Cloning and Expression of a Type 1 Fimbrial Subunit of Actinomycyes viscosus T14V

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The type 1 fimbriae of Actinomycyes viscosus mediate the adherence of this organism to saliva-treated hydroxyapatite. The gene encoding a putative subunit of this fimbrial adhesin was cloned in Escherichia coli, and its product was examined. A. viscosus T14V chromosomal DNA was partially restricted with Sau3A1 and cloned into E. coli JM109 by using the plasmid vector pUC13. Two clones, each containing a different DNA insert with a common 4.1-kilobase region, reacted in colony immunassays with specific polyclonal antibodies directed against A. viscosus T14V type 1 fimbriae. Western blot analysis revealed the expression of a 65-kilodalton protein that migrated slightly behind an antigenically similar protein from native type 1 fimbriae. Deletion analysis showed that the gene encoding the cloned protein was localized on a 1.9-kilobase PstI-BamHI fragment and that transcription was dependent on the lac promoter of the vector. The cloned fimbrial protein was purified from the E. coli cytoplasmic fraction by ion-exchange, immunoaffinity, and gel permeation chromatography. Rabbit antibodies prepared against the cloned protein and against purified A. viscosus type 1 fimbriae gave similar patterns with partially dissociated type 1 fimbriae after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. The data therefore provide evidence that the gene cloned encodes a subunit of this fimbrial adhesin.

Two immunologically distinct types of fimbriae on the gram-positive oral bacterium Actinomycyes viscosus T14V have been identified (5). Those designated as type 1 are thought to be the principal adhesin for bacterial attachment to the tooth surface. This has been inferred from the ability of specific type 1 fimbria antibody to inhibit the attachment of A. viscosus to saliva-treated hydroxyapatite (8) and by the demonstration that mutants lacking type 1 fimbriae fail to attach (J. O. Cisar, A. E. Vatter, W. B. Clark, S. H. Curl, S. Hursi-Calderone, and A. L. Sandberg, manuscript in preparation). In contrast to type 1, the type 2 fimbriae are the sites of a lactose-sensitive lectin activity involved in the interactions of Actinomycyes spp. with certain streptococci (5) as well as with sialidase-treated mammalian cells (4, 24).

While functional activities have been associated with each type of A. viscosus fimbria, these adhesins have not been structurally characterized because isolated fimbriae are resistant to complete dissociation by various means (5). Therefore, cloning of A. viscosus genes in Escherichia coli has been initiated as an approach to the identification of the fimbrial subunits. In this respect, a type 2 fimbrial gene has recently been cloned into a cosmid vector by Donkersloot et al. (10) and the encoded protein (molecular weight, 59,000) has been identified by its reaction with anti-type 2 fimbria antibody. The cosmid library was also screened for recombinant clones that expressed the type 1 fimbrial antigen, but none were detected. The present study describes the cloning of the type 1 fimbrial subunit gene of A. viscosus T14V by using pUC as the expression vector.

MATERIALS AND METHODS

Bacteria and plasmids. A. viscosus T14V (5) was the source of target DNA. E. coli JM109 [Δ(lac-pro) endA1 gyrA96 thi hsdR17 supE44 relA1 recA1F trd36 proA1Δ7 lacX74 lacZΔM15] was the host strain in all experiments. Plasmids pUC13, pUC18, and pUC19 (28) were used as cloning vectors.

Preparation of A. viscosus T14V clone bank in E. coli. The procedures for the isolation of A. viscosus chromosomal DNA were similar to those described by Donkersloot et al. (10). Purified chromosomal DNA was partially digested with 0.28 U of Sau3A1 (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) per µg of DNA at 37°C for 15 min. This condition resulted in the enrichment of DNA fragments in the 5- to 30-kilobase (kb) range as determined by agarose (0.4%) gel electrophoresis of the digested product. The digested sample was applied to a linear gradient of 10 to 40% sucrose in 1 M NaCl–5 mM EDTA–20 mM Tris (pH 8.0) and centrifuged overnight at 27,000 rpm at 25°C in an SW41 rotor. Fractions of 200 µl were collected, and 25 µl of each fraction was analyzed by agarose gel electrophoresis. Fractions containing DNA fragments of less than 10 kb were pooled, concentrated, and dissolved in 10 mM Tris–1 mM EDTA (pH 8.0) (TE) for ligation.

