Purification and Characterization of Fimbriae from Fimbriate *Vibrio cholerae* O1 Strain Bgd17

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Abstract: Fimbrillin (or pilin) of *Vibrio cholerae* Ol, purified both from an El Tor strain and a classical strain was shown to have the indentical *N*-terminal amino acid sequence which is extensively homologous to those of the *N*-methylphenyl alanine (NMePhe) pilin molecules and partly homologous to that of TcpA (Taylor *et al.*, 1987; Show and Taylor, 1990). The *N*-terminal amino acid residue of the fimbrillin was modified and has not been determined. Haemagglutinin (HA) activities of the purified fimbriae were completely inhibited by D-mannnose and D-glucose, but not by L-fucose. Interestingly, pellicle formation of fimbriate cells was also inhibited by D-mannnose and D-glucose but not by L-fucose as shown previously. Based on the hypothesis that fimbriae of *V. cholerae* Ol function as the colonization factor and the cell associated haemagglutinin, this correlation in inhibition by monosaccharides between HA activity and pellicle formation strongly suggests that D-glucose in Oral Rehydration Salts (ORS, recommended for treatment by World Health Organization) can reduce the clinical symptom (diarrhoea) by inhibiting the colonization of vibrio cells to the epithelial cells of the upper small intestine. The presence of fimbrial antigens among enteropathogenic *V. cholerae* O1 strains was confirmed by western blot− and dot blot-analyses. The fimbrillin of the strain Bgd17 was shown to be a simple protein. Immunoelectron microscopy of the fimbriae with a specific monoclonal antibody revealed that fimbriae of *V. cholerae* Ol function as fimbrial adhesins necessary for the cell-cell interaction.

Key words: *Vibrio cholerae* Ol, Haemagglutinin, Fimbriae, Cholera vaccine

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INTRODUCTION

Although it is known both from epidemiological studies of cholera in endemic areas (Mosley, 1969; Woodward, 1971; Glass, *et al.*, 1982; Ehara *et al.*, 1985) and from experiments in human volunteers (Cash *et al.*, 1974; Levine *et al.*, 1981) that natural infection with *Vibrio cholerae* associated with disease gives rise to long-lasting protective immunity against a second attack of cholera, the factors elicting the protective immunity in humans remain elusive. The ability of vibrio cells to adhere to and colonize the mucosal surface of small intestine is a crucial step in an infectious process. The answer for the above problem is thought most likely to be the cell-associated haemagglutinin. We have reported previously the purification of fimbriae from an El Tor strain (Ehara *et al.*, 1987) and from a classical strain (Ehara *et al.*, 1989). Both types of fimbriae were shown to possess HA activity sensitive to D-mannose, although the HA titres were not so high. In the accompanying paper, we have shown the selective induction of fimbriate vibrios. Here we report the purification and characterization of fimbriae of a classical vibrio strain together with *N*-terminal amino acid sequences of fimbrillins purified from both biotype strains. Taken together, we will show that fimbriae of *V. cholerae* O1 are fimbrial adhesins as those of type 4 fimbriae.

MATERIALS AND METHODS

Chemicals

Natural N-Glycanase and Peroxidase-Lectin-KitA were purchased from SEIKAGAKU KOGYO CO. LTD. The other chemicals used were all analytical grade.

Bacterial strains

Vibrio strains used were VC12, VC90 (classical, Ogawa), Bgd10, Bgd16, Bgd17 (classical, Inaba), 82P4, 82P5 (El Tor, Ogawa), and 83K3, 83K6 (El Tor, Inaba) for Western blot and 86B series of strains for dot blot analysis. Fimbriate Bgd17 strain kept at −80℃ was used for the purification of fimbriae. These strains isolated from stool specimens of cholera patients were enterotoxigenic and were selected from our laboratory collection.

Media and culture condition

Vibrio cells were grown on TCG agar (Ehara *et al.*, 1987) or in AT-broth (Ehara *et al.*, 1991) under static conditions at 37℃ overnight.

Purification of fimbriae

Fimbriate strain Bgd17 (kept at −80℃) organisms were inoculated in a Roux bottle containing 100 ml of AT-broth and cultured at 37℃ overnight. The pellicle formed by the fimbriate cells was carefully recovered after removing the liquid medium by pipet. The seed for a large scale culture was prepared by mixing the pellicle with 50 ml of fresh AT-broth. A few droplets of the seed was inoculated in 150 Roux bottles containing 60 ml of AT-broth.

Vibrio cells were cultured at 37℃ overnight and harvested by centrifugation at 6,000 rpm for 30 min. All centrifugations were done at 4℃. The resultant pellet was suspended in phosphate-buffered saline (PBS, 20 mM, pH 7.4) then sheared 3 times for 5 min with 1 min cooling intervals using a Sorvall Omnimixer, followed by centrifugation at 10,000 rpm for 30 min. The supernatant was designated as a shear-fraction. Solid ammonium sulfate was added to the shear fraction to a concentration of 0.2 M. The solution was mixed for 30 min at 4℃, then centrifuged at 10,000 rpm for 30 min. The pellet was solubilized with PBS and dialyzed against PBS at 4℃ overnight. The dialysate was centrifuged at 25,000 rpm for 30 min to remove membrane debris. The supernatant was loaded onto a linear sucrose gradient (20% to 50%) solution, then centrifuged at 25,000 rpm for 20 hr using a Beckman SW41 rotor. Fractionation was performed by taking 1.5 ml from the top of the tube. Each fraction was monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and by electron microscopy (EM). Fimbriae-rich fractions were combined and further purified by gel filtration through Sepharose CL-4B column using PBS as a buffer.

