Purification and characterization of CS2, a sialic acid-specific haemagglutinin of enterotoxigenic *Escherichia coli*

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CS2 fimbriae of enterotoxigenic *Escherichia coli* were purified and characterized. The surface haemagglutinins (fimbriae) were detached by sonication from a strain producing only the CS2 fimbriae. Isolation was carried out by gel filtration on a Sepharose 4B column. After depolymerization, the fimbriae subunits were purified on a Sephacryl S-300 column in 8.0 M-guanidinium chloride. From 1 litre of medium, 4–6 mg of purified fimbriae was obtained. We found that CS2 fimbriae were completely dissociated by saturated guanidinium chloride into subunits with a molecular mass of 16.5 kDa. CS2 fimbriae were sialic acid-specific, since sialic acids were the most potent inhibitors, and neuraminidase treatment of erythrocytes abolished haemagglutination. Both fimbriae and fimbrial subunits were found to bind to bovine erythrocytes. The binding of subunits to erythrocytes could be inhibited with low concentrations of sialyl-lactose.

INTRODUCTION

Enterotoxigenic *Escherichia coli* causes diarrhoea in humans and young animals (Smith & Linggood, 1971; Evans *et al.*, 1977; Guerrant, 1985). Filamentous surface proteins (fimbriae) on the bacterial cells possess lectin-like properties that enable adhesion to the small-intestinal epithelium. Such proteins are homopolymers of hydrophobic proteins. Morphologically CS2 fimbriae are similar to type 1 fimbriae (Brinton, 1965) and P fimbriae of *E. coli* (Korhonen *et al.*, 1982). They protrude 0.5–2 µm from the cell and are 5–7 nm in diameter. Haemagglutination was the first adhesive property observed of this hairlike structure (Duguid *et al.*, 1955). Several fimbriae bind specifically to carbohydrate, and different carbohydrate-binding specificities have been discovered in CFA/I (Evans *et al.*, 1979) and CFA/II (Evans *et al.*, 1979; Wadström & Trust, 1984).

The CS2 fimbriae belong to an antigenically heterogeneous group of fimbriae referred to as 'CFA/II' and preferentially agglutinate bovine erythrocytes of individual animals (Faris *et al.*, 1982). Within the entity of CFA/II, CS1-, CS2- and CS3-fimbriae have been identified (Smyth, 1984). However, the molecular nature of the host-cell glycoconjugates recognized by CS1-, and CS2- and CS3-fimbriae are still unknown. Studies of the fimbriae-carbohydrate interaction have been greatly hampered by difficulties encountered in the solubilization and dissociation of fimbriae into biologically functional subunits. Attempts to use affinity chromatography for purification and characterization have thus failed, owing to the large size and filamentous shape (up to 2 µm in length) of intact fimbriae or to irreversible denaturation of monomers (Eshdat *et al.*, 1981). The purpose of the present study was to purify and characterize the CS2 fimbriae and define their carbohydrate specificity.

MATERIALS AND METHODS

Bacterial strain

A well-characterized enterotoxigenic *E. coli* strain (C91f) (Bäck *et al.*, 1980) isolated in Ethiopia from human diarrhoea stools was used throughout the present study. This strain of the 06:K15:H16 serotype has the capability to produce type 1 and CS2 fimbriae and is serologically negative for CS1 and CS3 antigen (Smyth, 1984). The strain was cultivated on modified CFA (colonization-factor antigen) agar medium for 18 h at 37 °C to prevent production of type 1 fimbriae and to optimize production of CS2 antigens (Evans *et al.*, 1977a). CS2 fimbriae were assayed by coagglutination and haemagglutination tests.

