Immunochemical and functional studies of *Actinomyces viscosus* T14V type 1 fimbriae with monoclonal and polyclonal antibodies directed against the fimbrial subunit

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(Received 10 January 1991; revised 8 April 1991; accepted 15 May 1991)

Each of five monoclonal antibodies (mAbs) prepared against the type 1 fimbriae of *Actinomyces viscosus* T14V reacted with a 54 kDa cloned protein previously identified as a fimbrial subunit. This purified protein completely inhibited the reaction of a specific anti-type-1-fimbria rabbit antibody with *A. viscosus* whole cells. Maximum values for the number of antibody molecules bound per bacterial cell ranged from 7×10^3 to 1.2×10^4 for the different ¹²⁵I-labelled mAbs and was approximately 7×10^4 for ¹²⁵I-labelled rabbit IgG or Fab against either type 1 fimbriae or the 54 kDa cloned protein. Although the different mAbs, either individually or as a mixture, failed to inhibit the type-1-fimbria-mediated adherence of *A. viscosus* T14V to saliva-treated hydroxyapatite, each rabbit antibody gave 50% inhibition of adherence when approximately 5×10^4 molecules of IgG were bound per cell. However, binding of each corresponding rabbit Fab had no significant effect on bacterial attachment unless much higher concentrations were used. These findings suggest that antibodies directed solely against the 54 kDa fimbrial subunit do not react with the putative receptor binding sites of *A. viscosus* T14V type 1 fimbriae. Instead, inhibition of attachment by the polyclonal antibodies may depend on an indirect effect of antibody binding that prevents the fimbria–receptor interaction.

Introduction

Actinomyces viscosus, a Gram-positive member of the indigenous oral flora, participates in the microbial colonization of teeth, the formation of dental plaque and the initiation of gingival inflammation (Ellen, 1982). The affinity of this organism for teeth has been correlated with its attachment to saliva-treated hydroxyapatite (SHA) (Qureshi & Gibbons, 1981; Clark *et al.*, 1981). This interaction was inhibited by a rabbit antibody

against *A. viscosus* type 1 fimbriae but not by an antibody against type 2 fimbriae or by one against whole bacteria from which specific anti-type-1-fimbriae antibody had been removed by absorption (Clark *et al.*, 1984). Mutant cells with only type 1 fimbriae were adherent to SHA whereas those with only type 2 fimbriae did not attach (Cisar *et al.*, 1988). Similar results, obtained using beads of hydroxyapatite coated with purified proline-rich salivary proteins, suggested that the adherence of *A. viscosus* T14V to SHA depends on a specific fimbria–receptor interaction (Gibbons & Hay, 1988; Gibbons *et al.*, 1988; Clark *et al.*, 1989).

Studies to establish the subunit structure of *A. viscosus* T14V type 1 fimbriae have been limited by the resistance of these fimbriae to various methods of dissociation, including SDS-PAGE. However, a 54 kDa protein that reacted with antibody against *A. viscosus* type 1 fimbriae was expressed in *Escherichia coli* from a

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Abbreviations: Fab, papain-hydrolysed IgG fragment; mAb, monoclonal antibody; SHA, saliva-treated hydroxyapatite.

cloned *A. viscosus* T14V gene (Yeung *et al.*, 1987). An antibody raised against the cloned protein also reacted with type 1 fimbriae, and the pattern observed by Western blotting was comparable to the ladder of bands seen with antibody against intact fimbriae. Recent results (Yeung & Cisar, 1990) have shown that the N-terminal amino acid sequence of the type 1 fimbriae and the mature form of the cloned protein are identical, indicating that the cloned *A. viscosus* gene encodes the structural subunit of these fimbriae.

To assess the role of the 54 kDa type 1 fimbrial subunit in bacterial attachment, monoclonal and polyclonal antibodies directed against type 1 fimbriae and the cloned protein were characterized, and their effects on bacterial adsorption to SHA were compared.

