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Assembly of a Chemically Synthesized Peptide of *Escherichia coli* Type 1 Fimbriae into Fimbria-Like Antigenic Structures

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***Escherichia coli* type 1 fimbriae are composed of subunits, each of which comprises 158 amino acids. We synthesized a copy of a 13-residue peptide, located near the NH_2 terminus of the fimbrial subunit, that assumed some of the properties of type 1 fimbriae. At pH 5.5 the synthetic peptide autoassembled into fibrillar structures that resembled type 1 fimbriae except that they appeared less rigid and rodlike. A quaternary structure-specific monoclonal antibody against type 1 fimbriae recognized the synthetic peptide in the assembled but not the unassembled state. Furthermore, when the synthetic peptide was injected in its fimbrial conformation into rabbits, it evoked antibodies that reacted with type 1 fimbriae isolated from *E. coli*.**

Type 1 fimbriae on the surface of various members of the family *Enterobacteriaceae* have been shown to mediate the attachment of these microorganisms to mannose-containing receptors on eucaryotic cells (1, 2, 7, 9, 20, 23, 24). Purified type 1 fimbriae agglutinate guinea pig erythrocytes and bind to mucosal epithelial cells in a D-mannose-sensitive manner. Brinton (7) has shown that type 1 fimbriae are composed of 17-kilodalton protein subunits that are assembled into a right-handed helix that is 7 nm in diameter. Each turn of the helix consists of 3 1/8 subunits. The subunits are held together by hydrophobic bonds which are highly resistant to disruption but can be dissociated by boiling in acid (7) or treating with saturated solutions of guanidine hydrochloride (11). The dissociated subunits have been shown to reassemble in vitro into fimbrial structures (2, 11). This property is not unique to type 1 fimbriae; the fimbriae of *Pseudomonas aeruginosa* (27) and 987P of *Escherichia coli* (24a) have been shown to have similar properties.

The mechanism of fimbrial autoassembly is not clear, although results of studies of the primary structure of several different types of fimbriae have demonstrated a high degree of homology in their NH_2-terminal amino acid sequences (12–16, 21, 22, 26). Because of these homologies, we decided to synthesize several overlapping peptides of the first 35 residues of the NH_2-terminal region of *E. coli* type 1 fimbriae. Here we report that one of these peptides, containing residues 23 to 35, assembles itself into fibrillar structures resembling native type 1 fimbriae. We show that antibodies raised against the assembled synthetic peptide react both with fimbrial subunits and assembled type 1 fimbriae isolated from *E. coli*. Moreover, monoclonal antibodies directed against quaternary structural determinants of native type 1 fimbriae react with the assembled, but not the unassembled, synthetic fimbriae.

MATERIALS AND METHODS

Synthesis of peptides. Peptides were synthesized by solid-phase techniques (19) and purified as described previously (6). The purity of the peptide product was determined by high-performance liquid chromatography on a reverse phase column and by quantitative amino acid analyses (6).

Immunization of rabbits. To determine the immunogenicity of the synthetic peptides, New Zealand White rabbits (weight, 2 kg) were injected subcutaneously with 300 μg of synthetic peptide suspended in either saline (pH 7.0) or 0.1 M sodium acetate (pH 5.5) and emulsified in complete Freund adjuvant as described previously (4, 6). Rabbits were bled before and at 2-week intervals after the initial injection. At 4 and 8 weeks, each rabbit was injected subcutaneously with another 300-μg dose of the respective form of the synthetic peptide in incomplete Freund adjuvant, and serum samples were collected at 2-week intervals (4).

Assays of antibody reactivity. Antibodies were detected by an enzyme-linked immunosorbent assay (ELISA) (2). Isolated type 1 fimbriae (5 μg/ml) or synthetic peptides (10 μg/ml) were absorbed onto ELISA trays as the solid-phase antigen as described previously (2). Aggregated peptides in 0.1 M acetate buffer (pH 5.5) were adsorbed onto walls of ELISA trays by drying at 37°C.

Purification of bacterial fimbriae. Fimbriae were purified from bacteria as described previously (2).