Production of CS2 specific IgG

Rabbits were immunized by four intramuscular injections of 0.5 ml of bacterial suspension (10^{10} cells/ml suspended in 0.15 M-NaCl, heat-treated for 15 min at 121 °C). Bacteria (strain C91f) were grown on CFA agar at 37 °C. At 4-week intervals the rabbits were bled (40 ml). Antisera were absorbed with the appropriate fimbriae-negative strain (C91f-29) (Smyth, 1982) by using non-heated live cells grown under the same conditions as the parent strain used for rabbit immunization (Evans & Evans, 1978). The CS2-negative variants (10^{10} cells/ml) was incubated with 10 ml of antiserum (15 min, 20 °C). The sera were then centrifuged (3000 g, 30 min, 20 °C). Adsorption was terminated when no visible agglutination was observed with cells lacking CS2 fimbriae. The antisera were filtered (MF-Millipore membrane type GSWP; pore size 0.22 µm; obtained from Millipore, Molsheim, France) and was further purified on a protein A-Sepharose column (2.5 cm ×

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10 cm) equilibrated with 0.1 M-phosphate buffer, pH 7.0. Antibodies were desorbed with 0.1 M-glycine/HCl, pH 3.0, and dialysed against 0.01 M-sodium phosphate-buffered saline (0.145 M-NaCl), pH 7.4.

Preparation of coagglutination reagent

The methodology has been described elsewhere (Faris *et al.*, 1984). Briefly, 1 ml of a 10% (w/v) suspension of heat-killed and formalin-treated *Staphylococcus aureus* (strain Cowan I; A.T.T.C. 12598) was washed with phosphate-buffered saline. The cells were suspended in 1 ml of phosphate buffered saline with 0.1 mg of anti-CS2 IgG. The mixture was incubated for 2 h (20 °C) with continuous mixing. The cells were then centrifuged, washed and resuspended in phosphate-buffered saline to 2% (v/v). To facilitate the reading of the test, the reagent was stained with Methylene Blue (4%, 1 h, 20 °C) and finally washed five times to remove excess stain. This coagglutination reagent was stored at 4 °C in the presence of NaN₃ (0.1%).

Determination of CS2 antigens by coagglutination tests

A 30 µl portion of coagglutination reagent was mixed with 30 µl of fimbriae solution or bacterial suspension (10⁷ cells/ml) for 2 min on a glass slide. The coagglutination test was also performed with purified CS2 fimbrial antigens. The appearance of clearly visible clumps of coagglutination reagent after continuous mixing indicated the presence of CS2 fimbriae in the suspension.

Purification of fimbriae

Strain C91f possessing CS2 fimbriae was grown on CFA medium for 18 h at 37 °C in five aluminium pans (25 cm × 35 cm). Bacterial cells were suspended to a concentration of 10¹¹ cells/ml in 50 mM-sodium phosphate buffer, pH 8.0, containing 0.04% NaN₃ and 0.2 mM-phenylmethanesulphonyl fluoride and sonicated (15 s, six times, in an MSE sonicator, 20 µm amplitude) with the probe end close to the surface of the suspension (to detach fimbriae). After centrifugation (thrice) at 10000 g for 20 min at 4 °C to sediment bacterial cells, the supernatant was incubated at 20 °C for 1 h with deoxyribonuclease I (27 µg/ml), ribonuclease A (17 µg/ml) and 0.1% sodium deoxycholate, to eliminate aggregates of impurities and nucleic acids which would otherwise be eluted in the void volume of the Sepharose 4B-CL column. The supernatant was filtered, and the material was applied to a Sepharose 4B-CL column (3.2 cm × 100 cm) (Fig. 1 below). Eluted fractions were assayed for the presence of fimbriae by the coagglutination test described above. Fimbriae-containing fractions were combined and concentrated to 1 mg/ml with a PM 10 filter membrane (10000-M_r exclusion limit; Amicon Corp., Lexington, MA, U.S.A.), and detergent was removed by extensive dialysis.