Methods

Bacterial antigens. All strains of *A. viscosus* and *Actinomyces naeslundii* were grown in complex media (Cisar *et al.*, 1979). Fimbriae were removed from *A. viscosus* T14V by sonication, then applied to a column of Agarose 5M (Bio-Rad Laboratories) and recovered in fractions collected at the void volume (Cisar *et al.*, 1981). Type 1 fimbriae were precipitated in the presence of 20% saturated ammonium sulphate (Wheeler & Clark, 1980; Revis *et al.*, 1982), dissolved in Tris-buffered saline (TBS; 0.15 M-NaCl; 10^{-4} M-CaCl₂, 10^{-4} M-MgCl₂, 0.02% sodium azide and 0.02 M-Tris/HCl, pH 7.8), dialysed against this buffer to remove ammonium sulphate and passed through a column of affinity gel prepared by coupling the anti-type-2-fimbriae mAb 2A (Cisar *et al.*, 1981) to CNBr-activated Sephacryl S1000 (Pharmacia) (Cuatrecasas, 1970). In most cases, the amount of coupled immunoglobulin was approximately 1 mg per ml of gel. Type 1 fimbriae also were isolated from *A. viscosus* PK455, a mutant strain that does not express type 2 fimbriae (Cisar *et al.*, 1983). The fimbriae from this strain were applied to immunoaffinity gels prepared with a mAb described in this paper, and eluted with either NaSCN (3 M) or guanidine HCl (3 M to 6 M). Purified fimbriae were ¹²⁵I-labelled with Bolton-Hunter reagent (NEN Research Products) according to instructions provided by the manufacturer.

The cloned *A. viscosus* T14V type 1 fimbrial subunit was isolated from a soluble extract of *E. coli* MY3833 by immunoaffinity chromatography as described above and further purified by Sephacryl S200 (Pharmacia) gel filtration (Yeung *et al.*, 1987). All buffers contained 0.1 mM-PMSF (Sigma) and 0.25% CHAPS (Sigma) rather than Tween 20 which had been used previously (Yeung *et al.*, 1987).

Rabbit antisera. Antiserum R32 was prepared by repeated intravenous injections of *A. viscosus* T14V cells (Cisar *et al.*, 1981). Antisera R59 against *A. viscosus* PK455 type 1 fimbriae and R85 against the cloned type 1 fimbrial protein were prepared by subcutaneous injections of 50 µg antigen in Freund's adjuvant (Difco) at multiple sites on days 1 and 22. Antisera were obtained 2 to 4 weeks later and the IgG purified by methods described previously (Cisar *et al.*, 1983; Clark *et al.*, 1984). The production of Fab fragments by papain digestion of IgG and their purification by gel filtration and ion exchange column chromatography have also been described (Clark *et al.*, 1984).

Hybridomas and monoclonal antibodies. Female 8-week-old BALB/c mice each received 60 µg type 1 fimbriae from *A. viscosus* T14V administered in complete Freund's adjuvant at multiple sites on days 1 and 25. On day 35, immune splenocytes were adoptively transferred to

X-irradiated syngeneic recipients (Fox *et al.*, 1981) and one day later, each recipient was boosted with 50 µg fimbriae administered intravenously. On day 40, the spleens of recipient animals were removed and the cells hybridized with plasmacytoma cell line X63-Ag8.653 (Kearney *et al.*, 1979) as described previously (Cisar *et al.*, 1981). The selection, growth and cloning of hybridomas has also been described (Cisar *et al.*, 1981). Specific antibody in cell culture supernatants was detected by passive haemagglutination of fimbria-sensitized sheep erythrocytes and by agglutination of strain T14V. Clones positive for antibody activity were transferred to pristane-primed BALB/c mice for ascites production and the immunoglobulin purified from ascites fluid by methods described previously (Cisar *et al.*, 1981).