Plasmid pUC13 (restricted with BamHI and dephosphorylated with bacterial alkaline phosphatase) was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Sau3A1-restricted A. viscosus DNA and BamHI-restricted pUC13 were combined (3:1 molar ratio) and incubated at 14°C with 2 U of T4 DNA ligase (Bethesda Research Laboratories) in 20 µl of ligation buffer (18). The ligation mixture was used to transform E. coli JM109 competent cells prepared by the method of Hanahan (12). Portions of transformed cells were plated onto LB (18) agar plates containing ampicillin (50 µg/ml), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; 0.005%), and isopropyl-β-D-thiogalactopyranoside (IPTG; 0.25 mM).

Plasmid isolation and DNA mapping. Isolation of plasmid DNA was as described by Birnboim and Doly (3). For large-scale DNA preparation, the plasmids were further purified by centrifugation to equilibrium in CsCl-ethidium bromide gradients at 35,000 rpm for 48 h (18). Endonuclease maps were obtained by single and double restrictions with
enzymes obtained from Bethesda Research Laboratories or Boehringer Mannheim, and the digestion conditions were as suggested by the suppliers. The digested products were electrophoresed on a 1% agarose gel in Tris-acetate buffer (18).

**Antigens and antibodies.** Type 1 and type 2 fimbriae were purified as previously described (6, 7). Rabbit antisera R32 against *A. viscosus* T14V, R55 against type 2 fimbriae, R59 against type 1 fimbriae, R66 against strain PK455-2, a mutant lacking type 1 and type 2 fimbriae, and each of five monoclonal antibodies against the type 1 fimbriae of strain T14V have also been described (6, 7; J. O. Cisar, A. L. Sandberg, E. L. Barsumian, R. P. Siraganian, S. H. Curl, W. B. Clark, and A. E. Vatter, manuscript in preparation). Rabbit anti-serum R85 against the *A. viscosus* cloned type 1 fimbrial protein was prepared by immunization at multiple subcutaneous sites with 120 μg of purified cloned protein mixed with incomplete adjuvant. A boost with the same amount of antigen in incomplete adjuvant was given at 21 days, and antisera was obtained 10 days later. Prior to use in immunological assays, immune immunoglobulin G (IgG) was prepared (8) and extensively absorbed with *E. coli* JM109 cells and with an affinity gel prepared by coupling a sonic extract of *E. coli* cells to CNBr-activated (9) Sepharose CL-4B (Pharmacia, Inc., Piscataway, N.J.).

**Immunological screening.** *E. coli* colonies were transferred from LB (18) agar plates to detergent-free nitrocellulose (type HATF; Millipore Corp., Bedford, Mass.). The filters were then placed onto fresh LB agar plates and incubated at 37°C overnight. The colonies on replicate filters were lysed with chloroform by the method of Helfman et al. (14), and the filters were processed as described previously (10, 14). For Western blot analysis of *E. coli* lysates, cells from a 5-ml overnight culture in L broth containing 50 μg of ampicillin per ml and 0.25 mM IPTG were washed, suspended in 500 μl of Laemmli buffer (17) containing 2 mM EDTA, and sonicated for 1 min. The samples were heated in a boiling-water bath for 5 min and centrifuged at 12,000 × g for 5 min, and 20 μl of the supernatant fluid was applied to sodium dodecyl sulfate (SDS)-polyacrylamide gels (5 to 12% gradient or 10% gel). After SDS-polyacrylamide gel electrophoresis (PAGE), the proteins were electroblotted to nitrocellulose (BA85; Schleicher & Schuell, Inc., Keene, N.H.) in 25 mM phosphate buffer (1). The transfers were incubated sequentially in blocking buffer (10), rabbit or mouse immunoglobulins at 1 μg/ml, and 125I-labeled protein A prior to autoradiography. Alternatively, after the reaction with primary antibody, the filters were incubated in peroxidase-conjugated anti-rabbit or anti-mouse IgG (Bio-Rad Laboratories, Richmond, Calif.) and developed with hydrogen peroxide and 4-chloro-1-naphthol (Bio-Rad).