SDS-PAGE and Western blot analysis of fimbriae. Samples of type 1 fimbriae were dissociated before electrophoresis by heating in hydrocholoric acid as described previously (2). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) were performed on 12% slab gels by the method described by Laemmli (18). After SDS-PAGE the bands of fimbrial protein were electrophoretically transferred onto nitrocellulose paper by the method described by Towbin et al. (25) as described previously (2). After transfer, the nitrocellulose strip was exposed to synthetic fimbriae-specific antiserum, horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G IgG; Cappel Laboratories, West Chester, Pa.), and 4-chloro-1-napthol (Bio-Rad Laboratories, Richmond, Calif.) as the chromophore.

Electron microscopy. Samples of synthetic peptide or type 1 fimbriated *E. coli* suspended in 10 mM Tris (pH 7.0) or 0.1 M acetate buffer (pH 5.5) were applied to Formvar-coated copper grids and negatively stained with 0.5% phosphotungstic acid (pH 4.0). For immunoelectron microscopy, bacteria-coated grids were placed on a drop of antiserum that was diluted 1:50 for 20 min and then washed several times with deionized water. The grids were finally stained with phosphophosphotungstic acid. Electron micrographs were made

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TABLE 1. Covalent structure of synthetic peptides of type 1 fimbriae

Synthetic peptides[a]	Amino acid sequence
S-T1(1-13)C	1 AATTVNGGTVHFKC 13
S-T1(13-35)C	13 KGEVVNAACAVDAGTVDQTVQLGC 35
S-T1(23-35)C	23 VDAGTVDQTVQLGC 35

[a] The peptides selected for synthesis were based on the amino acid sequences of the subunit of type 1 fimbriae reported by Klemm (13). The sequence of each peptide was confirmed by Edman degradation, as described in the text.

