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Sequence and Structural Characteristics of the Trypsin-Resistant T6 Surface Protein of Group A Streptococci

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The gene for the trypsin-resistant surface T6 protein of *Streptococcus pyogenes* D471 (M type 6) was cloned and expressed in *Escherichia coli*. The complete nucleotide sequence of the gene (*tee6*) and its flanking regions was determined and found to include only one major open reading frame coding for a protein of 537 amino acids (M_r, 57,675). The N terminus of the deduced protein sequence exhibits features of a typical signal sequence, and the C-terminal segment was found to have a high degree of homology with the membrane anchor region of other gram-positive surface proteins, such as streptococcal M protein, wapA protein from *Streptococcus mutans* and staphylococcal protein A. A hexapeptide having the consensus sequence LPSTGE and located immediately upstream of the C-terminal hydrophobic segment showed the highest degree of conservation at both the protein and DNA levels, with nearly all reported surface proteins from gram-positive cocci. The amino acid composition of the T6 protein revealed 21% serine and threonine residues distributed nearly regularly throughout the molecule, and analysis of the secondary structure predicted a conformation composed of >70% β-sheet potential interrupted by β-turns or random coils. Localization experiments in *E. coli* show very little T6 protein in the periplasmic space. When found here, however, this T6 protein had a molecular mass of 55 kilodaltons, similar to that extracted from the streptococci by nonionic detergent. Most of the T6 protein was found localized in the membrane fraction, where it was composed of a triple band of 60, 58, and 57 kilodaltons. The coexistence of streptococcal surface proteins which are either resistant (T protein) or sensitive (M protein) to proteolytic enzymes may offer a new dimension to the modulation of these antigens under specific biological conditions.

Group A streptococci are the causative agent for several human diseases, including acute streptococcal pharyngitis, rheumatic fever, nephritis, and wound infection, among others. Most recently, several outbreaks of rheumatic fever and highly lethal wound infections were reported in the United States (43) where, in contrast to developing countries, such streptococcal diseases generally play a minor role, except for acute streptococcal pharyngitis. Among several molecules identified on the surface of group A streptococci, the M molecule is recognized as the major virulence determinant because of its ability to confer resistance against phagocytic attack (24). Type-specific antibodies to the M molecule (of which >80 serotypes have been identified) allow for opsonization and phagocytosis of M-positive streptococci. The M protein is an α-helical coiled-coil molecule whose structure and function have been studied extensively (recently reviewed in reference 9).

The T protein, a surface molecule found on pathogenic groups A, B, C, and G streptococci (22) was first identified by Lancefield and Dole (25), who named it for its resistance to trypsin digestion. During streptococcal infections, high levels of T-specific antibodies are produced; however, these and antibodies raised against partially purified T protein are unable to prevent subsequent streptococcal infections (37). Because of this, most vaccine-related streptococcal research has been primarily directed towards the M protein. However, in contrast to the M protein, whose presence on the cell surface is highly variable (1), the T antigen is stable and rarely lost under either laboratory or clinical conditions (23, 25). Unlike the M molecule, T proteins are not associated with surface fibrils (45).

More than 25 serologically different T antigens have been described (29) and are found to be expressed independently of M protein, but certain T proteins occur only in association with specific M serotypes (i.e., T6 protein is always found on M6 streptococcal strains). In some instances, strains with more than one T protein on their surface are found, with certain strains having as many as 3 or 4 (16). T proteins have proved to be epidemiologically valuable for subclassifying strains of group A streptococci by an agglutination assay in which T-specific sera are used (16, 43). This has been particularly important in those streptococci producing no, or a nontypeable, M protein. T typing is performed after the streptococci are treated with trypsin, which digests the trypsin-sensitive protein molecules on the cell surface, including the M protein, leaving the T protein exposed for interaction with T-specific antibody. Thus, the streptococcus has positioned at least two proteins on its surface, with differing susceptibility to proteolytic digestion.