Dot-blot assays were performed by spotting 1 to 3 μl of purified *A. viscosus* fimbriae, *E. coli* cell extracts, or column fractions directly onto nitrocellulose. The filters were allowed to dry for 15 to 20 min at room temperature and then incubated with blocking buffer (10) and processed for antigen detection.

**Purification of cloned and native *A. viscosus* T14V type 1 fimbrial subunits.** Cells from a 3-liter overnight culture of *E. coli* MY3833 in L broth containing ampicillin and IPTG were harvested by centrifugation at 12,000 × g, washed twice in phosphate-buffered saline at 4°C, and converted to protoplasts with EDTA and lysozyme by the method of Weiss (29). The protoplasts were lysed, and the resulting suspension was centrifuged at 400,000 × g at 4°C for 1 h. The supernatant was removed and applied to a DEAE-Sepharose (Pharmacia) column (1.5 by 22 cm) equilibrated with 0.02 M Tris (pH 8.0). Proteins were eluted with a linear gradient of KCl (0.1 M to 0.25 M) in 0.02 M Tris. Fractions were monitored for the type 1 fimbrial antigen by dot-blot assays, and antigen-containing fractions were pooled, concentrated, and dialyzed against Tris-buffered saline (TBS; 20 mM Tris hydrochloride [pH 7.8], 150 mM NaCl, 1 mM MgCl2, 1 mM CaCl2, 0.02% sodium azide) containing 0.05% Tween 20. The sample was applied to an affinity column prepared by coupling anti-type 1 monoclonal antibody 8A (Cisar et al., in preparation) to CNBr-activated (9) Sephacryl S-1000 (Pharmacia). The column was washed extensively with TBS-0.05% Tween 20 to remove unbound material, and bound antigen was eluted with 3 M guanidine hydrochloride in TBS-0.05% Tween 20. The eluate was dialyzed against TBS, concentrated, and applied to a Sephacryl S-200 (Pharmacia) column (0.75 by 90 cm), and antigen was eluted with TBS. Protein was determined by the method of Pierce Chemical Co., Rockford, Ill.) with bovine serum albumin as the standard. The purity of preparations was evaluated by silver staining of SDS-polyacrylamide gels by the method of Wray et al. (30).

The monoclonal anti-type 1 affinity gel also was used to isolate native type 1 fimbrial subunits from a sonic extract of *A. viscosus* T14V (6) enriched for proteins of approximately 60 kilodaltons (kDa). The 60-kDa fraction, obtained by sequential gel filtration of the extract through Agarose 5 M (Bio-Rad) and Sephacryl S-300 (Pharmacia), was applied to the affinity gel, and bound antigen was eluted with 3 M guanidine hydrochloride.

**RESULTS**

**Identification of antigen-producing clones.** Approximately 50,000 recombinant clones from the genomic library of *A. viscosus* DNA in *E. coli* JM109 were screened by colony immunoassays for reactivity with rabbit antibody against *A. viscosus* T14V type 1 fimbriae. Two clones, MY1001 and MY2101, containing recombinant plasmids pMY1001 and pMY2101, respectively, were positive upon repeated screening for antigen expression. These recombinants also reacted with anti-type 1 monoclonal antibodies but failed to react with rabbit antibody against *A. viscosus* type 2 fimbriae (Fig. 1). In addition, both clones were positive with antibody against strain T14V (Fig. 1) but negative with antibody against strain PK455-2, a mutant that lacks both type 1 and type 2 fimbriae (data not shown).