To a solution of purified fimbriae, crystalline guanidinium chloride was added to saturation (8.6 M). Fimbriae were depolymerized by pH-adjustment to 8.0, followed by incubation for 18 h at 37 °C. The depolymerized material was applied on a Sephacryl S-300 column (1 cm × 95 cm) that had been equilibrated with 8.0 M-guanidinium chloride/5 mM-Tris/HCl buffer, pH 8.0, and calibrated with molecular-mass markers (Fig. 2 below). Purified fimbrial subunits were dialysed against

0.11 M-sodium phosphate buffer, pH 7.4, and maintained frozen (−20 °C) at a concentration of 0.10 mg/ml.

Preparation of affinity adsorbent

Human transferrin was coupled to CNBr-activated Sepharose 4B (March *et al.*, 1974). Briefly, to 20 ml of an aq. 50% (v/v) suspension of the gel, 40 ml of 2 M-Na₂CO₃ was added with cooling. The gel was activated by adding 1.3 g of CNBr in 2.3 ml of acetonitrile and gently stirred for 2 min. The gel slurry was poured on to a sintered-glass funnel, washed with cold water, 0.25 M-NaHCO₃, pH 9.0, and transferred into a beaker containing 50 mg of transferrin in 10 ml of 0.25 M-NaHCO₃. The coupling was allowed to continue for 4 h at room temperature with slow stirring. The gel was washed with water, 0.05 M-ethanolamine and phosphate-buffered saline. In a typical experiment, the amount of transferrin coupled to the settled gel was 1 mg/ml, as determined by subtracting the amount of transferrin in the filtrate and washings from that added initially.

Affinity chromatography

Affinity chromatography was performed at a flow rate of 10 ml·h^{−1} on a column (1 cm × 12 cm) of human transferrin coupled to Sepharose 4B. Both intact fimbriae, purified on Sepharose 4B-CL, and depolymerized fimbriae, peak 3 from Sephacryl S-300, were applied to the column. Each sample contained 1 mg of protein. Sample application and washing were done at room temperature with 5 mM-Tris/HCl, pH 8.0, and elution was done with the same buffer containing 0.1 M-sodium borate.

Electrophoresis

Polyacrylamide-gel electrophoresis was carried out in a 10–18%-(w/v)-polyacrylamide gradient gel as described by Blobel & Dobberstein (1975). All samples were boiled in 2% SDS and dithiothreitol for 5 min and alkylated with iodoacetamide before application on the gel. The following proteins from Pharmacia (electrophoresis calibration kit) were used as M_r standards: phosphorylase b (M_r 94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000) soybean trypsin inhibitor (20100), and α-lactalbumin (14400). Gels were stained with Coomassie Brilliant Blue R-250.

Electron microscopy

Suspensions of bacteria were made directly from agar in distilled water, and the cell density was adjusted to $A_{540}^{10\text{ mm}} = 0.5$. One drop of the suspension was applied to a grid covered with carbon-coated Formvar, and the excess fluid was removed with a filter paper after 1 min. CS2 fimbriae were also examined at a concentration of 1 mg/ml after the first gel-filtration step. The samples were negatively stained with ammonium molybdate and examined with a Philips transmission electron microscope operated at 60 kV.

Amino acid analysis

Amino acid analysis was performed on an LKB 4151 'alpha plus' amino acid analyser with duplicate samples after hydrolysis for 24 h at 110 °C in 6 M-HCl containing 2 mg of phenol/ml.

Protein determination

The method of Lowry *et al.* (1951) was used, with bovine serum albumin as a standard.

Haemagglutination test

Haemagglutination was performed on glass depression slides (Faris *et al.*, 1982) or in a Payton 300 A aggregometer (Payton Associates, Buffalo, NY, U.S.A.) (Lindahl *et al.*, 1987). Bovine erythrocytes were collected in a heparinized syringe, washed five times in 10 vol. of 0.01 M-phosphate-buffered saline, pH 7.4, and diluted to 5×10^7 cells/ml. Erythrocyte suspension (3%, v/v) was incubated with clostridial sialidase (1 unit/ml) for 30 min in 37 °C and washed five times in phosphate-buffered saline. Monosaccharides were mixed with suspensions of human erythrocytes (5×10^6 cells·ml⁻¹) in (1:1) 0.01 M-phosphate-buffered saline, pH 6.8, and 0.3 M-sucrose containing 0.1 M- α -methyl mannose in a cell aggregometer at 800 rev./min and 20 °C. Bacterial cells (4×10^7 fimbriated cells·ml⁻¹) were added and the increase in transmitted light ($\Delta T \cdot \text{min}^{-1}$) at 609 nm was measured. An increase in transmittance is a measure of the decrease in the number of particles (erythrocytes or clumps of erythrocytes) scattering light as the haemagglutination reaction proceeds. The increase in transmittance is constant for a few minutes after a lag phase of 1–2 min. The increase in transmittance/min, during the constant phase, in the presence of different concentrations of inhibitors, was plotted against log (concentration of inhibitor), giving linear plots for each inhibitor (Fig. 5 below). The concentration of monosaccharides giving half the value of the increase in transmittance/min for the reaction without inhibitor was regarded as the concentration giving 50% inhibition.