To understand better the structural properties of the T protein and its relationship to other streptococcal surface proteins, the gene for T6 protein was cloned and expressed in *Escherichia coli*. The complete nucleotide sequence of the T6 structural gene and analysis of the translated sequence reveal that except for the C-terminal anchor region, the T protein is a bacterial surface molecule that is unique in both structure and amino acid composition.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophage. Group A streptococcal strain D471 is typed as M6,T6 and was from the Rockefeller University collection. The *E. coli* strains used were K-12 XL1-Blue (2), LE392 (28), Q358, and Q359 (21). Plasmid pHSG399 (46) was a gift from the Japanese Cancer Research Resources bank. The λ phage EMBL4 (13) was the primary cloning vector.

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Chemicals and enzymes. Restriction enzymes, T4 DNA ligase, and Klenow fragment from DNA polymerase were purchased from New England BioLabs, Inc. Calf intestinal alkaline phosphatase was obtained from Boehringer Mannheim Biochemicals. The sequencing reaction was performed with Sequenase kit 2.0 (United States Biochemical Corp.). Deletions were generated from inserted DNA in M13mp18/19 (48) systems by using Cyclone 1 Biosystem (International Biotechnologies, Inc.), and [α-^{35}S]dATP from the Amersham Corp. Chemicals were from Sigma if not otherwise indicated.

Nonionic detergent extraction of streptococcal T6 antigen. Streptococcal strain D471 was grown overnight at 37°C in 50 ml of Todd-Hewitt broth supplemented with 0.3% yeast extract (Difco Laboratories). Cells were centrifuged for 20 min at 2,500 × *g*, and the supernatant was discarded. The cell pellet was suspended in 50 ml of 50 mM Tris–10 mM EDTA (pH 8.0), recentrifuged, and resuspended in 1.6 ml of the Tris-EDTA buffer. A 10% solution of the nonionic detergent Igepal 897 (GAF Corp.) was added, yielding a final concentration of 2%, and the suspension was rotated at room temperature for 4 h. Cells were removed by centrifugation as described above, and the supernatant was filtered to remove residual bacteria and stored at 4°C.

DNA preparation and cloning procedures. Chromosomal DNA from streptococcal strain D471 was prepared as follows: an overnight culture in Todd-Hewitt broth was centrifuged and washed in 1/10 volume of 50 mM phosphate buffer, pH 6.1, and finally suspended in 1/50 volume (1 ml) of lysin buffer (50 mM phosphate buffer, 50 mM EDTA, 30% raffinose). After 10 μl of phage lysin (10) was added to the cell suspension, the mixture was incubated for 20 min at 37°C with slow rotation. The resulting protoplasts were sedimented and lysed in DNA preparation buffer (50 mM glucose, 25 mM Tris [pH 8.0], 10 mM EDTA, 0.1 mg of inactivated RNase A per ml) and incubated at 37°C for an additional 60 min. The solution was finally extracted with phenol and chloroform and dialyzed against several thousand volumes of TE (10 mM Tris, pH 8.0, 1 mM EDTA) buffer.

Preparation of λEMBL4 vector arms followed the protocol of Frischauf et al. (13). Chromosomal DNA from streptococcal strain D471 was partially digested with *Sau*3A and size fractionated on a sucrose gradient to yield 10- to 20-kilobase (kb) fragments. The bacteriophage λ replacement vector λEMBL4 was treated with *Bam*HI, and the vector arms were dephosphorylated. Streptococcal DNA fragments and vector arms were ligated and packaged in vitro (Gigapack gold; Stratagene). The size of the streptococcal DNA library was 10^6 PFU with 90% recombinant phage. To screen the library for T6 expression, the unamplified library was plated with Q358 and immunologically reacting plaques were counted at a frequency of 1:1,000.