Immunoblot analysis of cell lysates with specific anti-type 1 antibodies showed that the proteins expressed by clones MY1001 and MY2101 were of similar size. After electrophoresis in linear SDS-polyacrylamide gels (10 or 12% polyacrylamide), the cloned protein migrated as one predominant band with an apparent molecular weight of 65,000 (data not shown), but in SDS-polyacrylamide gradient gels (5 to 12% polyacrylamide), two closely spaced bands were observed (Fig. 2). The upper band of the doublet was the predominant species in freshly prepared samples, while the two bands were approximately equal in samples previously stored at 4°C. The doublet detected from the recombinant lysates appeared similar to a pair of bands observed when type 1 fimbriae were subjected to SDS-PAGE followed by Western blotting with polyclonal as well as monoclonal anti-type 1 antibodies (Fig. 2). However, the cloned protein had a slightly slower migration rate than the corresponding bands from native fimbriae.

**Localization of the cloned gene.** Endonuclease restriction maps showed that plasmids pMY1001 and pMY2101, al-
though not identical, share a common 4.1-kb region of *A. viscosus* DNA (Fig. 3). Plasmid pMY1001 contained additional *SalI* partial fragments that presumably arose from random ligation of unrelated fragments into the insert present in pMY2101. Analysis of deletion mutants of pMY2101 for expression of the type 1 antigen further localized the gene for the 65-kDa type 1 fimbrial protein on a 1.9-kb *PstI-BamHI* fragment (pMY3833; Fig. 4 and 5). Deletion of the 0.5-kb *BamHI-SalI* fragment from pMY3833 resulted in plasmid pMY4807, which encoded the 47-kDa N-terminal segment of the cloned protein (Fig. 3A). Subclones such as MY5034 carrying the 0.5-kb *SalI-BamHI* fragment of pMY3833 were also obtained, but these did not express an immunoreactive product (Fig. 4). Expression of the type 1 antigen was not detected when the DNA inserts from pMY3833 and pMY4807 were re ligated to the *pUC* vector in an orientation opposite from that shown in Fig. 5B. Thus, expression of the *A. viscosus* protein in *E. coli* is controlled by the *lac* promoter of the vector with the direction of transcription as indicated (Fig. 5B). In addition, expression of the 65-kDa protein directed by pMY3833 and of the 47-kDa protein directed by pMY4807 was induced by the addition of IPTG to the culture medium (data not shown).

**Heat modifiability of the cloned protein.** The cloned type 1 protein was heat modifiable, as shown by its electrophoretic migration properties in SDS-PAGE. The apparent molecular weight was approximately 50,000 when cell lysates of *E. coli* MY3715 or MY3833 were solubilized in SDS-sample buffer at room temperature or 37°C for 30 min and 65,000 when heated at 50, 70, or 100°C for 15 to 30 min (Fig. 6). Identical results were obtained with or without 2-mercaptoethanol in the SDS-sample buffer and when samples were heated in buffer containing 0, 0.25, or 2.5% SDS. Significantly, fimbriae isolated from *A. viscosus* T14V exhibited similar changes in electrophoretic mobilities when examined under the same conditions (data not shown). In contrast, the truncated protein expressed by *E. coli* MY4807 was readily denatured by SDS even at room temperature. The apparent molecular weight was 47,000, irrespective of the temperature at which the sample was prepared (Fig. 6).