Immunological and biochemical methods. Concentrations of rabbit and mouse IgG or Fab were determined by measurements of ultraviolet absorbance using an absorption coefficient ($A_{1\%}^{1\text{cm}}$) of 15.0 at 278 nm. The techniques used for immunodiffusion, crossed immunoelectrophoresis and agglutination of bacteria or fimbria-sensitized erythrocytes have been described (Cisar *et al.*, 1981).

SDS-PAGE was performed by the method of Laemmli (1970) with a 3% (w/v) polyacrylamide stacking gel and a 3% to 15% (w/v) polyacrylamide gradient resolving gel. Samples were diluted in double strength sample buffer containing 2-mercaptoethanol and heated at 100 °C for 10 min. Gels containing ¹²⁵I-labelled fimbriae (3×10^5 c.p.m. per lane) were fixed, dried and exposed for autoradiography with XAR-5 film (Eastman Kodak). Immunoblotting of unlabelled type 1 fimbriae (6 µg per lane), fimbrial subunit (0.6 µg per lane) and ¹⁴C-labelled protein standards (Amersham) was performed as previously described (Donkersloot *et al.*, 1985). Nitrocellulose transfers were incubated with primary antibody (1 µg IgG ml⁻¹) for 2 h and subsequently with ³⁵S-labelled goat anti-mouse IgG (Amersham), ³⁵S-labelled donkey anti-rabbit IgG (Amersham) or ¹²⁵I-labelled goat anti-mouse IgG (NEN Research Products) for 2 h prior to washing and autoradiography. Bands were not observed on autoradiographs of transfers when nonimmune rabbit or mouse IgG was used in place of the primary antibody.

Electron microscopy. Type 1 fimbriae (50 µg ml⁻¹) were applied to grids, negatively stained with phosphotungstic acid, examined under a Philips 300 electron microscope and photographed (Cisar & Vatter, 1979). Immunoelectron microscopy of bacteria following their reaction with different mAbs was also performed as previously described (Cisar *et al.*, 1981).

Antibody binding. IgG and Fab fragments were labelled with ¹²⁵I [100 mCi ml⁻¹ (3.7 GBq ml⁻¹), ICN Biomedicals] by use of Iodo beads (Pierce Chemical Co.). Following removal of unincorporated iodine, the labelled immunoglobulin was assayed for protein using the bicinchoninic acid (BCA) Protein Assay Reagent (Pierce) with rabbit IgG as the standard, and for the percentage of radioactivity precipitated by 30% (w/v) trichloroacetic acid in the presence of carrier protein (0.2 mg bovine serum albumin ml⁻¹). Specific activities of approximately 3×10^5 c.p.m. (µg protein)⁻¹ were generally obtained when reaction mixtures contained 1 mg protein and 1 mCi (37 MBq) ¹²⁵I. In all experiments, more than 90% of the radioactivity was protein-associated. Total specific antibody was determined from the results of binding assays performed with a constant amount of labelled immunoglobulin (0.01 µg mAb or 0.35 µg rabbit IgG or Fab) and increasing numbers of bacteria in a total volume of 0.15 ml TBS containing 0.05% Tween 20. A modification of the technique described by Cuatrecasas & Hollenberg (1976) was used to rapidly separate bound from free antibody. Following 30 min incubation at room temperature with continuous mixing, triplicate 40 µl aliquots of each reaction mixture were layered above cushions of dibutyl phthalate (Eastman Kodak), bis(2-ethylhexyl)phthalate (Eastman Kodak) and Tween 20 (1.1/1.0/0.001 by volume, respectively) in 0.3 ml microtubes (no.