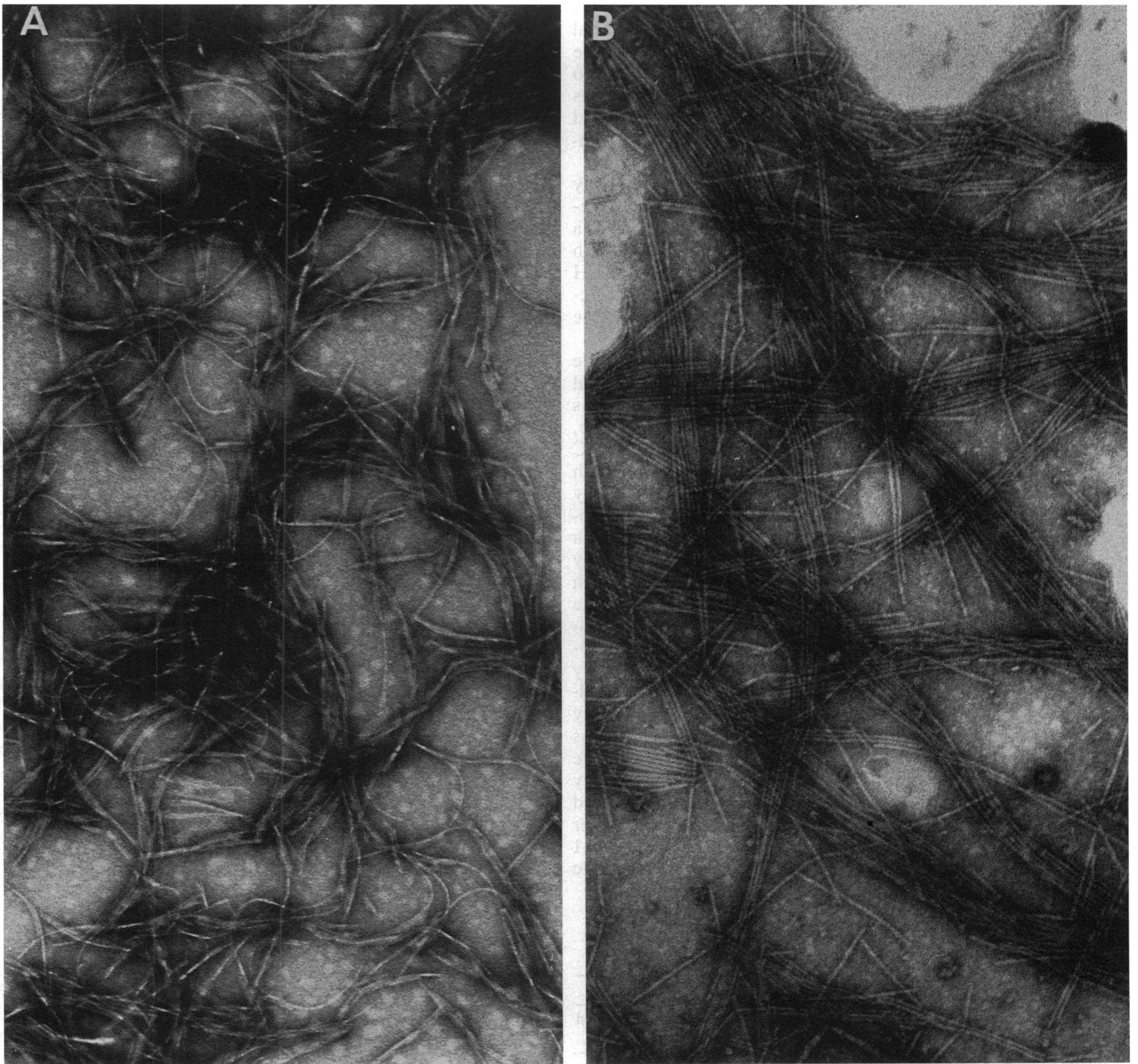


FIG. 1. Electron micrographs of assembled synthetic peptide S-T1(23-35)C at pH 5.5 (A) and of native type 1 fimbriae (B). The assembled peptides form curly fibrillar structures in contrast to the rigid rodlike structure of native type 1 fimbriae. Magnification, ×64,000.

by using a transmission electron microscope (AEI EM 6B) (2).

RESULTS

Synthesis and chemical properties of amino-terminal peptides of type 1 fimbriae. The synthetic peptides S-T1(1-13)C, S-T1(13-35)C, and S-T1(23-35)C were purified by high-performance liquid chromatography and their sequences were confirmed by automated Edman degradation (Table 1). They were found to be identical to the corresponding regions of the subunit of *E. coli* type 1 fimbriae as reported by Klemm (13).

Formation of fimbrial structures by synthetic peptide S-T1(23-35)C. In the course of our studies, we found that at pH 5.5 the synthetic peptide S-T1(23-35)C, but not peptides S-T1(1-13)C or S-T1(13-35)C, assembled itself into fimbrial structures (Fig. 1). In contrast to the rigid rodlike structure of type 1 fimbriae (Fig. 1B), the synthetic peptide gave rise to a curly fibrillar structure (Fig. 1A). The other two peptides failed to assemble into fimbrial structures at any pH tested between pH 5.0 and 10.0, even though S-T1(13-35)C encompassed the active peptide S-T1(23-35)C.

Because the addition of $MgCl_2$ enhances the autoassembly of dissociated type 1 fimbrial subunits into fimbrial structures (11), we incubated each of the synthetic peptides at a concentration of 1 mg/ml with 10 mM $MgCl_2$. Although S-T1(23-35)C again formed fimbriae at pH 5.5, at neutral pH none of the synthetic peptides assembled into fimbrial structures that were detectable by electron microscopy in the presence of $MgCl_2$.

Antigenic properties of S-T1(23-35)C. Because the peptide S-T1(23-35)C aggregated at pH 5.5 and assumed a fimbrial conformation, we compared its immunochemical properties with those of native type 1 fimbriae. For this purpose, synthetic fimbriae and unassembled forms of S-T1(23-35)C were used as the immobilized antigen and were reacted with monoclonal antibodies specific for type 1 fimbriae by ELISA. In a previous study (2) we described several monoclonal antibodies against type 1 fimbriae, some of which reacted with the fimbrial subunit but not with the fully assembled fimbriae (subunit specific) and others of which reacted with the fully assembled fimbriae but not with the subunits (quaternary structure specific). To study the antigenic reactivity of the molecular conformations of S-T1(23-35)C, we employed subunit-specific (D1) and quaternary structure-specific (BD4) monoclonal antibodies in the following studies. The subunit-specific monoclonal antibody (D1) was found to react with both the unassembled form as well as the assembled form of the peptide by ELISA (Table 2). The quaternary structure-specific monoclonal antibody (BD4) reacted with the assembled but not with unassembled form of the peptide (Table 2), indicating that the former contains quaternary structure-specific epitopes of type 1 fimbriae. These results indicate that the assembled synthetic fimbriae contain some conformational epitopes of type 1 fimbriae.