**Purification of and preparation of antibody against the cloned protein from *E. coli*.** Following cell fractionation of *E. coli* MY3833, the 65-kDa cloned type 1 protein was detected in the cytoplasmic and inner membrane fractions but not in the periplasmic fraction (data not shown). The cloned antigen was isolated from the cytoplasmic fraction by sequential ion-exchange, immunoaffinity, and gel permeation chromatographic procedures (see Materials and Methods). Although the fraction from the DEAE-Sephalac purification (Fig. 7, lane 3) contained mostly *E. coli* proteins, the material bound to the monoclonal immunoaffinity column and eluted with 3 M guanidine hydrochloride was greatly enriched for the cloned type 1 protein (Fig. 7A, lane 4). Final purification was achieved by Sephacryl S-200 column chromatography, which separated residual low-molecular-weight proteins from the cloned protein (Fig. 7A; compare lanes 4 and 5). Significantly, the major and minor bands observed by silver staining of the purified protein in SDS-gels (Fig. 7A, lane 5) were reactive with monoclonal antibody 8A, as shown by Western blotting (Fig. 7B, lane 5).

Rabbit antibody prepared against the affinity-purified cloned protein reacted in Western blotting with partially dissociated *A. viscosus* type 1 fimbriae to give a pattern (Fig. 8A, lane 2) indistinguishable from that observed with polyclonal antibody against the purified fimbrial antigen (Fig. 8B, lane 2). Both antibodies also reacted with the 65- and 35-kDa proteins present in the native subunit fraction prepared from *A. viscosus* as described in Materials and Methods (Fig. 8, lanes 3). Additional immunoreactive bands at 35 kDa as well as at approximately 50 kDa were detected in the purified cloned protein preparations (Fig. 8, lanes 1). These bands probably result from degradation of the 65-kDa species upon storage at 4°C, since they were not detected in fresh extracts prepared from cells carrying pMY3833.

**Discussion**

Molecular cloning of *A. viscosus* T14V genes in *E. coli* with *pUC* as an expression vector has resulted in the

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**FIG. 2.** Western blot analysis of MY1001 and MY2101 cell lysates. Crude cell extracts were electrophoresed in an SDS-polyacrylamide gradient (5 to 12%) gel and transferred to nitrocellulose, and the transfer was overlaid with antibody directed against *A. viscosus* T14V type 1 fimbriae followed by 125I-labeled protein A prior to autoradiography. Lanes: 1, 14C-labeled protein standards with molecular masses indicated; 2, cell extract from *E. coli* MY1001; 3, cell extract from *E. coli* MY2101; 4, cell extract from *E. coli* JM109(pUC13); 5, purified *A. viscosus* type 1 fimbriae.

**FIG. 1.** Dot-blot analysis of cell lysates of *E. coli* MY1001 and MY2101 with various antibodies. A 1-μl portion of concentrated *E. coli* cell lysates prepared as described in Materials and Methods was applied to each of six separate nitrocellulose strips. Purified *A. viscosus* fimbriae (0.3 μg of type 1 or type 2) and 104 *A. viscosus* T14V cells were also applied. Each strip was incubated with a different antibody (1 μg of IgG/ml) followed by 125I-labeled protein A prior to autoradiography. Antibodies: 1, rabbit anti-type 1 IgG; 2, monoclonal anti-type 1 IgG (protein 8A); 3, rabbit anti-type 2 IgG; 4, rabbit anti-*A. viscosus* T14V IgG; 5, normal rabbit IgG; 6, normal mouse IgG.
isolation of a gene that encodes a structural subunit of \textit{A. viscosus} type 1 fimbriae. The 65-kDa cloned type 1 protein was expressed from a 1.9-kb DNA insert, and thus the corresponding gene must contain greater than 90\% of the cloned DNA. The cloned protein reacted with specific polyclonal as well as monoclonal antibodies directed against \textit{A. viscosus} type 1 fimbriae (Fig. 2 and 5). On Western blots of SDS-gels, these antibodies bound a series of bands from partially dissociated type 1 fimbriae, and these bands were also reactive with antibody against the cloned 65-kDa protein (Fig. 2 and 8; Cisar et al., in preparation). Taken together, these findings strongly suggest that the cloned protein is the principal subunit of the \textit{A. viscosus} type 1 fimbriae, although they do not exclude the existence of additional minor components.