Table 1. Agglutination of *A. viscosus* and *A. naeslundii* strains by mAbs against *A. viscosus* T14V type 1 fimbriae

Minimum concentration of mAb required for agglutination relative to that for agglutination of *A. viscosus* T14V: ●, 0.33–3 ×; ○, 10–100 ×; ○, > 300 ×; –, no agglutination with ≤ 250 µg IgG ml⁻¹. The endpoints for agglutination of *A. viscosus* T14V (5 × 10⁸ cells ml⁻¹) ranged from 0.3 to 3 µg ml⁻¹ with mAbs 8A and 8B, respectively, and were determined from the settling pattern of bacteria after overnight incubation at 4 °C.

Monoclonal antibody	<i>Actinomyces viscosus</i> strains									<i>Actinomyces naeslundii</i> strains									
	W1528	W859	W1557	M100	5-5s	W1628	MG1	A1B1	A32B1	W752	I	W1544	W1527	WVU820	ATCC12104	W1096	WVU45	W826	WVU398
7B(IgG3)	●	●	●	○	●	●	—	—	—	—	—	—	—	—	—	—	—	—	—
8B(IgG1)	●	●	●	○	—	—	—	—	—	●	○	○	—	—	—	—	—	—	—
7A(IgG2b)	●	●	●	○	●	○	○	●	●	●	●	●	—	—	—	—	—	—	—
8A(IgG2a)	●	●	●	○	●	○	○	●	●	●	●	○	—	—	—	—	—	—	—
6A(IgG3)	●	●	●	●	●	●	●	●	●	●	●	●	—	—	—	—	—	—	—

72.702, Sarstadt Inc., Princeton, NJ). The tubes were centrifuged at 12000 g for 3 min in a Beckman Microfuge 11 to pellet bacteria; the tip of each tube was then removed, and cell-associated radioactivity was determined. Similar amounts of radioactivity were specifically bound to 1 × 10⁹ and 2 × 10⁹ cells and the mean of these values was used to estimate total specific antibody. Antibody affinity and the maximum number of molecules bound per cell were estimated from binding experiments performed with 1.5 × 10⁷ cells and different amounts of labelled antibody in a total volume of 0.15 ml. In all experiments, controls were performed by incubating bacteria with radiolabelled normal rabbit or mouse IgG and Fab fragments to estimate nonspecific binding, which was generally less than 10% of the total amount bound. Similar estimates of nonspecific binding were obtained when unlabelled antibody was included in reaction mixtures at concentrations that were 100 times those of the homologous, labelled antibody. Free antibody was determined as the difference between specific antibody added and the amount specifically bound.

Bacterial adherence. The assays for adsorption of [³H]thymidine labelled actinomyces to saliva-treated Spheroidal Hydroxylapatite (BDH Chemicals) and inhibition of bacterial adsorption by antibody have been described by Clark *et al.* (1978, 1984). In the present study, 1 × 10⁷ labelled bacteria were preincubated for 30 min with buffer and either specific antibody or normal rabbit or mouse immunoglobulin in a total volume of 0.1 ml. Reaction mixtures were transferred to 250 µl microfuge tubes (Beckman Instruments) containing 5 mg SHA beads or control tubes with no beads. After 90 min of continuous mixing, the radioactivity in supernatants (i.e. unadsorbed bacteria) of experimental and control tubes was determined and the percentage adsorption of bacteria to SHA calculated.

Results

Five hybridomas secreting mAbs that agglutinated *A. viscosus* T14V were derived from splenocytes of BALB/c mice immunized with type 1 fimbriae. Each mAb also agglutinated certain heterologous bacterial strains as indicated in Table 1. The strain specificities of mAbs 7B and 8B differed from each other and from those

of mAbs 7A, 8A and 6A. The latter three were similar in that each reacted with the same nine *A. viscosus* and three *A. naeslundii* strains. Significantly, *A. naeslundii* WVU45 (ATCC 12104) and certain similar strains which express type 2 but not type 1 fimbriae (Cisar *et al.*, 1984) were not agglutinated by any of the mAbs.