TABLE 2. Reactivities of monoclonal antibodies against type 1 fimbriae with unassembled and assembled forms of synthetic peptide S-T1(23-35)C

Monoclonal antibody	Structural specificity of antibody	ELISA titers against:	
		Unassembled peptide	Assembled peptide
BD4	Quarternary	<200	1,600
D1	Subunit	3,200	1,600

TABLE 3. Immune responses in rabbits immunized with unassembled and assembled synthetic peptide S-T1(23-35)C

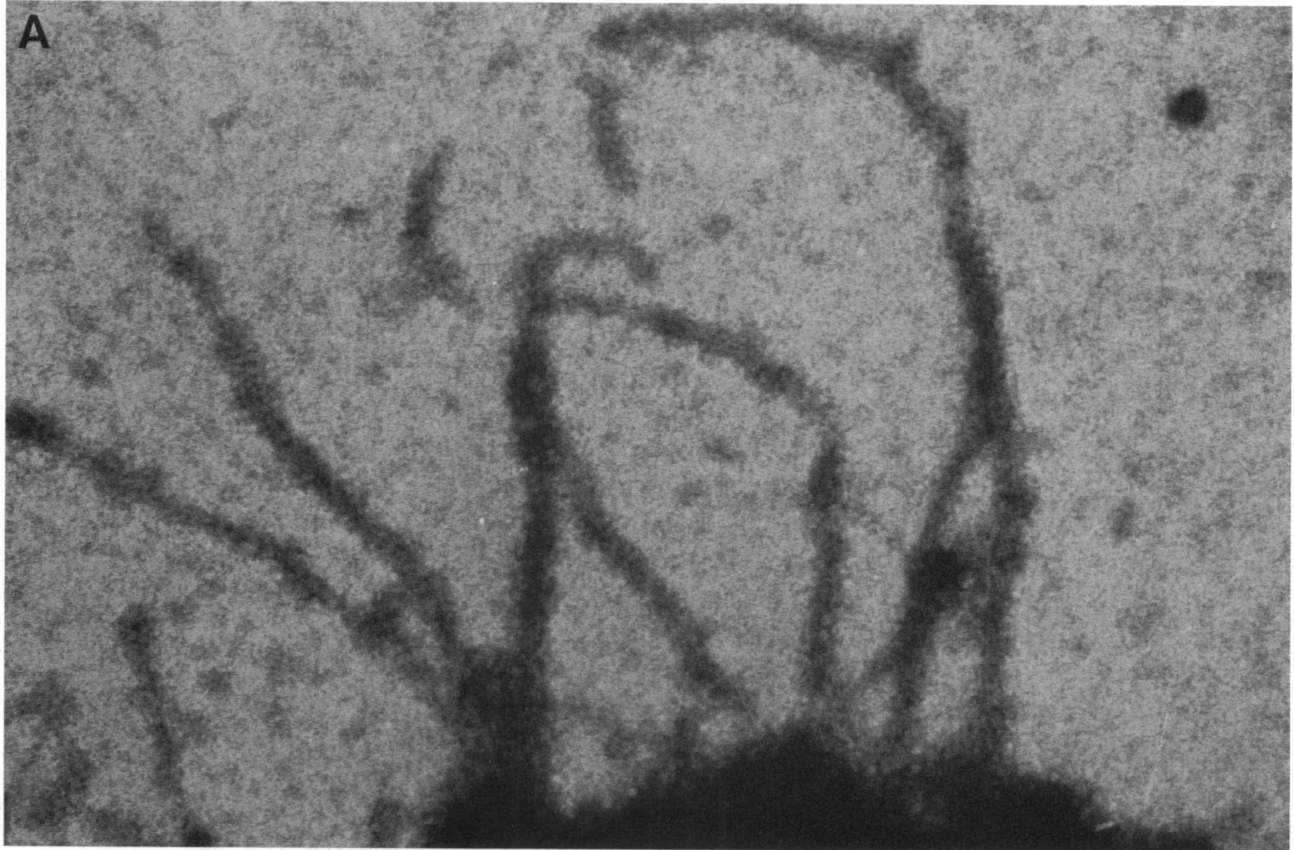
Antiserum of rabbits immunized with:	ELISA titer against[a]:	
	Unassembled synthetic peptide	Native type 1 fimbriae
Assembled synthetic peptide		
Rabbit 1	12,800	102,400
Rabbit 2	51,200	1,600
Rabbit 3	1,600	1,600
Preimmune pool	<200	<200
Unassembled synthetic peptide		
Rabbit I	25,600	800
Rabbit II	25,600	400
Preimmune pool	<200	<200

[a] Although ELISA titers against assembled synthetic peptide are not included in this table, they were identical to those of unassembled synthetic peptides.