Subcloning procedures. The method of Struhl (44) was used for subcloning DNA fragments from agarose gels. Cloning procedures are described elsewhere (28). The plasmid pHSG399 was used as a subcloning vector, and recombinants were transformed into *E. coli* XL1-Blue; expression of T6 protein was identified by colony blot. Clone λTEE6.4 was selected for subcloning experiments because of its small insert size and central location of the common *Eco*RI restriction site. After digestion with *Xba*I, a 7-kb fragment of λTEE6.4 was subcloned into the corresponding vector site and the recombinant plasmid, called pTEE6.1, expressed T6 protein while stably maintaining the insert. Expression proved to be independent of the inserted DNA fragment with relation to the *lacZ* promotor of the vector (pTEE6.1a/b); in addition, isopropyl-β-D-thiogalactopyranoside had no effect on the level of T6 protein production by plasmids pTEE6.1a and pTEE6.1b (data not shown). From an internal *Kpn*I site of pTEE6.1a, a 3-kb DNA fragment was removed along with the *Kpn*I site from the multiple cloning site of the vector to yield pTEE6.2, which still produced T6 protein. The central 2.3-kb *Hin*dIII fragment of pTEE6.1a was subcloned into pHSG399 cut with *Hin*dIII; the plasmid obtained expressed T6 protein and was designated pTEE6.3. A *Bsp*MI-*Bsm*I 2.2-kb fragment of pTEE6.2, after filling of the overlapping ends with DNA polymerase and recombination with the *Sma*I site of pHSG399, also expressed T6 protein and was named pTEE6.4. A subclone having the 1.3-kb *Eco*RI-*Xba*I fragment of pTEE6.1 was inserted into pHSG399 cut with the same enzymes and was found to be T6 negative (pTEE6.5). (See Fig. 3 for a summary of the subcloning data.)

DNA sequence and sequence analysis. DNA sequence was established by the method of Sanger et al. (38). Sequence data were aligned by using the Staden program package (Cambridge, United Kingdom). The structural features of the T6 protein sequence were analyzed by using the EuGene program (Baylor College of Medicine, Houston, Texas).

Cell fractionation and Western blots. *E. coli* XL1-Blue was grown overnight at 37°C in L broth, diluted 1:100 and grown to an optical density at 600 nm of 0.2 at the same temperature. Cell fractionation was performed with a modification of the protocol of Davis et al. (3). The cells were recovered by centrifuging 1.0 ml of culture in a microcentrifuge tube for 1 min. The cell pellet was suspended in 500 μl of cold TSE (100 mM Tris [pH 8.0], 5 mM EDTA, 20% sucrose containing 100 μg of freshly dissolved lysozyme per ml) and incubated for 10 min on ice. The resulting spheroplasts were centrifuged for 2 min, and the supernatant representing the periplasmic contents was saved. Protoplasts were suspended in 50 μl of ice-cold TSM (100 mM Tris [pH 8.0], 10 mM $MgCl_2$, 1.0 mg of DNase per ml, 20% sucrose) and lysed with the addition of 200 μl of cold water and five rapid freeze-thaw cycles. The membrane fraction was separated from the cytoplasmic fraction by a 10-min centrifugation at 13,000 × *g*. All samples were precipitated with trichloroacetic acid to 5%, washed with acetone, dried under vacuum, and solubilized in 25 μl of 4% sodium dodecyl sulfate (SDS) with 3 min of boiling before SDS-polyacrylamide gel electrophoresis (PAGE) and Western blot (immunoblot).

Antisera. T6-specific antiserum (generously supplied by Richard Facklam, Centers for Disease Control, Atlanta, Ga.) was prepared against heat-killed T6 whole group A streptococci, absorbed with T-negative streptococci, and used at a 1:250 dilution. This antibody is routinely used for the T typing of streptococcal isolates. Goat ($F(ab')_2$ anti-rabbit immunoglobulin G (IgG) was obtained from Sigma and diluted 1:1,000. The procedures for colony immunoblotting and crude phage lysate preparation are described elsewhere (40).

RESULTS

Extraction of the T6 protein from *Streptococcus pyogenes*. T6 protein extracted from strain D471 with nonionic detergent was separated by SDS-PAGE and identified by Western blot. This form of the T6 protein migrated with an apparent molecular mass of 55 kilodaltons (kDa) and was used as a standard for comparison to the cloned T6 molecule (Fig. 1).