Whereas rabbit antiserum against whole bacteria precipitated the type 1 and type 2 fimbrial antigens of *A. viscosus* T14V (Fig. 1a), mAb 8A (Fig. 1b) and each of the other four (results not shown) reacted only with the type 1 antigen. Moreover, preincubation of each mAb with fimbriae prior to electrophoresis removed the type 1, but not the type 2, component from the pattern developed by precipitation with the rabbit antiserum (Fig. 1c). Consistent with these results, immunodiffusion (Fig. 2) with isolated fimbriae revealed reactions of at least partial identity between different anti-type-1 mAbs and a reaction of nonidentity between an anti-type-1 and an anti-type-2 mAb (Cisar *et al.*, 1981).

The reaction of each mAb with fimbrial structures on the bacterial surface was verified by immunoelectron microscopy (results not shown). In addition, type 1 fimbriae from *A. viscosus* PK455 were retained on an affinity column prepared by coupling mAb 8B to Sephacryl S1000, and were eluted with 6 M-guanidine HCl (Fig. 3a) as shown by electron microscope examination of a dialysed sample (Fig. 3b).

Radioiodinated type 1 fimbriae subjected to SDS-PAGE migrated as a series of closely spaced bands extending from the top of the resolving gel to the 30 kDa region with additional radioactivity near the dye front (i.e., below the 21 kDa marker in Fig. 4). Nitrocellulose transfers of unlabelled fimbriae probed with each mAb or with rabbit (R59) antibody against type 1 fimbriae

Fig. 1. Crossed immunoelectrophoresis showing the reaction of mAb 8B with the type 1 but not the type 2 fimbriae of *A. viscosus* T14V. (a) Fimbriae in the well with rabbit antiserum against *A. viscosus* T14V in the agar (arrows indicate precipitated type 1 or type 2 fimbriae); (b) fimbriae in the well with mAb 8B in the agar; (c) fimbriae preincubated with mAb 8B in the well with rabbit antiserum against *A. viscosus* T14V in the agar. Anode is to the left.

Fig. 2. Immunodiffusion with mAbs against *A. viscosus* T14V fimbriae. Wells 1 to 5, respectively, anti-type-1 mAbs 8B, 8A, 7A, 6A and 7B; well 6, anti-type-2 mAb 2A; well 7, rabbit anti-*A. viscosus* T14V. Ag wells, *A. viscosus* T14V fimbriae.

resulted in similar patterns of immunoreactive bands down to the 60 kDa region (Fig. 4). The reactions of mAb 8A or 8B and that of the rabbit antibody also revealed additional faint bands near the 35 kDa region of autoradiographs exposed six times longer than those shown in Fig. 4 (results not shown). Each of the five

mAbs reacted with the cloned type 1 fimbrial subunit, which has a calculated molecular mass of 54000 Da based on the amino acid sequence deduced from the DNA sequence (Yeung & Cisar, 1990). This protein appeared as a doublet on nitrocellulose transfers overlaid with rabbit antibody against type 1 fimbriae (Yeung *et al.*, 1987) or each individual mAb (Fig. 4). In similar immunoblotting experiments, reactions were not observed between the anti-type-1 antibodies and *A. viscosus* T14V type 2 fimbriae (results not shown).

The binding of each ^{125}I -labelled mAb to *A. viscosus* T14V gave linear Scatchard plots (correlation coefficient < -0.98) that intersected the x-axis at values of n ranging from 7.0×10^3 molecules of mAb 7A to 1.2×10^4 molecules of mAb 6A bound per cell (Table 2). Association constants (K_a) for the binding of different antibodies to bacteria varied from $9 \times 10^8 \text{ M}^{-1}$ for protein 7B to $4.5 \times 10^9 \text{ M}^{-1}$ for protein 8B (Table 2). Whereas near maximum binding was observed with $1.5 \mu\text{g}$ of each mAb per ml, $100 \mu\text{g ml}^{-1}$ gave no significant inhibition of bacterial adsorption to SHA, and in addition, a mixture of the different mAbs, each at $25 \mu\text{g ml}^{-1}$, also failed to inhibit (results not shown).