Immunogenic properties of S-T1(23-35)C. Having demonstrated the presence of epitopes in the assembled and unassembled S-T1(23-35)C peptides that are shared with type 1 fimbriae, it was of interest to determine if either conformation of the peptide was capable of evoking immune responses against type 1 fimbriae in rabbits. Polyclonal antisera against the assembled and unassembled synthetic fimbriae were raised by immunizing rabbits with peptides emulsified in complete Freund adjuvant. Both forms of the peptide elicited strong specific immune responses in most rabbits, as judged by ELISA against the corresponding peptide (Table 3). However, the animals immunized with the unassembled form of the peptide developed only weak immune responses against type 1 fimbriae (Table 3). In contrast, one of three rabbits (rabbit 1) immunized with the assembled synthetic fimbriae developed a high titer of antibodies against *E. coli* type 1 fimbriae (Table 3). Moreover, the antibodies bound in a periodic manner to intact fimbriae on the surface of *E. coli* (Fig. 2) in a manner similar to that previously found with quaternary structure-specific antibodies raised against type 1 fimbriae (2).

To determine if the antiserum to the synthetic fimbriae also recognized individual subunits of type 1 fimbriae, the fimbriae were dissociated by boiling in acid, electrophoresed on a 12% SDS-polyacrylamide gel, transferred onto a sheet of nitrocellulose, and reacted with the antiserum obtained from rabbit 1. The antibody labeled the fimbrial protein corresponding to the 17-kilodalton subunit as well as to higher polymers of this subunit (Fig. 3). These findings confirm the specificity of the antiserum for both the polymeric and subunit forms of type 1 fimbriae. When synthetic peptide S-T1(23-35)C was employed to inhibit the binding of polyclonal type 1 fimbriae-specific antiserum to native type 1 fimbriae, no significant inhibition was detected (data not shown), suggesting that this peptide, in its unassembled state, does not constitute an immunodominant region of the native fimbrial structure.

Cross-reactions of antibodies to synthetic fimbriae with *E. coli* P and 987P fimbriae *Haemophilus influenzae* C54 and C47 fimbriae. Because of recent reports in which conserved regions in the amino-terminal ends of fimbrial subunits from several gram-negative bacteria have been described (3, 12–16, 21, 22), we examined the reactivity of the antiserum from rabbit 1 against heterologous fimbriae. Isolated *H.*