Haemagglutination tests

Haemagglutination was performed by the method of Jones and Freter (1976). Serial two-fold dilution of the purified fimbriae (0.12 mg/ml) was prepared in a U-bottomed microtitre plate in 25 μl of PBS. Equine-red blood cells (2% in PBS) were added to 25 μl. The plates were shaken to mix the ingredients and the erythrocytes were allowed to settle at 25℃ for 30 min. The titre is defined as the reciprocal of the highest dilution in which HA was clearly visible. To test whether or not the HA reaction was inhibitable by specific monosaccharides, erythrocytes with 1% (W/V) monosaccharides were added to the wells containing serially diluted-fimbriae and HA reactions were allowed for 30 min at 25℃.

N-Glycanase treatment of the fimbriae

To determine whether the fimbrillin of *V. cholerae* Ol is a simple protein or a glycoprotein, purified fimbriae were digested by incubating overnight (18 hr) at 37℃ with N-Glycanase following the Manual by the manufacturer (Genzyme), then, analyzed on a SDS-PAGE, in parallel with an undigested control. Deglycosylation was confirmed by blotting and probing with lectins (Lectin-binding assay).

Immunological cross-reactivity of fimbrillins

Fimbrial preparations from different sources were probed with antiserum to the fimbriae purified from strain K23 (El Tor, Ogawa) (Ehara *et al.*, 1987). For the preparation of samples for SDS-PAGE, vibrio organisms were cultured on TCG agar at 37℃ overnight. After harvesting, the cells were suspended in PBS and adjusted to pH 2.5 with 0.1 N HCl, then boiled for 3 min. After cooling, the cell suspensions were neutralized with 2 M Tris and centrifuged at 10,000 rpm for 10 min. The supernatants were mixed with Laemmli's sample buffer containing 2.5% (W/V) SDS and 5% (V/V) 2-mercaptoethanol. For dot blot analysis, vibrio cells (biotype classical and El Tor) were grown in AT-broth and *Escherichia coli* strain

H10407 (a negative control) were grown in L-broth at 37℃ overnight under a static condition. Each strain was cultured in a small test tube containing 4 ml of the media. After harvesting by centrifugation, the cell pellets were suspended with a small volume of PBS and adjusted to pH 2.5 with 0.1 N HCl, then, boiled for 3 min. After cooling, the suspensions were neutralized with 2 M Tris and centrifuged at 10,000 rpm for 10 min. The supernatants were loaded onto a nitrocellulose membrane. Dot blot analysis was performed using Bio-Dot Microfiltration Apparatus (Bio-Rad Laboratories) according to the Instruction Manual as recommended by the manufacturer.

Preparation of the fimbrillin-specific monoclonal antibodies (McAbs)

A BALB/c mouse was immunized with 1% formalin-killed fimbriate cells of Bgd17 strain emulsified in complete Freund adjuvant (manuscript in preparation).

Preparation of IgG

Purified monoclonal immunoglobulin (IgG, McAb42) was prepared by DEAE-Sephacel (Pharmacia-LKB) ion exchange column chromatography.

Electron microscopy

For negative staining, one drop of the sample was placed on a sheet of PARAFILM and a carbon-coated copper grid was floated on the drop for 1 min. The excess liquid was removed with the tip of a filter paper, and washed three times with distilled water, then stained with 1% uranyl acetate for 30 sec. The excess stain was removed with the tip of a filter paper. For shadowing, the negative stain was omitted, and the air-dried grids were shadowed with platinum. For immunoelectron microscopy of the fimbriae with McAb42, fimbriate Bgd17 strain organisms grown overnight in AT-broth were allowed to adhere to a copper grid carring a hydrophilic carbon film. In this procedure, hydrophilic vibrios are able to adhere to the grid. Hydrophobic fimbriate vibrios are rather difficult to attach to the hydrophilic carbon film. Therefore, hydrophobic cells (pellicle) were suspended in PBS containing 1% D-glucose, then quickly allowed to adhere to the grid. Labeling of bacteria with immunogold was carried out essentially as described by Faulk and Taylor (1971). Carbon-coated copper grids with fimbriate vibrios were washed with 3 serial drops of distilled water and reacted with the primary antibody (McAb42, 20 fold diluted with PBS) for 15 min. The specimens were washed with 3 serial drops of distilled water and reacted for 15 min with a drop of 15 nm-colloidal gold-labeled anti-mouse IgG (H+L) goat serum (E. Y. LABS., INC. SANMAEO, CA. 94401, USA). The specimens were stained with 1% uranyl acetate for 30 min and examined with a JEM 100CX electron microscope operated at 80 kV.

SDS-PAGE and western blot

SDS-PAGE was performed according to the system of Laemmli (1970), while western blotting was performed by the techniques of Towbin *et al.* (1979).

N-terminal amino acid sequences of fimbrillins

For the determination of *N*-terminal amino acid sequence, the fimbrial preparations purified from the fimbriate Bgd17 strain and from the K23 strain (previously reported, Ehara *et al.*, 1987) were denatured with 8 M urea in 0.1 M Tris-HCl, pH 8.5 at 50℃ for 3 hr, then gel-filtrated through PROTEIN PAK 300 (19 mm × 15 cm, Waters, Division of MILLIPORE) with 0.1 M Tris-HCl, pH 8.5 containing 6 M urea. The purity of the dissociated fimbrilin was monitored by SDS-PAGE and EM by shadowing with platinum. Fimbrial preparations were dialyzed against distilled water and lyophilized. The amino-terminal amino acid sequences of fimbrillins were determined with an Applied Biosystems model 470A automated sequencer. The sequence analysis was performed twice.

RESULTS

Purification of fimbriae

The fimbriae purified from the fimbriate strain Bgd17 are flexible filamentous structures 5 to 7 nm in diameter and are hydrophobic (Fig. 1). The fimbriae shown here are distinct from the TcpA (Taylor *et al.*, 1987; Herrington *et al.*, 1988; Sharma *et al.*, 1989; Shaw and Taylor, 1990) morphologically and biochemically. The molecular weight of the fimbrillin is 18 kDa estimated by a SDS-PAGE as shown in Fig. 2.