Fig. 3. Isolation of fimbriae using an affinity column prepared with mAb 8B. Trace-labelled type 1 fimbriae (300 μ g protein) from *A. viscosus* PK455 were applied to a 1.5 ml column of affinity gel: (a) elution profile showing one peak of radioactivity eluted with buffer and another eluted (↓) with 6 M-guanidine HCl; (b) electron micrograph of type 1 fimbriae that were eluted from the column with guanidine HCl, dialysed against buffer and negatively stained. Fimbriae were not observed in fractions that passed through the column with buffer. Bar, 0.1 μ m.

Fig. 4. SDS-PAGE of *A. viscosus* T14V type 1 fimbriae and the cloned type 1 fimbrial subunit. The electrophoretic pattern of iodinated fimbriae (125 I-F) was revealed by autoradiography of the dried gel and the immunoreactivity of unlabelled fimbriae (F) or fimbrial subunit (S) by Western blotting. Nitrocellulose transfers were treated with rabbit antibody against type 1 fimbriae (R59) or each mAb (8B, 8A, 7A, 6A or 7B), incubated with radiolabelled anti-rabbit IgG or anti-mouse IgG and exposed for autoradiography.

Table 2. Binding of mouse monoclonal and rabbit polyclonal antibodies to *Actinomyces viscosus* T14V

Immunoglobulin	Immunizing antigen	Antigen reactivity (% $\pm$ S.E.)*	$10^{-3} \times n$ (molecules per cell $\pm$ S.E.)†	$10^{-8} \times K_a$ (M^{-1} $\pm$ S.E.)†	Number of experiments
Monoclonal antibody					
8A(IgG2a)	fimbriae	99.4 $\pm$ 0.6	9.3 $\pm$ 0.4	21 $\pm$ 2	3
8B(IgG1)	fimbriae	78.9 $\pm$ 1.8	10.0 $\pm$ 0.5	45 $\pm$ 5	4
7A(IgG2b)	fimbriae	76.1 $\pm$ 0.6	7.0 $\pm$ 1.1	17 $\pm$ 2	3
6A(IgG3)	fimbriae	87.1 $\pm$ 1.5	12.0 $\pm$ 0.9	13 $\pm$ 2	3
7B(IgG1)	fimbriae	82.5 $\pm$ 2.1	7.7 $\pm$ 0.6	9 $\pm$ 0.6	4
Polyclonal antibody					
R59 IgG	fimbriae	7.3 $\pm$ 0.05	65 $\pm$ 1	1.9 $\pm$ 0.2	4
R59 Fab	fimbriae	4.5 $\pm$ 0.3	79 $\pm$ 7	0.8 $\pm$ 0.1	3
R85 IgG	subunit	7.7 $\pm$ 0.3	75 $\pm$ 4	1.7 $\pm$ 0.2	3
R85 Fab	subunit	4.4 $\pm$ 0.1	72 $\pm$ 3	1.0 $\pm$ 0.2	3

* Percentage of total ^{125}I -labelled IgG or Fab specifically bound to excess *A. viscosus* T14V.

† Maximum number of molecules bound per cell (n) and association constant (K_a , reciprocal of concentration of free antibody for half maximum binding) determined by Scatchard analysis of ^{125}I -labelled IgG or Fab binding to *A. viscosus* T14V (10^8 cells ml^{-1}).

Fig. 5. Effects of rabbit IgG and Fab against *A. viscosus* T14V type 1 fimbriae or fimbrial subunit on adsorption of 1×10^7 *A. viscosus* T14V cells to 5 mg SHA in 0.1 ml total volume. The binding curve of each immune IgG and Fab to *A. viscosus* T14V (10^8 cells ml^{-1}) is shown in the inset. Values of r at very high (∞) concentrations of total IgG or Fab were estimated from Scatchard plots. ●, Anti-fimbria subunit IgG; ■, anti-fimbria IgG; ◆, normal rabbit IgG or Fab; ○, anti-fimbria subunit Fab; □, anti-fimbria Fab.