Binding of ¹²⁵I-labelled fimbriae and subunits to bovine erythrocytes

Purified CS2 fimbriae, as well as fimbrial subunits, were ¹²⁵I-labelled. Labelling was performed in 0.11 M-sodium phosphate buffer, pH 7.4, as described by Hunter (1978), using Chloramine-T as oxidizing agent. To 7.5 µg of protein, 0.5 mCi of Na¹²⁵I was added and incubated for 90 s in the presence of Chloramine-T (0.2 mg/ml). After reduction with Na₂S₂O₅ (0.2 mg/ml) and addition of KI (0.5 mg/ml), labelled protein was separated from free iodine by gel filtration on a PD-10 column (Pharmacia Fine Chemicals).

Binding experiments were carried out in plastic tubes, previously soaked with 2% (w/v) bovine serum albumin in 0.01 M-phosphate-buffered saline, pH 7.4, for 45 min. The reaction mixture (300 µl) contained 0.1% (w/v) bovine serum albumin in 0.01 M-phosphate-buffered saline, pH 7.4, 1.2×10^8 bovine erythrocytes/ml and ¹²⁵I-labelled fimbriae or subunits (7×10^{-2} µg and 5×10^{-2} µg). The specific radioactivities of the iodinated proteins were 1.3×10^9 c.p.m./mg and 1.4×10^9 c.p.m./mg for whole fimbriae and subunits respectively. For inhibition, sialyl-lactose was included in the reaction mixture at concentrations from 0.5 mM up to 15 mM.

Tubes were rotated at room temperature for 2 h. After incubation, 3.0 ml of 0.01 M-phosphate-buffered saline pH 7.4, was added to each tube, followed by a brief mixing and centrifugation at 800 g for 30 min. Supernatants were thoroughly sucked off and discarded. The amount of labelled protein bound to erythrocytes was

Fig. 1. Gel filtration on a Sepharose 4B-CL column (3.2 cm × 100 cm) in 50 mM-sodium phosphate buffer/0.1% sodium deoxycholate/0.04% NaN₃, 5 mM-EDTA pH 8.0, of material detached by sonication of the bacterial suspension

The bar indicates the pooled material in the void volume containing CS2 fimbriae.

measured in a α -radiation counter. The average radioactivity of duplicate samples was calculated, and the background (i.e., radioactivity of samples without erythrocytes) was subtracted.

Inhibitors

Acidic carbohydrates were used as their ammonium salts. Equimolar amounts of sialic acids, or derivative thereof, and ammonia (0.1 M) were mixed and diluted with 0.2 ml of distilled water. The solution was freeze-dried. Freeze-dried ammonium salts of sialic acids, as well as neutral carbohydrates, were dissolved in 0.01 M-phosphate-buffered saline, pH 7.4.

Chemicals

Sialyl-lactose, *N*-glycolylneuraminic acid, *N*-acetylneuraminic acid, *N*-acetylgalactosamine, *N*-acetylglucosamine, L-fucose, D-(+)-galactose, D-(+)-glucose, L-(+)-arabinose, deoxyribonuclease I, ribonuclease A, clostridial sialidase type X and methyl α -D-mannoside were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Guanidinium chloride and reagents for polyacrylamide-gel electrophoresis were obtained from Fluka (Buchs, Switzerland). Sepharose 4B-CL, Sephacryl S-300, protein A-Sepharose and PD-10 columns were obtained from Pharmacia (Uppsala, Sweden). Human transferrin was a gift from Kabi (Stockholm, Sweden). Other chemicals, purchased from commercial sources, were of the highest purity available and were used without further purification.