The possibility that type 1 fimbriae are composed of a repeating 65-kDa subunit is not inconsistent with the complex pattern of the affinity-purified antigen in SDS-PAGE (Fig. 2 and 8). The 35-kDa protein observed in partially dissociated type 1 fimbriae and in the native subunit fraction also appeared in the sample of cloned 65-kDa subunit following storage at 4\(^\circ\)C (Fig. 8). Moreover, the 35- and 65-kDa proteins from various samples appeared to be antigenically related, since they reacted in Western blotting with rabbit antibody against the cloned subunit (Fig. 8B) and also with monoclonal antibodies against the type 1 fimbrial antigen (Cisar et al., in preparation). Thus, the 35-kDa band may represent a relatively stable degradative product derived from the 65-kDa subunit. Similar degradation of fimbriae or the retention of secondary structure under conditions of partial dissociation could also account for the appearance on Western blots of a series of high-molecular-weight bands at increments of less than 65 kDa (Fig. 2 and 8).

In SDS-PAGE, the cloned protein migrated as a doublet (Fig. 2). The presence of two bands appears to depend on a difference in molecular size rather than conformation, as indicated by analysis of Furgason plots (unpublished data). The synthesis of similar polypeptides with slightly different lengths from the same gene could result from ambiguities at the level of transcription or translation. However, the observations that the upper band of the doublet was most prominent in freshly prepared samples of the cloned protein and that both bands were of similar intensity in samples stored at 4\(^\circ\)C for some time suggests that the appearance of two bands depends on secondary structure or posttranslational cleavage of the primary gene product. The mobility of the cloned protein was slightly slower than that of antigen-

\begin{figure}
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\includegraphics[width=\textwidth]{fig3}
\caption{Endonuclease restriction maps of pMY1001 and pMY2101. Symbols: , \textit{A. viscosus} DNA sequence common to both pMY1001 and pMY2101; , DNA present in pMY1001 but not in pMY2101.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{Restriction endonuclease maps of deletion mutants derived from pMY2101. The size of the antigen expressed by each subclone is indicated in kilodaltons. Symbols: , vector DNA; , \textit{A. viscosus} DNA; , deleted DNA.}
\end{figure}

\begin{figure}
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\includegraphics[width=\textwidth]{fig5}
\caption{Antigen expression by recombinant clones MY3833 and MY4807. (A) Western blot analysis of \textit{E. coli} MY3833 (lane 1) and MY4807 (lane 2) cell extracts. Proteins were electrophoresed on an SDS-10\% polyacrylamide gel and transferred to nitrocellulose, and the transfer was overlaid with monoclonal antibody 8A prior to reaction with peroxidase-conjugated goat anti-mouse IgG and development with substrate. Molecular masses of the immunoreactive bands are indicated in kilodaltons. (B) Physical map of pMY3833 showing (arrows) the direction of transcription of the fimbrial gene from the lac promoter (Plac) of the vector and the position and direction of transcription of the ampicillin resistance gene (\textit{Amp}'). The 65-kDa antigen encoded by pMY3833 and the 47-kDa antigen encoded by pMY4807 are aligned with the corresponding DNA regions of pMY3833.}
\end{figure}
cally similar bands from *A. viscosus* fimbriae (Fig. 2) and from the native subunit fraction (Fig. 8). This difference may be due to a leader peptide on the *A. viscosus* protein synthesized by *E. coli*. Indeed, the presence of a leader is suggested by preliminary observations involving the secretion of the 47-kDa N-terminal segment of the cloned protein into the periplasm of *E. coli*. Clearly, additional proposals could be advanced to explain the migration properties of the cloned and native proteins, and further results based on structural studies of these proteins and DNA sequence analysis of the cloned gene are needed to resolve this problem.