The binding curves of an ^{125}I -labelled rabbit antibody against type 1 fimbriae (R59) and another against the cloned fimbrial subunit (R85) were similar over a range of 4 to 100 μg of added IgG per ml (Fig. 5, inset). Scatchard plots of these data were linear (correlation

coefficients < -0.98) and values of n for these polyclonal antibodies were 6.5×10^4 and 7.5×10^4 molecules of IgG bound per cell, respectively (Table 2). Comparable values were obtained with the labelled Fab of each rabbit antibody. In addition, the estimated K_a of each

Fig. 6. Inhibition of binding of 50 µg ml⁻¹ ¹²⁵I-labelled R85 anti-fimbrial subunit Fab (a) or R59 anti-fimbria Fab (b) to *A. viscosus* T14V (10⁸ cells ml⁻¹) by either type 1 fimbriae (●) or the cloned type 1 fimbrial subunit (○).

IgG was only twofold greater than that of the corresponding Fab, a finding that suggested monovalent binding by the majority of anti-fimbria IgG molecules. The epitope specificities of each rabbit antibody appeared to be similar but not identical since, on a weight basis, type 1 fimbriae and the cloned subunit, though nearly equivalent inhibitors of binding of the ¹²⁵I-labelled anti-subunit Fab (Fig. 6a), differed by a factor of three as inhibitors of binding of the anti-fimbria Fab (Fig. 6b). In spite of this difference, binding of the anti-fimbria Fab to cells was inhibited at least 99% by the cloned fimbrial subunit.

Unlike the different mAbs, immune rabbit IgG against the fimbriae and fimbrial subunit inhibited adsorption of *A. viscosus* T14V to SHA (Fig. 5). Fifty percent inhibition of bacterial attachment was observed when each antibody was added at concentrations that resulted in binding of approximately 5×10^4 IgG molecules per cell (i.e. 60 µg R59 IgG and 40 µg R85 IgG ml⁻¹). In contrast, similar binding of each corresponding rabbit Fab had no significant effect on bacterial attachment (Fig. 5). A concentration of Fab (100 µg ml⁻¹) that resulted in approximately 85% saturation of the cells inhibited attachment by only 10% or less. Although 50% inhibition could be achieved, it required the addition of each immune rabbit Fab at a final concentration of almost 1000 µg ml⁻¹.

Discussion

The present findings demonstrate that each of five mAbs for *A. viscosus* T14V type 1 fimbriae is directed against the 54 kDa protein previously identified as a fimbrial subunit by cloning and expression of an *A. viscosus* gene in *E. coli* (Yeung *et al.*, 1987). The apparent specificities of mAbs 6A, 7A and 8A were similar, since each agglutinated the same group of heterologous bacterial strains (Table 1) and competed with the other two for binding to *A. viscosus* T14V (Sandberg *et al.*, 1990). In contrast, mAbs 7B and 8B reacted with nonidentical sets of heterologous strains (Table 1) and each failed to compete with any of the other four mAbs for binding to strain T14V (Sandberg *et al.*, 1990). Thus, the panel of five mAbs was directed against at least three different epitopes of the 54 kDa fimbrial subunit.

The results from SDS-PAGE of radioiodinated type 1 fimbriae and immunoblotting of unlabelled fimbriae with each mAb showed that epitopes of the 54 kDa cloned subunit were associated with virtually every band of fimbrial protein. Even those bands in the 30 to 35 kDa region that were readily detected following electrophoresis of ¹²⁵I-labelled fimbriae appeared to be antigenically related to the 54 kDa subunit, since they were weakly reactive with two different mAbs. The detection of these reactions, like the demonstration of a 35 kDa band following immunoblotting of type 1 fimbriae with rabbit antibody against the 54 kDa cloned protein (Yeung *et al.*, 1987), may indicate that at least some of these bands are derived from the 54 kDa subunit. Thus, the present immunochemical findings strongly support the identification (Yeung *et al.*, 1987; Yeung & Cisar, 1990) of this protein as the structural subunit of *A. viscosus* T14V type 1 fimbriae.