RESULTS

Purification of the CS2 fimbriae

By SDS/polyacrylamide-gel electrophoresis, bacterium-free sonicated samples were found to contain large amounts of impurities (Fig. 3 below, lane 3). Fimbriae were found to be eluted in the void volume of a Sepharose 4B-CL column (Fig. 1). Transmission electron microscopy of the coagglutination-positive

Fig. 2. Gel filtration on a Sephacryl S-300 column (1 cm × 95 cm) in 5 mM-Tris/HCl/8.0 M-guanidinium chloride, pH 8.0, of depolymerized material.

Molecular-mass markers used were: Dextran Blue (DB, M_r 2000000), ovalbumin (OA, 43000), α -chymotrypsinogen (CH, 25700), ribonuclease A (R, 13700) and $K_2Cr_2O_7$ (K, 294). The bar indicates the pooled material containing CS2 fimbrial subunits.

Fig. 3. Gradient-gel electrophoresis in an SDS/polyacrylamide slab gel of (1) molecular mass markers, (2) CS2 fimbriae purified from *E. coli* C91f by two gel-filtration steps and (3) material detached by sonication of the bacterial suspension

void peak showed the presence of fimbriae (Fig. 5 below). As a final purification step, the fimbriae were depolymerized in 8.6 M-guanidinium chloride, and the subunits were purified by gel filtration on a Sephacryl S-300 column as depicted in Fig. 2. Guanidinium chloride of lower concentration could not depolymerize the fimbriae completely. The material that eluted in peak 3

Table 1. Amino acid composition of purified fimbriae from *E. coli* strain C91f

Amino acid	Content (mol/100 mol)	Residues per fimbrial subunit*
Aspartic acid	11.3	17
Threonine	9.6	15
Serine	8.0	12
Glutamic acid	9.8	15
Proline	3.7	6
Glycine	17.9	27
Alanine	8.5	13
Half-cystine	0.1	0
Valine	7.0	10
Methionine	1.1	2
Isoleucine	5.0	8
Leucine	6.4	10
Tyrosine	1.5	2
Phenylalanine	2.0	3
Histidine	0.8	1
Lysine	4.9	7
Arginine	2.0	3
Tryptophan	—†	—†

* Subunit M_r 16500 by SDS/polyacrylamide-gel electrophoresis.

† Not determined.

Table 2. Inhibition of CS2 haemagglutination by monosaccharides

Compound	IC ₅₀ * (mM)
NeuAc-Lac	6.2
NeuGc	17.3
NeuAc	32.9
GalNAc	> 100†
GlcNAc	> 100†
Fuc	> 100†
Glu	> 100†
Gal	> 100†
Ara	> 100†

* Concentration of monosaccharides giving 50% haemagglutination inhibition.

† No inhibition of haemagglutination reaction was detected at this concentration.

was highly purified as shown by SDS/polyacrylamide-gel electrophoresis (Fig. 3, lane 2) and was positive in the co-agglutination test. From 1 litre of medium, 4–6 mg of purified fimbriae was obtained.

Attempts to use affinity chromatography for purification failed: most of the material did not bind to the transferrin-coupled column, although transferrin was a potent inhibitor in the aggregometer assay.

Structure of fimbriae

The fimbriae retained their morphology after the first gel-filtration step. Note the aggregation to bundles in the absence of detergent. The fimbriae with a diameter of 6 nm (Fig. 4) can be dissociated completely into identical subunits with a molecular mass of 16.5 kDa (Fig. 3) by treatment with saturated guanidinium chloride (Fig. 2).

Fig. 4. Electron micrograph of CS2 fimbriae purified from *E. coli* C91f by gel filtration on Sepharose 4B-CL

The horizontal bar represents 100 nm.

Fig. 5. Haemagglutination by CS2 fimbriated bacterial cells (strain C91f) in the presence of sialyl-lactose (■), *N*-glycolylneuraminic acid (●) and *N*-acetylneuraminic acid (▲) at different concentrations

K represents the calculated haemagglutination rate constant in the absence of inhibitor.