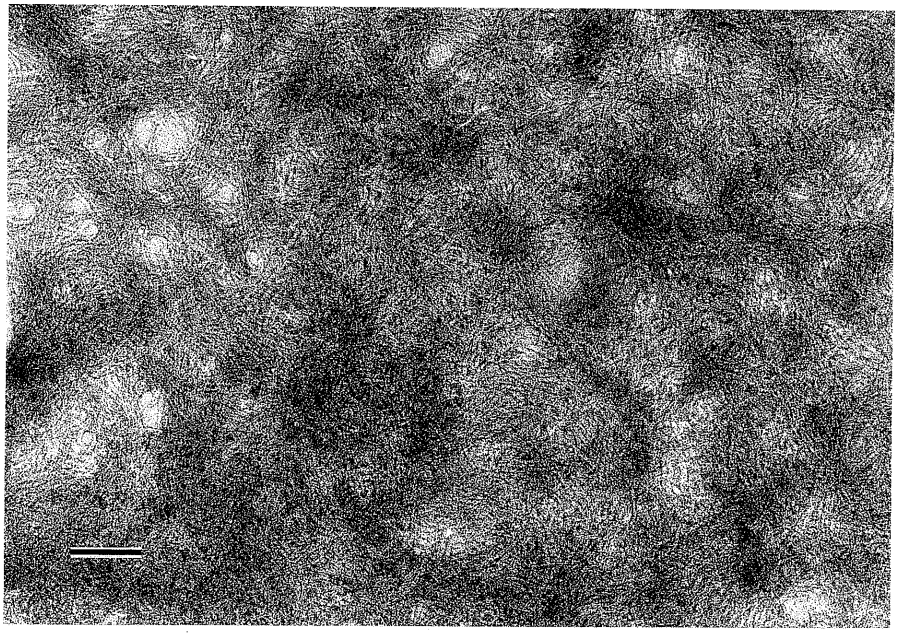

Fig. 1. An electron micrograph showing the fimbriae purified from the fimbriate strain Bgd 17. The bar indicates 100 nm. Note that the fimbriae are highly hydrophobic as shown as a aggregated mass.

Haemagglutination test

The purified fimbriae agglutinated all kinds of red blood cells tested (human type O, chicken, sheep, horse, data not shown). Therefore, fimbriae of *V. cholerae* are involved in the cell-associated haemagglutinin. Effects of monosaccharides on the HA activity of the fimbriae

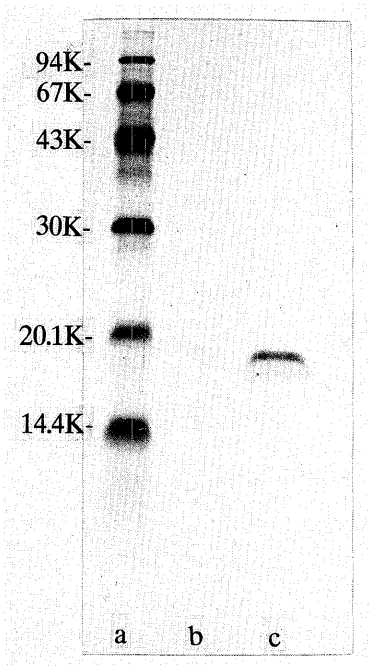

Fig. 2. SDS-PAGE analysis of the fimbrillins purified from strains of non-fimbriate and fimbriate Bgd17. Lanes: a; LMW Calibration Kit proteins (Pharmacia-LKB), phosphrylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), lactalbumin (14.4 kDa), b; fimbrillin purified from the non-fimbriate Bgd17 strain (Ehara *et al.*, 1989), c; fimbrillin purified from the fimbriate Bgd17 strain.

Table 1. The effects of monosaccharides on pellicle formation and haemagglutinin (HA) activity. Note the same pattern in inhibition between pellicle formation and the HA activity of the purified fimbriae

	Pellicle* formation	HA activity of purified fimbriae (120 μg/ml)
AT broth/2% horse-RBCs in the presence of 1%−	+	1,024
D-mannose	−	0
α−methyl D-mannoside	−	0
L-fucose	+	1,024
D-glucose	−	0
methyl-α-D-glucoside	−	0
N-acetyl D-glucosamine	−	0
D-galactose	+	1,024

* Ehara *et al.* (1991).

were examined using similar monosaccharides as used in the previous study. The purified fimbriae clearly showed a D-mannose sensitive HA activity (Table 1), and this HA activity was inhibited even by D-glucose, in parallel with the pellicle formation, as shown in the previous study.

N-Glycanase treatment of the fimbriae

There was no difference in the mobility between undigested control and digested fimbrial sample (Fig. 3A, lanes b and c). The protein concentration of the digested fimbrillin (Fig. 3A, lane C) decreased clearly, possibly due to the contamination of zinc ion dependent protease, although 0.5 mM EDTA was used as a protease inhibitor. The amount of EDTA was not sufficient to inhibit the protease activity. In the lectin-binding assay, both bands of undigested and digested fimbrillin showed a negative response (Fig. 3B, lanes b and C). The lectin used was active because it bound to ovalbumin (glycoprotein), (Fig. 3B, lane a). The reactivity of the digested fimbrillin to anti-fimbriae antiserum decreased possibly due to digestion by contaminating protease. Based on the above data, we concluded that fimbrillin of Bgd 17 strain is a simple protein.