The complete inhibition of binding of a rabbit anti-fimbria Fab to whole bacteria by the 54 kDa cloned protein (Fig. 6b) showed that under non-denaturing conditions, this protein and intact type 1 fimbriae shared a similar set of epitopes. That the two antigens were not equivalent inhibitors of the anti-fimbria Fab, on a mass basis, may be related to a difference between the quaternary structures of the intact fimbriae and the cloned protein that influences the exposure and spatial arrangement of common epitopes. Electron microscopy of the cloned protein did not reveal fimbriae. However, elution of the protein at or near the void volume of a Sephacryl S200 gel filtration column (result not shown) suggested that it does not exist as a monomer in solution. Indeed, SDS-PAGE and immunoblotting of the unheated subunit resulted in the appearance of bands with apparent molecular masses of 47, 85 and 108 kDa (M. K. Yeung, unpublished). The 47 kDa band was previously identified as the undenatured monomer (Yeung *et al.*,

1987), and consequently, the slower migrating proteins may be dimers and trimers. These oligomers were fully dissociated by heating in the presence of SDS (Fig. 4) and therefore were maintained by subunit-subunit interactions that differed from those of mature fimbriae. The structural basis of these observations and their relationship to the biogenesis of *A. viscosus* fimbriae remains to be determined.

The Fab of a rabbit antibody, prepared previously by immunization with type 1 fimbriae, appeared to be a potent inhibitor of bacterial adherence to SHA (Clark *et al.*, 1984); i.e. the amount of Fab required for 50% inhibition was twice that of the corresponding IgG (6 µg Fab vs 3 µg IgG ml⁻¹). In contrast, comparable inhibition by rabbit antibody R59 against type 1 fimbriae or antibody R85 against the 54 kDa cloned protein required approximately 20 times more Fab than IgG on a weight basis (Fig. 5). With these antibodies, 50% inhibition of adherence occurred when the amount of IgG bound was somewhat greater than 50% of saturation and the amount of Fab bound approached saturation. Consequently, the antibodies under study were probably not directed against putative receptor binding sites of the type 1 fimbriae. A simple effect of bacterial agglutination also seemed unlikely since bacterial adherence to SHA was not affected by other antibodies with agglutinating activity for *A. viscosus* T14V, including a rabbit antibody against type 2 fimbriae (Clark *et al.*, 1984) and each anti-type-1 mAb (Table 1). Instead, other mechanisms such as changes in the charge, hydrophobicity or cell-surface orientation of the type 1 fimbriae that indirectly prevent the fimbriae-receptor interaction may account for inhibition of adherence by the intact anti-type-1 polyclonal antibodies (Fig. 5).

The present findings with antibodies against the 54 kDa type 1 fimbrial subunit do not necessarily exclude the participation of this protein in receptor binding since weakly immunogenic or nonimmunogenic regions of the protein may be involved. A similar possibility has been proposed in studies of gonococcal pili (Rothbard *et al.*, 1985). Alternatively, the functional activity of *A. viscosus* type 1 fimbriae may depend on a minor and as yet unidentified protein, analogous to the various adhesins associated with different *E. coli* pili (Lindberg *et al.*, 1986; Moch *et al.*, 1987; Maurer & Orndorff, 1987; Abraham *et al.*, 1987). Further studies of *A. viscosus* type 1 fimbriae are in progress to examine these possibilities and also to define better the mechanisms by which different antibodies against these structures inhibit bacterial adherence.

We thank Jacob Donkersloot for his helpful suggestions and critical review of this manuscript.

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