Amino acid analysis

The amino acid composition of CS2 is given in Table 1. Calculation of the number of residues of each amino acid was based on a molecular mass of approx. 16.5 kDa for each subunit. CS2 has very low amounts of aromatic

Fig. 6. Binding of ¹²⁵I-labelled subunits to erythrocytes

The binding of subunits in the absence of inhibitor was maximally 4142 c.p.m. The highest inhibitory effect (85 %) was observed with sialyl-lactose at a concentration of 5 mM or greater.

amino acids: only two residues of tyrosine and three of phenylalanine, and 45 % of the amino acids are hydrophobic. Only 7 % of the amino acids were basic (arginine and lysine). The CS2 protein does not contain any cysteine or cystine residues. No hexosamines were detected.

Haemagglutination-inhibition test

Haemagglutination of strain C91f was found to be sensitive to *N*-acetylneuraminic acid (Table 2). The trisaccharide sialyl-lactose was found to be a five times stronger inhibitor than neuraminic acid (Fig. 5). D-(+)-Galactose, D-(+)-glucose, *N*-acetylglucosamine, L-(+)-arabinose, L-fucose and *N*-acetylgalactosamine were not inhibitory at a concentration of 0.1 M. Transferrin was also tested before preparation of the affinity adsorbent

and showed a strong inhibitory effect (1.5×10^{-2} M). Purified CS2 fimbriae with a concentration of 1 mg/ml do not cause haemagglutination in the aggregometer. Furthermore, sialidase-treated bovine erythrocytes are not agglutinated by bacteria possessing CS2 fimbriae.

Binding of ^{125}I -labelled fimbriae and subunits to bovine erythrocytes

Low amounts of ^{125}I -labelled fimbriae (5.7%) and subunits (6.0%) bound to bovine erythrocytes. The binding of subunits to erythrocytes was inhibited with low concentrations of sialyl-lactose. Concentrations above 5 mM resulted in maximal inhibition (85%) of binding. Inhibition was concentration-dependent up to 5 mM-sialyl-lactose (Fig. 6).

DISCUSSION

CS2 fimbriae from sonicated cells of a well-defined strain can be purified by two gel-filtration steps: the first step in the absence, and the second in the presence, of a dissociating agent (guanidinium chloride). In the second gel-filtration step, fimbriae subunits were eluted in a single peak corresponding to a molecular mass of about 16 kDa. The material in this peak migrated as a single band in SDS polyacrylamide-gel electrophoresis. Only the material in this peak was positive in the CS2 co-agglutination test.

However, if the fimbriae subunits still have carbohydrate-binding properties after solubilization, affinity chromatography may provide a useful method to separate different types of fimbriae. However, we were unable to demonstrate any reversible specific binding to immobilized transferrin either before or after solubilization, in spite of the fact that transferrin is a potent inhibitor in the haemagglutination test.

It is possible that fimbriae with a length of approx. $1\text{ }\mu\text{m}$ and an M_r of 10^6 are too large to enter the gel to form, within the matrix interstices, complexes with a ligand of the molecular size of transferrin, and thus only a small amount would adsorb to the column. Furthermore, it is also possible that only a portion of the monomers in each intact fimbriae has a carbohydrate-binding activity with a low affinity attributable to sterical hindrance (Brinton, 1965). Binding of ^{125}I -labelled fimbriae showed that less than 10% of the material bound to erythrocytes. This implies that CS2 fimbriae have a low affinity for sialic acid present on bovine erythrocytes. The fact that ^{125}I -labelled subunits showed the same low degree of binding capacity as the intact fimbriae further indicates the low affinity of the protein-carbohydrates interaction and may also explain the failure of affinity chromatography. Another possible explanation of the low amount of fimbrial subunits bound to erythrocytes could be that 90% of the material did not renature upon dialysis (Eshdat *et al.*, 1981).

The binding of ^{125}I -labelled subunits to bovine erythrocytes was inhibited by low concentrations of sialyl-lactose. This shows that the binding is truly specific, and is additional evidence for a low affinity for sialic acid.