Immunological cross-reactivity of fimbrillins

In a previous paper (Ehara, *et al.*, 1987), we showed that fimbrillins prepared from *V. cholerae* O1 strains of different biotypes as well as serotypes possess shared antigenic determinant. In the present study, we found that fimbrillins werer highly antigenic and confirmed

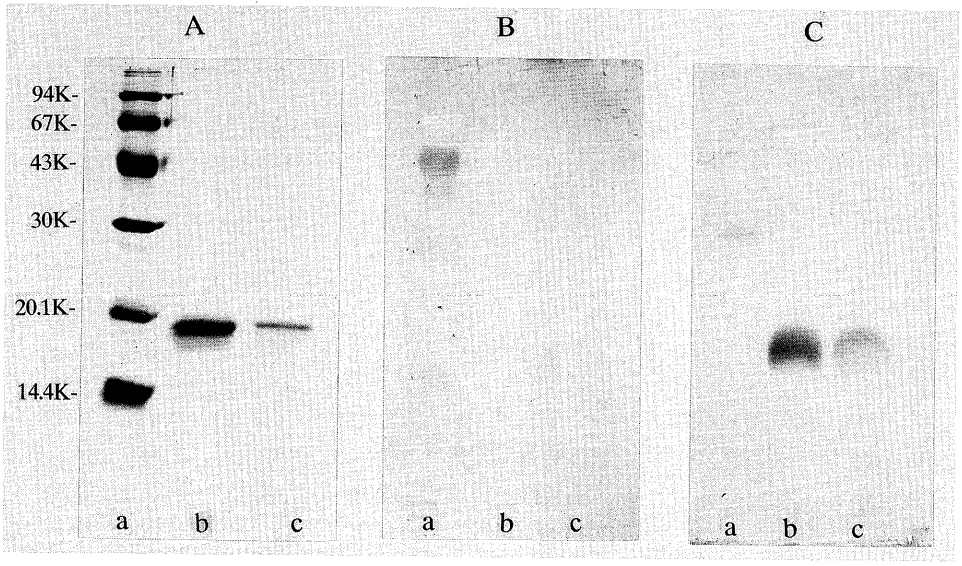

Fig. 3. N-Glycanase treatment of the fimbriae. A) SDS-PAGE, B) Lectin-binding assay, lectins used were ConA, LCA, PHA-E4, PNA, RCA120, WGA, C) Western blot probed with anti-fimbriae antiserum (developed against the fimbriae of strain K23). Lanes: a; LMW marker proteins, b, c; undigested, digested fimbriae.

that these were immunologically cross-reactive among *V. cholerae* O1 (Fig. 4). The presence of fimbrial antigens among vibrio strains isolated from the stool specimens at ICDDR, B. by Dr. Iwanaga, M. in 1986 was examined by dot blot analysis. All the vibrio specimens tested were shown positive (Fig. 5).

Preparation of monoclonal antibodies specific to the fimbrillin

Three monoclonal antibodies, McAb42, McAb64 and McAb58, were established and were found to be isotype IgG2a, IgG3 and IgM, respectively, as determined by microimmunodiffusion against antisera specific for various IgG and IgM isotypes (data not shown). These were shown to recognize the fimbrillin, respectively as shown in Fig. 6. These McAbs

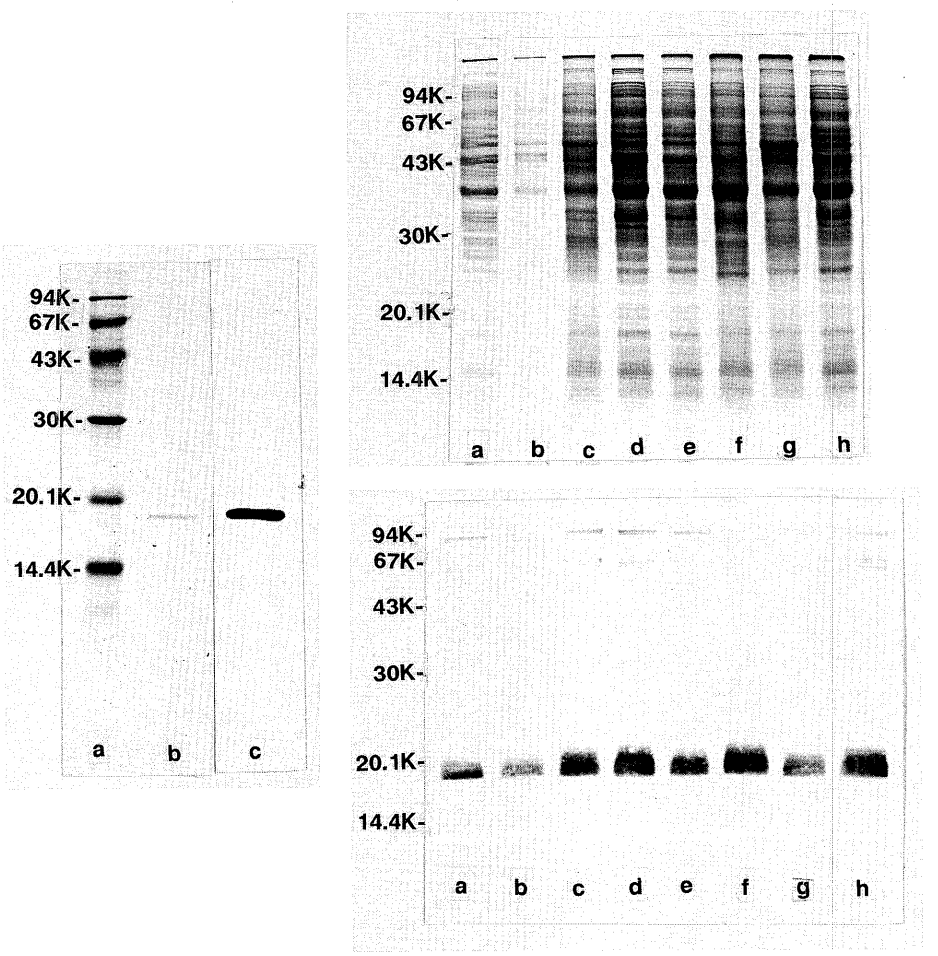

Fig. 4. Western blot analyses of fimbrial preparations from different sources probed with the antiserum to the fimbriae purified from strain K23 (El Tor, Ogawa).
a) lanes a; LMW marker proteins, b; SDS-PAGE of the fimbriae from strain Bgd 17, c; Western blot. b, c) SDS-PAGE, Western blot respectively. lanes a, b; VC12, VC90 (classical, Ogawa), c, d; Bgd10, Bgd16 (classical, Inaba), e, f; 82P4, 82P5 (El Tor Ogawa), g, h; 83K3, 83K6 (El Tor Inaba). Note the strong cross-reactivity at the 18 kDa band.