Cloning and expression of the T6 protein gene (*tee6*) in *E.*

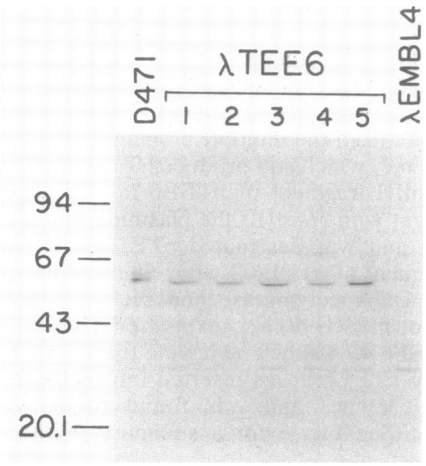

FIG. 1. Western blot analysis of T6 protein from streptococci and recombinant phage lysates. T6 protein extracted with nonionic detergent from streptococci (D471) is compared with that found in crude phage lysates of λTEE6.1 to λTEE6.5. A lysate of the λEMBL4 vector is included as a control. The blot was reacted with T6-specific antibodies.

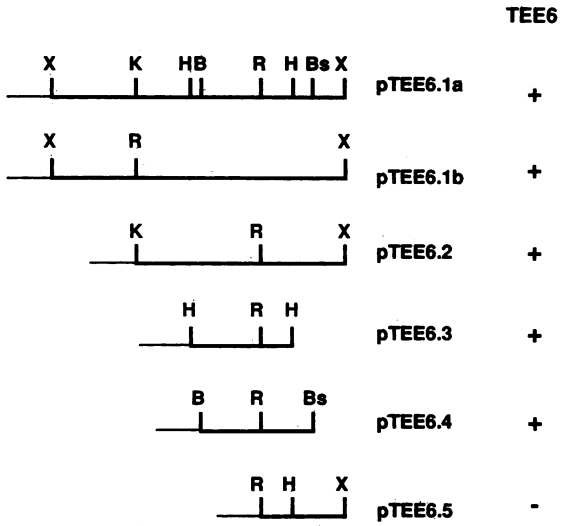

FIG. 3. Subcloning of the *tee*6 gene. Recombinant DNA from λTEE6.4 was subcloned into pHSG399, and the expression of T6 protein (TEE6) was monitored by immunoblot. The insert DNA is displayed as a heavy line, in contrast to vector DNA and T6 expression indicated to the right of each individual clone. Recognition sequences for restriction enzymes are as follows: B, *Bsp*MI; Bs, *Bsm*I; H, *Hin*dIII; K, *Kpn*I; R, *Eco*RI; and X, *Xba*I.

coli. By immunoblot, several phages were identified and isolated, and they were further analyzed by SDS-PAGE and Western blots of crude phage lysates. Phages λTEE6.1 to TEE6.5 express a 55-kDa protein corresponding in size to the T6 protein extracted from strain D471 by nonionic detergent (Fig. 1). This protein does not appear in a phage lysate preparation of λEMBL4. The DNA inserts of four recombinant phages expressing the T6 protein, named λTEE6.1 to TEE6.4, were mapped with restriction enzymes and aligned along common restriction fragments (Fig. 2).

Sequence and analysis of the *tee*6 gene. The results of the subcloning experiments are summarized in Fig. 3. The 2.3-kb *Hin*dIII insert of pTEE6.3 was subcloned into M13mp 18/19 and sequenced after generating a set of deletions. Additional data for the stop codon and transcription termination region were obtained from a subclone comprising sequences downstream of the internal *Eco*RI site of pTEE6.1a with an *Eco*RI-*Sau*3A fragment cloned into M13mp19 cut with *Bam*HI and *Eco*RI. The nucleotide sequence with the deduced amino acid sequence is shown in Fig. 4. The sequence begins at the *Hin*dIII site with position 1 and ends at the *Sau*3A site of position 2508; restriction sites *Bsp*MI and *Bsm*I are indicated in the figure. We found only one major open reading frame from position 719 to 2332 that codes for a protein of 537 amino acids with a size of 57,675 corresponding to the apparent size of the T6 protein extracted from the streptococcus (55 kDa).