These results support the idea that the fimbrial subunit can harbour the binding site for specific protein-carbohydrate interaction. Type 1 fimbriae of *E. coli* have previously been shown to be built up of a carbohydrate-binding protein (Eshdat *et al.*, 1981). Reports on the function of fimbrial subunits as well as the function of

other gene products (polypeptides) of the gene cluster responsible for fimbriae expression in enterotoxigenic *E. coli* have proposed the fimbrial subunit to be the adhesive protein (Mooi *et al.*, 1983; Jacobs *et al.*, 1985). On the other hand, another *E. coli* fimbria (P-fimbria) has been suggested to have a 'tip-protein', different from the fimbrial subunit with carbohydrate specificity (Norgren *et al.*, 1984). According to the literature, various kinds of fimbriae may be constructed differently.

Opposite to what was reported for type 1 fimbriae, the purified CS2 fimbriae permeate SDS/polyacrylamide gels without acid treatment (McMichael & Ou, 1979). The apparent molecular mass of the CS2 fimbrial subunit, as determined by SDS/polyacrylamide-gel electrophoresis, agrees well with the results calculated on the basis of amino acid composition (Table 1) and gel filtration (Fig. 2). Furthermore, the amino acid composition seems very similar to that of CFA 1 (Klemm *et al.*, 1985). Both proteins lack cysteine, and therefore the subunits must be held together by non-covalent forces. The amino acid analysis showed that CS2 has a very low content of aromatic amino acids and thus a very low absorbance at 280 nm.

Purification of CS2 fimbriae from a strain possessing both CS3 and CS2 fimbriae shows that only CS2 fimbriae are disrupted in guanidinium chloride (Klemm *et al.*, 1985). Their CS2 preparation was very similar to ours, except for the glycine content. Possible explanations for the difference in amino acid compositions obtained could be that we used a bacterial strain different from that used by Klemm *et al.* (1985), and a different purification procedure.

The binding specificity of CS2 fimbriae for sialic acids or sialoglycoconjugates was shown with the haemagglutination-inhibition assay. The CS2-possessing strain shows inhibition only by *N*-acetylneuraminic acid, sialyl-lactose and *N*-glycolylneuraminic acid. The strong inhibition by sialyl-lactose might be due to an increased binding conferred by Gal- β -(1-4)-Glc and indicates a receptor of size larger than that of a monosaccharide. Furthermore, the carboxy group in the free *N*-acetylneuraminic acid molecule is in an equatorial position, in contrast with the α -linked *N*-acetylneuraminic acid, which has the carboxy group in an axial position. This could also be an explanation for the different inhibitory potencies of these compounds. Similar studies have been done with other types of fimbriae (Firon *et al.*, 1983). Moreover, these studies also showed that trisaccharides were up to 30 times stronger inhibitors than the monosaccharide. Most interestingly, some uropathogenic *E. coli* strains cause sialidase-sensitive haemagglutination and possess an NeuAc- β -(2-3)-Gal-specific lectin (Parkkinen *et al.*, 1983).

Colonization factor antigen I (CFA I) (Evans *et al.*, 1979) and K99 of *E. coli* (Lindahl *et al.*, 1987) have also been shown to have sialic acid specificity. Purified fimbriae did not give haemagglutination in the aggregometer assay, which might be due to the strong stirring or indicate that only a portion of the fimbriae polymer has exposed carbohydrate-binding sites.

The number and location of binding sites on fimbriae is not yet known for any fimbrial type. However, it seems obvious that the binding is due to a protein-carbohydrate interaction mediated by hydrogen bonds. To clarify the biological activity and involvement of fimbriae in bacterial adhesion, pure preparations of biological active

fimbrial monomers are required. CS2 fimbriae are sialic acid-specific homopolymer proteins which dissociate totally in guanidinium chloride. However, a more detailed investigation to clarify the structure-function relationships of fimbriae and the process involved in the assembly of fimbriae structures in general is needed before these fimbriae can be used as lectins and biochemical tools.

We are grateful to Dr. David Eaker for the amino acid analyses. This work was supported by a grant from the Swedish Medical Research Council (16X-04723).

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Received 6 November 1987/16 March 1988; accepted 3 June 1988