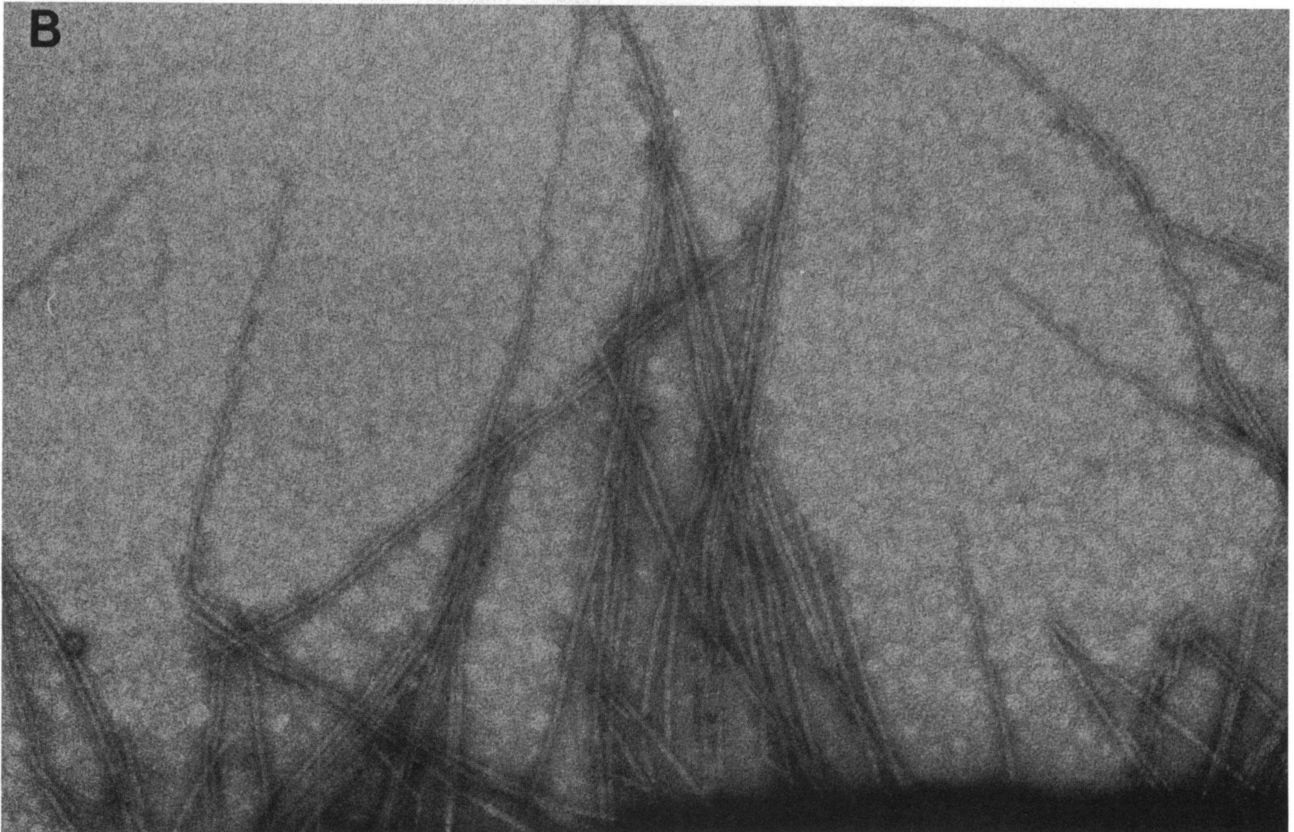


FIG. 2. Electron micrographs of type 1 fimbriated *E. coli* stained with a 1:50 dilution of antiserum against assembled synthetic peptide S-T1(23-35)C (A) or with a 1:50 dilution of antiserum against unassembled peptide S-T1(23-35)C (B). Note that the antibody against the assembled peptide binds along the length of the fimbriae, whereas that against the unassembled peptide does not bind at all. Magnification, ×72,000.

influenzae C54 and C47 fimbriae (kindly provided by Marilyn Loeb), enterotoxigenic *E. coli* 987P fimbriae (strains kindly supplied by Richard Isaacson), *Actinomycetes naeslundii* fimbriae (kindly supplied by Jegdish Babu), and P fimbriae from a clinical strain of *E. coli* 101 were adsorbed onto ELISA plates and reacted with the antiserum. Each of the fimbrial types, with exception of *A. naeslundii* fimbriae, reacted with antiserum to the synthetic fimbriae, although the reactions with the heterologous fimbriae were weaker than that with *E. coli* type 1 fimbriae (Table 4).

DISCUSSION

Results of this study demonstrate that antibodies specific for type 1 fimbriae can be evoked by immunization with a chemically synthesized peptide fragment of the fimbriae. Perhaps even more interesting is the observation that this 13-residue peptide, S-T1(23-35)C, autoassembles and assumes a conformation that gives rise to quaternary structural epitopes that are shared with authentic type 1 fimbriae. It is as yet unclear how this fimbrial conformation is brought about, but it is likely that hydrophobic interactions between the several hydrophobic amino acids in the peptide sequence play a role. Hydropathy plots and secondary structural analyses reported by Klemm (13) demonstrated that the NH_2-terminal region of the type 1 fimbrial subunit is hydrophobic and exhibits strong secondary structural potential. Because of the conformational potential, it is not unreasonable to speculate that assembly of fimbrial subunits is initiated at this region.