The cloned protein and its antigenic counterpart from *A. viscosus* fimbriae exhibit similar heat-modifiable properties when examined by SDS-PAGE. This characteristic appears to be a common property of *Actinomyces* sp. fimbrial subunits, since similar findings were observed with native and cloned *A. naeslundii* type 2 fimbrial proteins (M. K. Yeung, unpublished data) and with a cloned *A. viscosus* type 2 protein (J. A. Donkersloot, personal communication). In all cases, substantial changes in electrophoretic mobility are affected by solubilization temperature but not by the reducing agent, which has been shown to modify certain cell surface proteins from other organisms (13, 26). While heat-sensitive outer membrane proteins of several gram-negative bacteria (2, 13, 15, 19, 22, 23, 25, 27) and others (11, 26), as well as the flagellin of *E. coli* K-12 (16), have been described, the structural basis for this property is not well understood. In the present study, since the truncated N-terminal segment of the type 1 fimbrial subunit was not heat modifiable (Fig. 6), it is possible that the C-terminal end of the protein contributes to noncovalent intramolecular interactions that are heat sensitive.

The interactions that maintain individual subunits in functional fimbriae are largely unknown, because isolated fimbriae are resistant to complete dissociation. Similarly, the manner in which subunits polymerize to form fimbriae is not clear. The purified cloned subunit exhibited no tendency to aggregate or self-assemble even under nondenaturing conditions (data not presented). This may indicate that the in vitro conditions used do not favor self-assembly. Alternatively, during in vivo biosynthesis, additional accessory proteins may be required for the polymerization of subunits in a manner similar to that hypothesized for biosynthesis of the K88ab fimbriae (20) or the digalactoside binding Pap pili (21) of *E. coli*. With gram-positive organisms, very little is known concerning the structure and biosynthesis of fimbriae. The present identification of a fimbrial subunit should facilitate

**FIG. 6.** Western blots developed with anti-type 1 monoclonal antibody 8A of cell extracts solubilized in SDS buffer at various temperatures. Samples were heated at (a) 100°C for 15 min or (b) room temperature for 30 min prior to electrophoresis on an SDS-10% polyacrylamide gel. Lanes: 1, cell extract from *E. coli* MY3715; 2, cell extract from *E. coli* MY3833; 3, cell extract from *E. coli* MY4807. Immunoreactive proteins in samples solubilized at 100°C (●) and at room temperature (△) are indicated.

**FIG. 7.** Purification of the cloned *A. viscosus* type 1 fimbrial protein from *E. coli* MY3833. Samples from the purification procedure (see Materials and Methods) were analyzed on SDS-polyacrylamide gradient (5 to 12%) gels. (A) Silver staining of the gels to reveal protein bands; (B) Western blots showing the reaction of anti-type 1 monoclonal antibody 8A with selected samples. Lanes: 1, molecular weight markers; 2, crude extract; 3, 4, and 5, type 1 antigen-containing fractions from DEAE-Sephacel, immunoaffinity, and Sephacryl S-200 column chromatography, respectively.

**FIG. 8.** Western blots developed with rabbit antibodies against (A) the cloned *A. viscosus* type 1 fimbrial protein or (B) *A. viscosus* type 1 fimbriae. Lanes: 1, cloned 65-kDa type 1 protein; 2, *A. viscosus* T14V type 1 fimbriae; 3, type 1 subunit fraction from *A. viscosus* T14V. Samples (5 μg of total protein per lane) in Laemmli buffer containing 2 mM EDTA were heated in a boiling-water bath for 5 min prior to electrophoresis in an SDS-polyacrylamide gradient (5 to 10%) gel.
future studies to biochemically and genetically characterize the type 1 fimbrial adhesin from *Actinomyces* spp.

**LITERATURE CITED**


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