Analysis of the amino acid composition of T6 protein revealed that 21% of the molecule is composed of serine and threonine residues distributed almost evenly throughout the molecule (Fig. 4). The hydropathicity pattern analyzed by the Eisenberg algorithm (4) reveals strongly hydrophobic regions at the N terminus, where the signal sequence is located, and at the C terminus, in the position of a possible membrane anchor segment (Fig. 5A). In addition, strong hydrophobic regions are also revealed around residues 110, 130, and 360 and strongly hydrophilic segments are revealed at residues 310 to 330 and at position 430. At the end of the C-terminal hydrophobic segment are four positively charged amino acids (two arginine and two lysine residues) and one uncharged terminal residue. Upstream of the *tee*6 gene, a typical ribosome-binding site was identified, while downstream of the *tee*6 open reading frame, a potential transcrip-

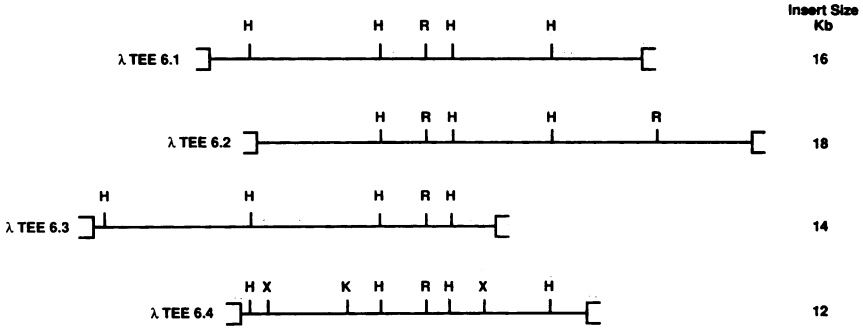

FIG. 2. Restriction maps of T6 protein expressing phages λTEE6.1 through λTEE6.4. The restriction maps are aligned along common restriction sites, and the respective insert size in kilobases (Kb) is indicated on the right. Vector DNA is portrayed by an open bar, in contrast to streptococcal insert DNA. Single letters represent recognition sequences for restriction enzymes: H, *Hin*dIII; K, *Kpn*I; R, *Eco*RI; and X, *Xba*I. The enzymes K and X were tested only for λTEE6.4.

