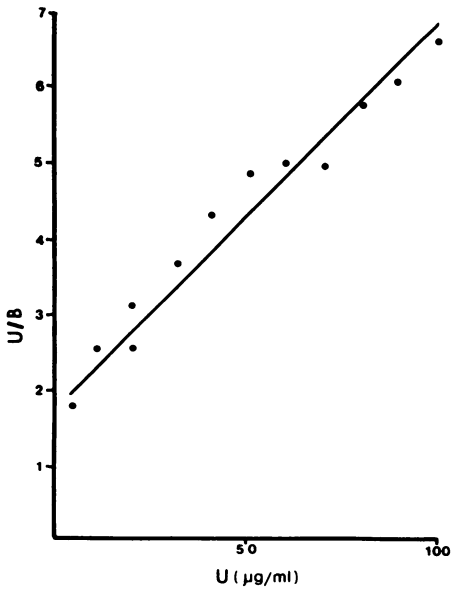

FIG. 4. Langmuir adsorption isotherm for binding of alginate from *P. aeruginosa* 492c to immobilized BECs. For the definitions of *U/B* and *U*, see the legends to Fig. 1 and 2.

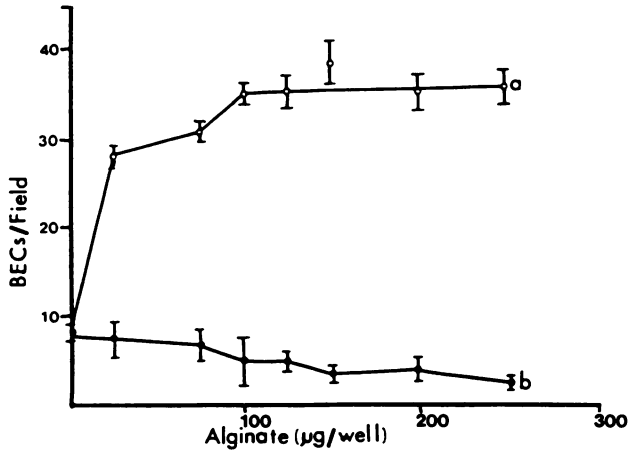

FIG. 5. Ability of alginate derived from *P. aeruginosa* 492c (a) and 492a (b) to bind BECs to a solid support. Shown are the mean ± 1 standard error for duplicate experiments.

Binding of alginate from strain 492a to BECs could not be detected over the range examined (<100 μg/ml).

Binding of BECs to alginate. BECs bound to tissue culture wells coated with alginate from strain 492c at a significantly higher level ($P < 0.001$) than to wells not coated with alginate. Coating the surface with increasing amounts of alginate resulted in an increase in the number of BECs bound (Fig. 5). Binding saturated at approximately 100 μg of alginate per well. This was probably due to the saturation of the solid phase with alginate. However, wells coated with alginate obtained from strain 492a did not bind BECs (Fig. 5). The observed differences in BEC binding to immobilized alginate from strains 492a and 492c were not due to differential coating of the wells because both alginates bound equally to the wells.

DISCUSSION

The adhesion of *P. aeruginosa* to an epithelial surface has proven to be complex (17, 24, 28), with multiple adhesion mechanisms being used, thus complicating the study of the process (17). Both pili and alginate have been implicated in the adhesion of this bacterium to BECs, although some controversy over the role of these potential adhesins exists (23, 25, 28, 30).

Alginate from strain 492c was found to be multivalent, and the alginate receptor on BECs was found to be trypsin sensitive. McEachran and Irvin (17) have reported that alginate is not important in the adhesion of strain 492c to trypsinized BECs but that it does act as an adhesin to untrypsinized BECs. Woods et al. (28) found that mucoid strains adhered poorly to trypsinized BECs. This may be attributable in part to the trypsin sensitivity of the alginate receptor.

Analysis of the binding of alginate from strain 492c to BECs, free of agglutination and its effects, was undertaken by using BECs immobilized on a solid matrix. By using this assay, the maximum amount of alginate that could bind per BEC was approximately one-half the amount that could bind to a free BEC since one surface of the BEC is in contact with the solid phase. This may reflect more accurately the binding of alginate to an epithelial surface.

Ramphal and Pier (23) have reported that the presence of cilia on rat tracheal cells prevents the binding of alginate to

TECs. This situation was not observed with human TECs. Indeed, alginate appears to interact directly with human cilia because TEC agglutination was usually by means of cilia-alginate-cilia bridges. This cilia-alginate interaction has also been observed with hamster tracheal epithelium (15).

The ability of alginate from strain 492a, a coisolate of 492c, to bind BECs was also examined. Unlike alginate from strain 492c, no binding of alginate from strain 492a to BECs could be detected. It has been reported that the adhesion of strain 492a to BECs is not mediated by its alginate (D. W. McEachran and R. T. Irvin, unpublished data). A similar diversity of alginate binding has also been reported by McArthur and Ceri (16) and Ceri et al. (2) by using a heparin-rat lung lectin. This lectin activity binds alginate from strain 492c but not alginate from strain 492a (2, 16). It is possible that a similar lectin is responsible for binding of alginate to BECs and TECs.

Alginate-based vaccines have been only partially successful (29). It now appears that a certain amount of the variability may be due to strain-specific variations in alginate structure and function. We have presented evidence that certain types or classes of alginate bind to epithelial cells in a specific manner, whereas others do not. Since there is a strain-specific diversity in alginate binding functions and structure, as reported here and previously (2, 16, 17), effective alginate-based vaccines which prevent adhesion, and hence colonization, will require the characterization of both epithelial cell alginate receptors and binding characteristics of alginate from multiple strains of *P. aeruginosa*.

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