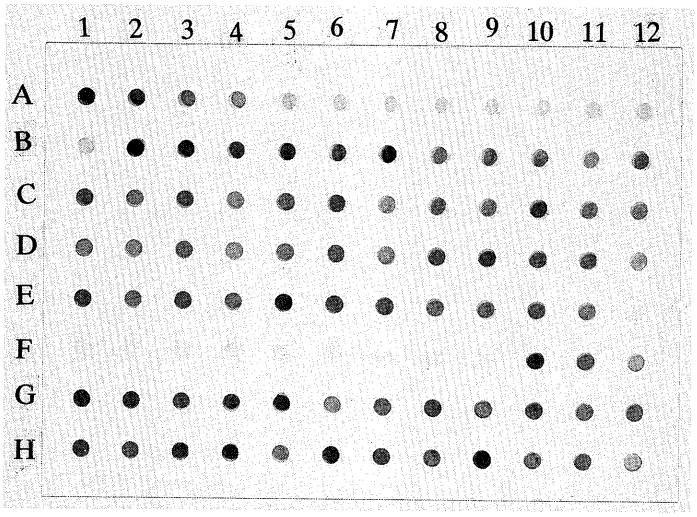

Fig. 5. Dot blot analysis of fimbrial preparations from stock strains with different biotypes.
Wells: A1 to A8; serial dilution of the fimbriae (40 μg/ml),
B1; negative control (*E. coli* strain H10407),
B2; positive control (fimbriate Bgd17 strain),
B3 to E11; classical biotype, F10 to H12; El Tor biotype, A9 to A12 and E12 to F9; blank.

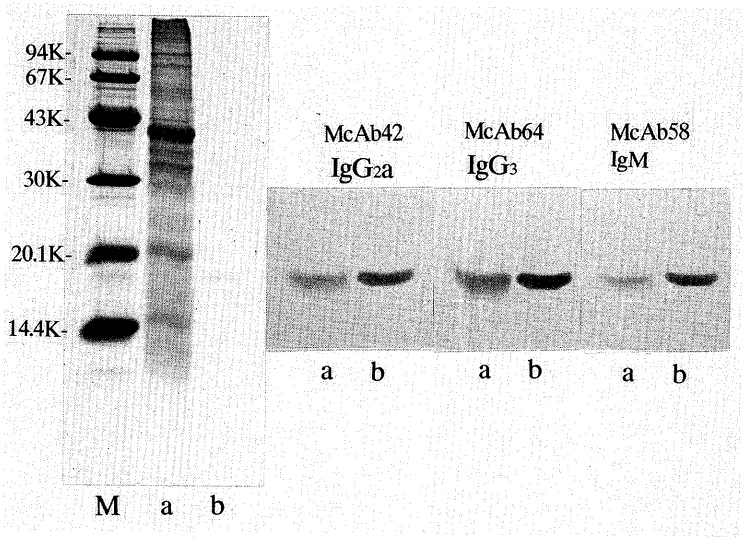

Fig. 6. Western blot analysis of fimbrial preparations from strain Bgd17 that were immunostained by separate McAbs. Lanes: M; LMW marker proteins, a; fimbriate whole cells used as a immunogen, b; fimbrial preparation separated by sucrose-density-gradient centrifugation.