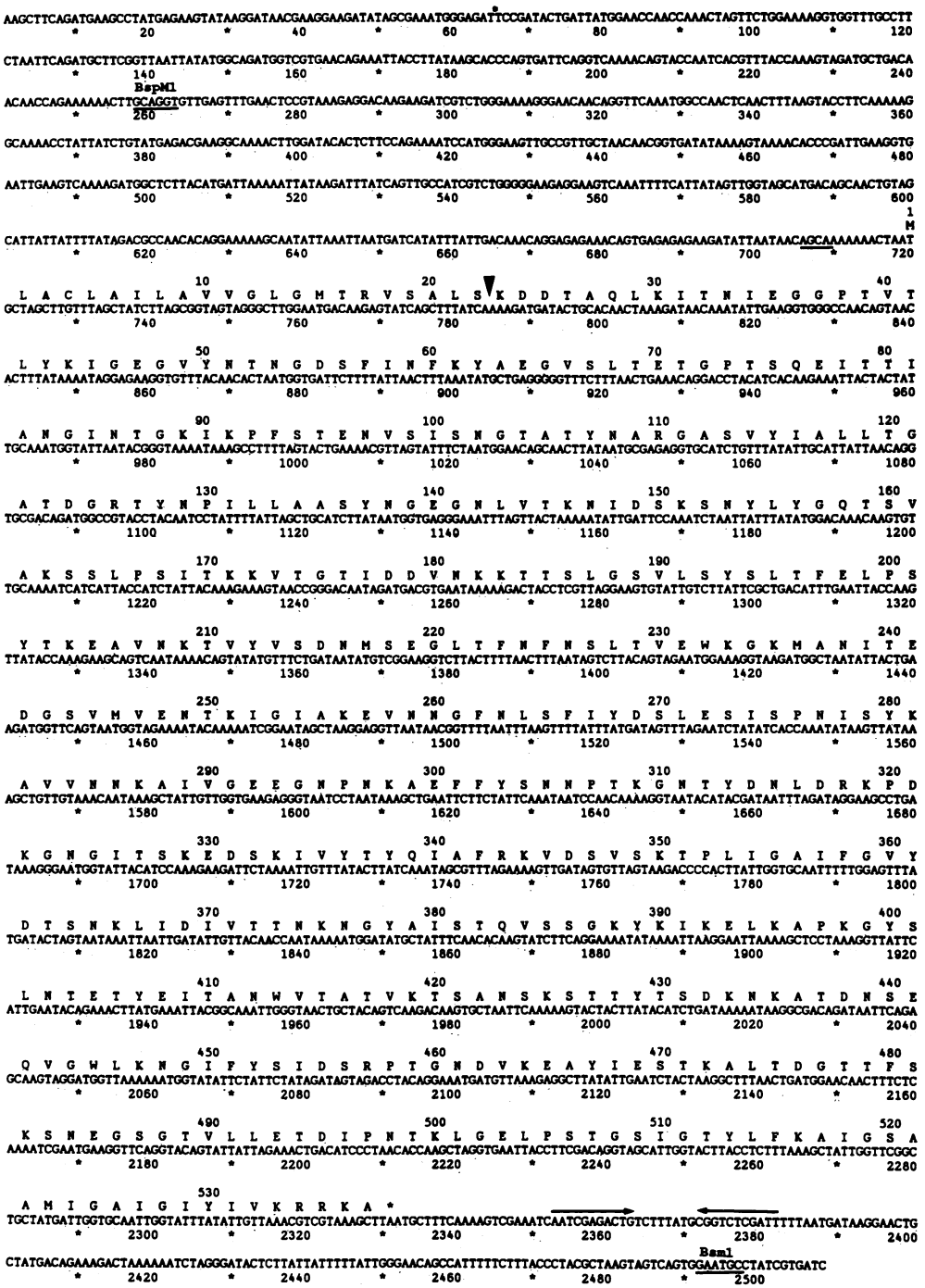

FIG. 4. Nucleotide sequence of the *tee*6 gene from streptococcal strain D471 and flanking regions. The deduced amino acid sequence defined by the open reading frame of 537 residues is indicated in single-letter code above the nucleotide sequence. The arrowhead designates the proposed site for the cleavage and removal of the signal peptide between amino acids 22 and 23. A putative ribosomal binding site upstream of the open reading frame is underlined, and a downstream transcription termination signal is indicated by two arrows. Restriction sites *Bsm*I and *Bsp*MI are indicated in the figure. The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank, and DDBJ nucleotide sequence databases under the accession number M32978.

tion termination signal was located (Fig. 4). The presence of a potential signal sequence cleavage site (indicated by the arrow in Fig. 4) was deduced by the method described by von Heijne (47).

Secondary structure analysis of the deduced T6 protein sequence by the algorithm of Garnier et al. (15) revealed that the molecule has a very high degree (71%) of β-sheet potential, with only 0.6% α-helix, 13.2% β-turn, and 13.9% random coil. The β-sheet characteristic is distributed nearly throughout the molecule, interrupted only by short segments of random coil or β-turns, suggesting a rather compact tertiary structure (Fig. 5B).

Sequence homology with known proteins. Comparison of the deduced amino acid sequence of the T6 protein with a

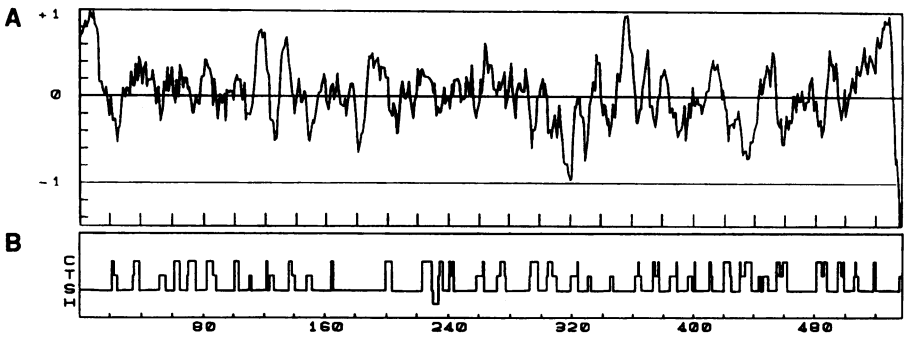

FIG. 5. Hydropathicity index (A) of the complete T6 sequence according to the algorithm of Eisenberg et al. (4). The *x* axis represents the amino acid number of the T6 sequence. Hydrophobic domains are located on the positive side of the graph, and hydrophilic domains are located on the negative side. Conformational analysis (B) of the T6 protein sequence by the algorithm of Garnier et al. (15). Conformational potential of the sequence is represented by the following: C, random coil; T, β-turn; S, β-sheet; and H, α-helix.