Type 1 fimbrial subunits that were obtained by dissociating fimbrial polymers under nondenaturing conditions can be induced to reassociate into their original conformation by the addition of magnesium chloride (11). We observed that the addition of magnesium chloride to the unassembled form of the synthetic peptide at pH 7.0 failed to induce assembly. This finding, together with the observed formation of less rigid, curly structures by the synthetic peptide and the discovery that the subunit-specific monoclonal antibody reacts equally well with assembled and unassembled synthetic peptide, suggests that in spite of the shared antigenic properties, the subunit assembly of the two polymers is

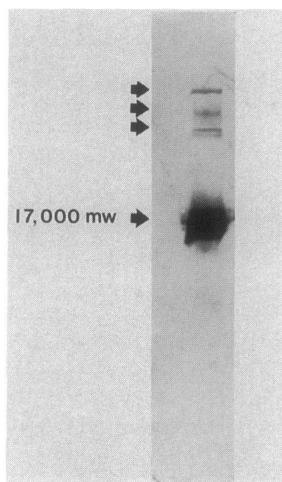


FIG. 3. Immunoelectroblot of a type 1 fimbrial preparation incubated with antiserum (rabbit 1) raised against the synthetic fimbriae. The lane was overloaded with fimbrial proteins so that fimbrial fragments other than the monomer could be detected. mw, Molecular weight.

TABLE 4. ELISA cross-reactions of antiserum to synthetic fimbriae with different types of fimbriae

Source of fimbriae	Antibody titer[a]
E. coli	
Type 1	102,400
P	1,600
987P	6,400
H. influenzae	
C54	3,200
C47	3,200
A. naeslundii	<20

[a] The antiserum was from rabbit 1, which was immunized with assembled synthetic peptide S-T1(23-35)C.

different. The extension of S-T1(23-35)C at its NH_2-terminal end by 10 residues that copied the corresponding region in its native fimbrial subunit to form S-T1(13-35)C abolished the ability of the peptide to form fimbrial structures at pH 5.5, even though the antigenicity of the molecule was retained, as shown by its reactivity with monoclonal antibody D1 (data not shown). Thus, the length and the nature of the amino acids in the sequence might be an important constraint in the aggregation of the synthetic peptide in fimbrial conformations at low pHs.

The observation that serum from one rabbit immunized with the synthetic fimbriae demonstrated high levels of reactivity with type 1 fimbriae agrees with the observation that the synthetic fimbriae share immunodeterminants with type 1 fimbriae, including at least one conformational epitope. Immunological characterization of this hyperimmune serum sample revealed its specificity for both intact type 1 fimbrial structure and fimbrial subunits. The observation that a rabbit immunized with the assembled form of the peptide evoked an immune response against type 1 fimbriae, together with the observation that rabbits immunized with the same peptide in its unassembled form showed poor reactivity with type 1 fimbriae, emphasizes the importance of quaternary structure in the immune response of the host to *E. coli* type 1 fimbriae.

It should be pointed out that the immune response to quaternary structural epitopes is dependent on the maintenance of the assembled form of the synthetic peptide in the immunized animal. Because of the requirement of pH 5.5 for the maintenance of the assembled form of the synthetic peptide, it is not surprising that only one of three instances did the immunogen consisting of assembled peptides maintain its assembled form long enough to evoke a specific immune response in vivo. The dissociation of the synthetic fimbriae may have been prevented by emulsification in complete Freund adjuvant. Alternatively, the synthetic fimbriae may have been prevented from breaking down as a result of the relatively low pH levels at the site of injection. According to Edlow and Sheldon (10), pH values as low as 5.6 are often found at sites of inflammation, such as those produced at the site of injection of complete Freund adjuvant.

The antiserum raised against the assembled form of S-T1(23-35)C demonstrated a weaker but definite reactivity with several heterologous fimbrial types. This observation is not surprising because NH_2-terminal sequence homologies have been reported in type 1, P, and *H. influenzae* fimbriae (12, 14). Although there appears to be no homology in the

NH_2-terminal sequence between 987P and the type 1 fimbrial subunit (21), common conformational epitopes have been described recently (S. N. Abraham, D. M. Schifferli, D. Banks, and E. H. Beachey, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, D109, p. 84). The apparent broad reactivity of antiserum with the synthetic fimbriae S-T1(23-35)C suggests that antigenic sites corresponding to the region between positions 23 and 35 on type 1 fimbriae exist on several fimbrial types. Alternatively, some epitopes formed by the conformations assumed by S-T1(23-35)C may be antigenically similar to conformational epitopes on other fimbrial types. It is as yet unclear if these antigenically similar epitopes on different gram-negative fimbriae play a role in fimbrial assembly. The cross-reactivity of our antiserum with strains of bacteria expressing heterologous fimbriae may have a bearing on the development of broadly protective vaccines against a variety of bacterial pathogens.

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