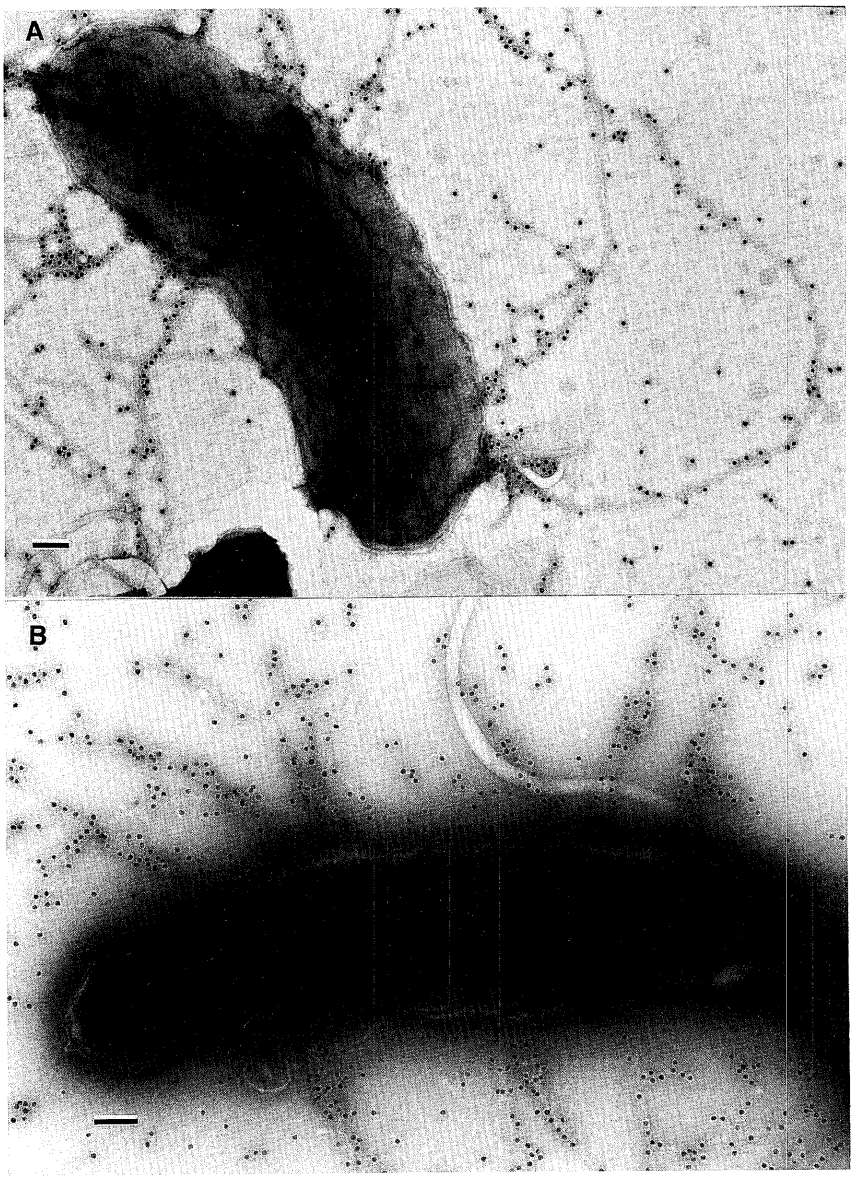

Fig. 7. Immunoelectron micrographs showing the transitional and fimbriate phase of vibrios of the Bgd17 strain reacted with McAb 42.
A, B: hydrophilic, transitional phase of vibrios, C, D and E: hydrophobic, fimbriate phase of vibrios suspended in PBS containing 1% D-glucose for avoiding cell aggregation. Note the difference in the reactivity of the fimbriae to McAb 42 between hydrophilic and hydrophobic organisms. Apparently, the fimbriae of the hydrophobic cells were heavily immunodecorated. Note also the surface of the fimbriate cells which show auto-agglutination is tightly coated with the fimbriae as indicated by arrows and arrow-heads (E). These (C, D and E) pictures, taken together with the previous picture of the pellicle (accompanying paper), prove morphologically that the fimbriae of the Bgd17 strain are the fimbrial adhesins necessary for cell-cell interaction. Bars indicate 100 nm.