protein sequence database (35) revealed the most striking homologies to be localized within the highly hydrophobic C-terminal end and a short upstream sequence of six amino acids (Fig. 6). Molecules such as the M protein (19) and IgA-binding protein (14) from *S. pyogenes* and wapA protein from *Streptococcus mutans* (8) in addition to protein A (17) and fibronectin-binding protein (41) from staphylococci exhibited the best homology in this region. The highest degree of sequence identity, however, was localized within the hexapeptide sequence LPSTGS, located 10 residues preceding the hydrophobic membrane anchor region (Fig. 6A). On the DNA sequence level, the coding sequence for these six amino acids is also highly conserved, which is not the case for the C-terminal hydrophobic region (Fig. 6B) or the remainder of the DNA sequence (not shown).

Localization of T6 protein in *E. coli*. Since a possible signal sequence and membrane anchor domain were identified in the T6 protein, we investigated whether the molecule would translocate and insert into the *E. coli* inner membrane. The clone pTEE6.4 in XL1-Blue was selected for the cell localization experiments because it contains the entire coding sequence with upstream and downstream sequences and is transcribed independently of the *lacZ* promotor. We followed an osmotic shock protocol to lyse the cells after releasing the periplasmic proteins with a combined lysozyme and EDTA treatment (3). The membranes were separated from the cytoplasm by centrifugation, and all samples were adjusted to the same volume before SDS-PAGE and Western blot (Fig. 7). In *E. coli* the T6 protein is located in the membrane fraction and migrates as a triple band of 60, 58, and 57 kDa similar to that seen for M protein (12). In the periplasmic space is found a small amount of protein which migrates at the same speed (55 kDa) as T6 protein extracted by nonionic detergent from the streptococci. A protein doublet of 58 and 55 kDa is found in small quantities in cytoplasmic preparations.

DISCUSSION

Group A streptococci elaborate several cell surface proteins (M protein [9], T protein [25], Fc receptor protein [18], and C5a peptidase [31]) and extracellular products (hemol-

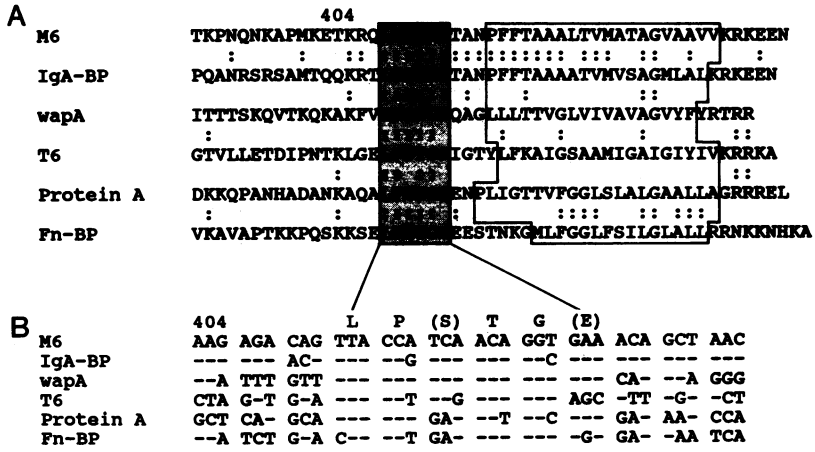

FIG. 6. Comparison of the amino acid and DNA sequence of the C-terminal end of T6 protein with M6 protein (M6) (19) and IgA-binding protein (IgA-BP) (14) from group A streptococci, wall-associated protein (wapA) (8) from *S. mutans*, and protein A (17) and fibronectin-binding protein (Fn-BP) (41) from *Staphylococcus aureus*. The sequences are aligned according to the hexapeptide consensus sequence LPSTGE (shaded box). (A) Identical residues between protein sequences are designated by colons. No attempt has been made to maximize the number of identities by the introduction of gaps in the sequences. The hydrophobic amino acids suggested to be a membrane anchor for these molecules are enclosed by a box. (B) Alignment of the DNA sequence of the LPSTGE sequence region and three flanking amino acids are presented with identical bases portrayed by a dash. 404 is Lys-404 of the M6 protein.