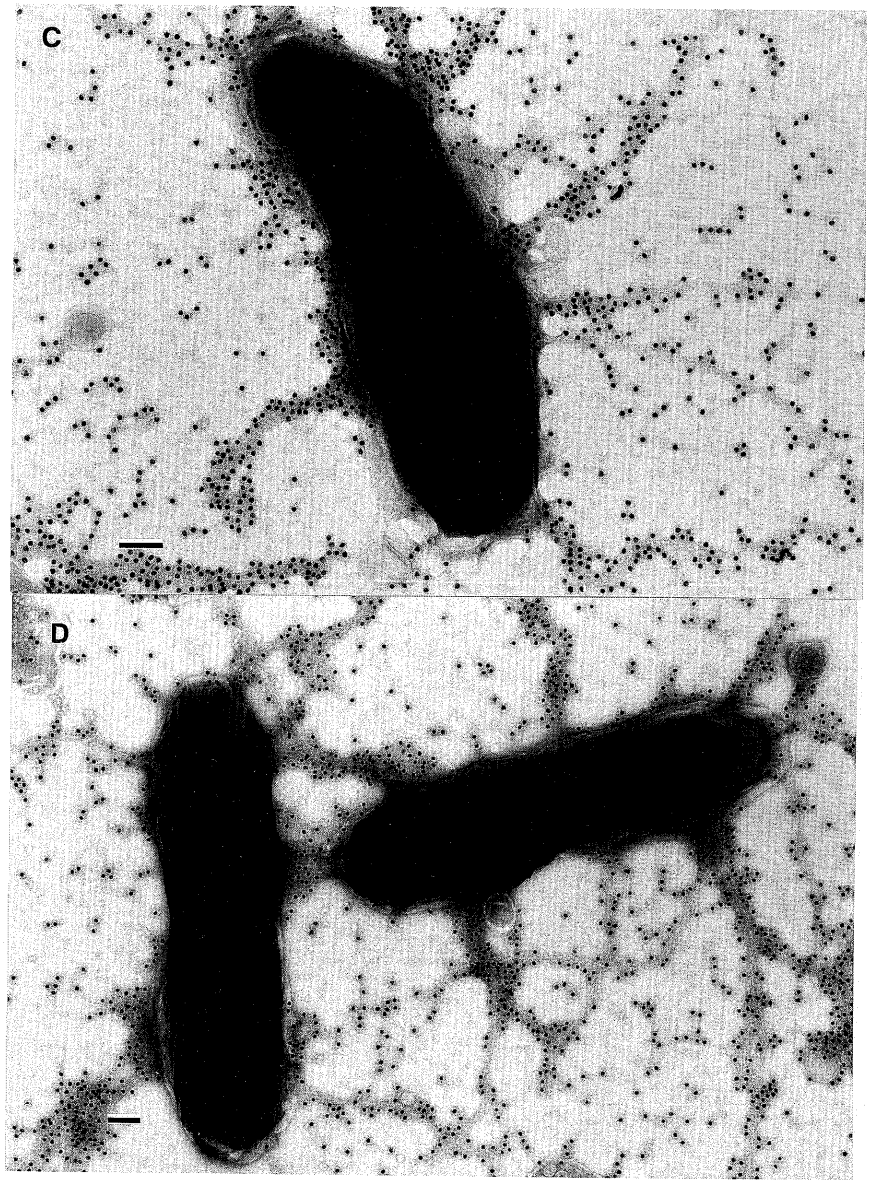

Fig. 7

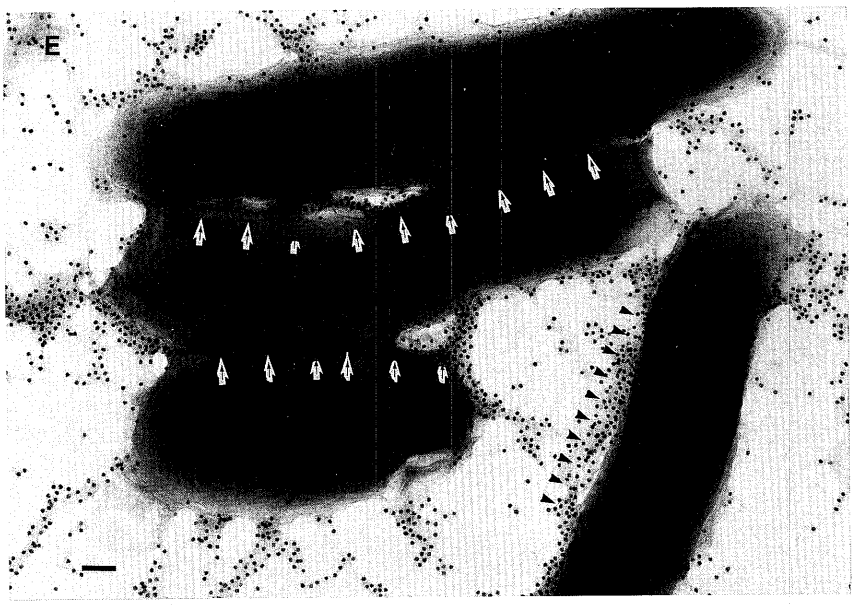

Fig. 7

have different HA neutralizing activities, however the detailed characterization of the antibodies and epitope analyses will be shown elsewhere. Out of these antibodies, the McAb42 was proven to recognize also the native fimbriae. Therefore, this clone was used to know the localization of fimbriae between vibrios and vibrios, possibly functioning as fimbrial adhesins necessary for cell-cell interaction and to compare the difference in reactivity between hydrophilic and hydrophobic fimbriae.

Immunoelectron microscopy of the fimbrillin with McAb42

Electron micrographs of the fimbriate whole cells treated with control sera indicated that there were no antibodies nonspecifically bound to the fimbriae (data not shown). The McAb42 bound weakly to the fimbriae of a hydrophilic cell, as compared to those of hydrophobic cells (Fig. 7, compare A with C and D). Fimbrial antigens were densely demonstrated between vibrio and vibrio as indicated by arrows (E). The surface of a detached cell was also coated with fimbrial antigens suggesting that the cell was interacting with another cell with the side as indicated by arrow-heads (E).

N-terminal amino acid sequences of the fimbrillins

The *N*-terminal amino acid sequences of the fimbrillins purified from strains of Bgd17 (classic, Inaba) and K23 (El Tor, Ogawa) were determined. The purified and dissociated molecules of the fimbrillins were shown to be electron-microscopically highly homogeneous (Fig. 8). The data obtained were compared with those of type NMePhe pilins (Dalrymple and Mattick, 1987; Paranchych and Frost, 1988) from *Neisseria gonorrhea* MS11 (Meyer *et al.*,

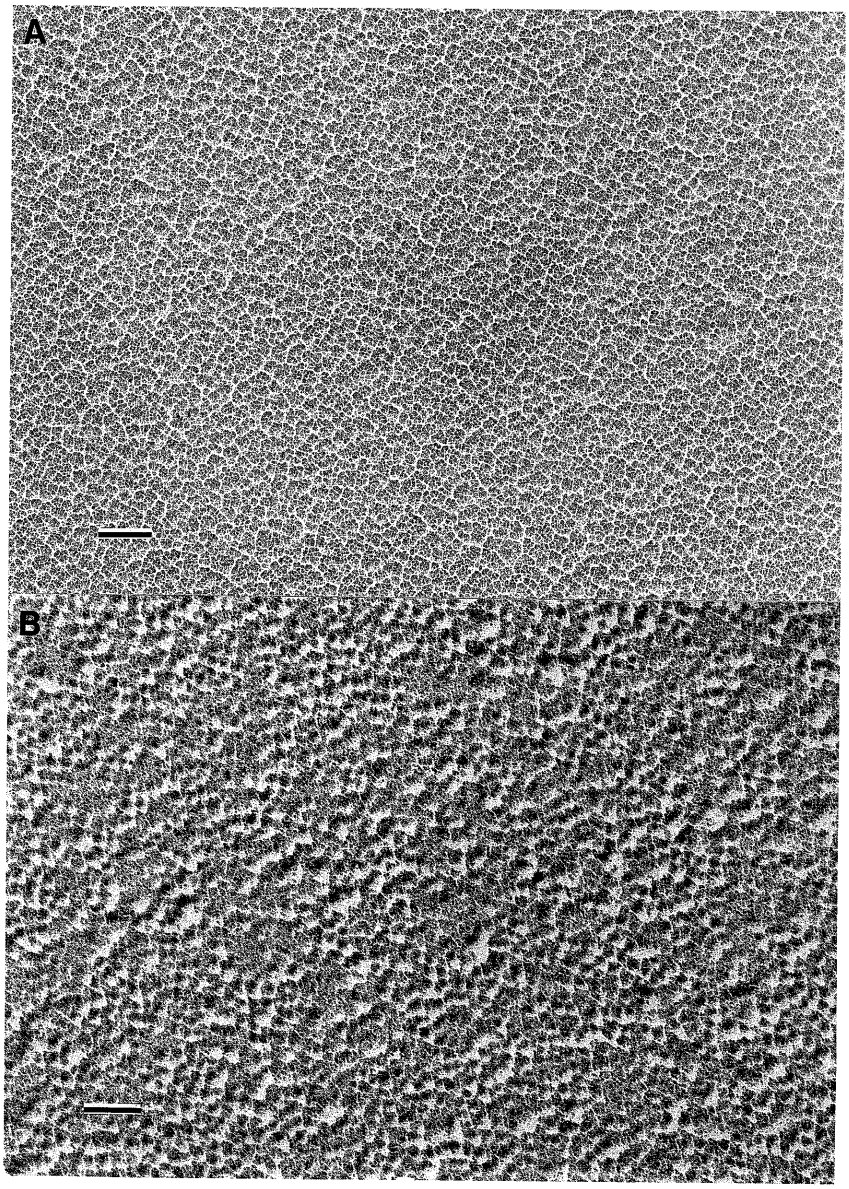

Fig. 8. Electron micrographs of the fimbrillins dissociated by the treatment with 8M urea.
A) dissociated fimbrillins of the strain Bgd17.
B) dissociated fimbrillin of the strain K23.

1984), *Pseudomonas aeruginosa* PAK (Sastry *et al.*, 1983), *Moraxella bovis* (Marrs *et al.*, 1985), *Bacteroides nodosus* (Elleman *et al.*, 1986) and with that of TcpA (Shaw and Taylor, 1990) of *V. cholerae* Ol, strain O395 (Fig. 9). The two sequences of fimbrillins from strains of Bgd17 and K23 were shown to be identical. The fimbrillin of *V. cholerae* Ol display extensive homology with a group of pilus major subunits of *N*-methylphenylalanine (NMePhe) pili in their hydrophobic *N*-terminal regions.

V. cholerae Bgd17	X	T	L	I	E	L	V	V	V	I	V	I	L	G	I	L	A	V	T	A	A	P
V. cholerae K23	X	T	L	I	E	L	V	V	V	I	V	I	L	G	I	L	A	V	T	A	A	P
N. gonorrheae MS11	F	T	L	I	E	L	M	I	V	I	A	I	V	G	I	L	A	A	V	A	L	P
P. aeruginoosa PAK	F	T	L	I	E	L	M	I	V	V	A	I	I	G	I	L	A	A	I	A	I	P
M. bovis B	F	T	L	I	E	L	M	I	V	I	A	I	I	G	I	L	A	A	I	A	L	P
B. nodosus	F	T	L	I	E	L	M	I	V	V	A	I	I	G	I	L	A	A	I	A	I	P
V. cholerae O395	M	T	L	L	E	V	I	I	V	L	G	I	M	G	V	V	S	A	G	V	V	T

Fig. 9. Comparison of the *N*-terminal amino acid sequences of the fimbrillins from strains of Bgd17 (classical, Inaba) and K23 (El Tor, Ogawa) with the sequences of type NMePhe pilins from *N. gonorrheae* MS11 (Meyer *et al.*, 1984), *P. aeruginosa* PAK (Sastry *et al.*, 1983), *M. bovis* (Marrs *et al.*), *B. nodosus* (Elleman *et al.* 1986), and with that of TcpA (Shaw and Taylor, 1990) of *V. cholerae* O1, strain O395. Residues that are identical in Bgd 17, K23 and all of the NMePhe pilins are boxed. Note also the homology in boxed residues among Bgd 17, K23 and TcpA.

DISCUSSION

Hydrophobic fimbriae of *V. cholerae* Ol, strain Bgd17, classical biotype and Inaba serotype, were purified and shown to be socalled cell-associated haemagglutinin sensitive to D-mannose, D-glucose and not to L-fucose. The fimbriae composed of 18 kDa fimbrillin are flexible, filamentous structures 5 to 7 nm in diameter. The inhibitory role of glucose in pellicle formation and in HA activity of the purified fimbriae has not been demonstrated for *V. cholerae* Ol. It is likely that the cell associated haemagglutinin of *V. cholerae* Ol displays the sensitivity to D-glucose clearly only when fimbriate cells are used as materials for the experiment. Glucose is an obviously important ingredient in ORS not only for Na^+−glucose cotransport but also for inhibiting the colonization of vibrios to the epithelial cells of the small intestine. The latter implies that malnutrition may affect the duration and the volume of diarrhoea due to *V. cholerae* Ol infection. In malnourished children, therefore, the mean duration of diarrhoea episodes may be longer and there may be a higher incidence of diarrhoea due to easy longer colonization of *V. cholerae* Ol to intestinal epthelia. The receptor for the fimbriae of *V. cholerae* Ol is considered to have an oligosaccharide which contains mannosyl and glucosyl residues.

Iwanaga *et al.* (1989), Nakasone and Iwanaga (1990) reported that fimbriae of *V. cholerae* Ol and non-Ol do not play a role in colonization. They failed to notice the follo-

wing points. They used non-fimbriate strains of *V. cholerae* Ol and non-Ol as the starting vibrios for their study. Their data showing the lost sensitivity of purified fimbriae to D-mannose and vibrio cells with intact flagella and a few fimbriae clearly show that vibrios used were in non-fimbriate. Even the fimbriae purified by them and vibrio cells appear to be hydrophilic under EM (compare the EM pictures with ours shown in Fig. 7).

When examined with the polyclonal anti-fimbriae antibody, all the strains of *V. cholerae* Ol tested, independent of biotype and serotype, were shown to have the immunologically cross-reactive fimbrial antigens. The antibody used can recognize even proteolyticaly digested fragments of the fimbrillin which are also highly antigenic. Therefore we cannot select the fimbriate strains by dot blot analysis, however the strong reaction of the fimbriate Bgd17 is suggestive.

As fimbriae (or pili) are originally polymers of a simple protein, it is a matter of course that the fimbrillin of *V. cholerae* Ol is a simple protein.

It is a new finding that the fimbrillins independent of biotype and serotype have the identical *N*-terminal amino acid sequence. Interestingly, the sequence of the fimbrillin is more similar to those of NMePhe or type 4 pilins than that of TcpA (Shaw and Taylor, 1990). These (NMePhe) pili are elaborated by a diverse group of gram-negative bacteria that have the common feature of colonizing mucosal surfaces (Meyer *et al.*, 1984) and also cause the piliate bacteria to auto-agglutinate, haemagglutinate, and to form a pellicle on the surface of broth cultures. The hydrophobic *N*-terminal regions are highly conservative in these species, but the hydrophilic *C*-terminal regions are variable (Meyer *et al.*, 1984). This latter region is the major target for the host immune response. As shown in Fig. 3, all enteropathogenic strains of *V. cholerae* Ol tested displayed a cross-reactivity at the 18 kDa subunit of the fimbriae. It is likely, therefore, that an extensive homology exists even in the central region and at the *C*-termini of the fimbrillin molecules.

The *N*-terminal amino acid residue of the fimbrillin was also modified and remains to be determined, possibly either methyl phenylalanine or modified methionine.

It is interesting to elucidate whether or not the expression of the fimbrillin is also co-regulated with cholera toxin by *Tox R* and whether or not there is any homology in the central regions of TcpA and that of the fimbrillin.

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