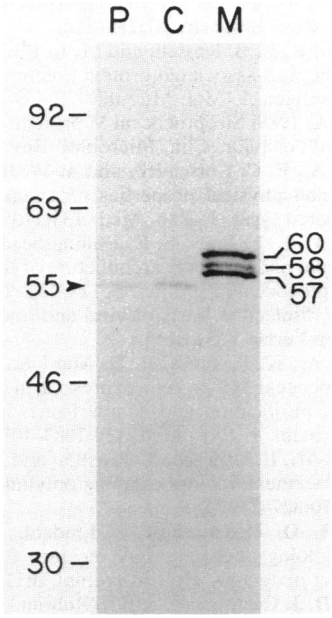

FIG. 7. Western blot analysis of the location of the T6 protein in *E. coli*. Periplasmic (P), cytoplasmic (C), and membrane (M) fractions of *E. coli* XL1-Blue containing the T6 gene were equalized to the same relative volume and analyzed by SDS-PAGE and Western blot by using a T6-specific antibody. Molecular mass standards (in kilodaltons) include: phosphorylase B, 92; bovine serum albumin, 69; ovalbumin, 46; and carbonic anhydrase, 30.

ysins, leukotoxins, hyaluronidase, proteinase, and DNase [30]), to name just a few. While many of these substances likely play a role in the pathogenesis of streptococcal diseases, the exact mechanisms by which these cellular and extracellular factors interrelate to accomplish a final disease outcome is poorly defined. Clearly, the antiphagocytic action of the M protein is important in maintaining the streptococci within infected tissues until type-specific antibodies are elaborated by the host to allow phagocytosis and clearance. Curiously, however, these organisms also elaborate a proteinase capable of destroying the M molecule on the cell surface (5). Elliott showed that most group A streptococcal strains producing low quantities of M protein produce active proteinase in the culture medium capable of destroying the M molecule (5). Passage of these strains through mice resulted in the isolation of virulent M-producing strains that do not elaborate active enzyme. The T protein, which is resistant to the action of proteinase, is exposed on the cell surface when the fibrillar M protein is enzymatically removed. Perhaps the ability to destroy preferentially one molecule to expose a second is an alternative means by which these organisms are able to modulate the molecules on their surface. Whether this event occurs during colonization and infection is unknown; however, a better understanding of the structure and properties of streptococcal surface molecules will be useful in establishing their importance in streptococcal pathogenesis.

The DNA sequence analysis of the *tee*6 gene disclosed only one major open reading frame whose deduced amino acid sequence showed a high degree of homology to other streptococcal and staphylococcal proteins in the region of the membrane anchor. This characteristic was described also for the anchor region of several different surface proteins from gram-positive cocci (19). An unusual feature of the T6 protein sequence not seen in other bacterial proteins is the presence of 21% serine and threonine amino acids and their almost even distribution throughout the molecule. This is in contrast to the wapA surface protein from *S. mutans* in which a threonine-serine-rich region was identified in a C-terminal segment predicted to be a cell wall-spanning region of the molecule (8). It is unlikely that this characteristic is a prerequisite for cell wall embedding, since the cell wall-spanning region of M protein contains a high concentration of proline and glycine amino acids (33). Whether the threonine-serine-rich region of the wapA protein is actually localized within the cell wall (8) will require further detailed analyses. A very high degree of serine and threonine residues was recently reported to occur in the eucaryotic surface molecules syndecan (39), versican (49), and α-agglutinin (26), all of which are involved in cell adhesion.

In sharp contrast to streptococcal M protein (36), protein G (6) and Fc-receptor protein (18), which are mainly α-helical in conformation and may be classified as fibrous molecules (34, 36), the >70% β-sheet potential predicted for the T6 protein, separated by short stretches of β-turns or random coils (Fig. 5), suggests a compact molecular configuration. The resistance of T protein to the action of trypsin despite 8 arginine and 48 lysine residues supports the view of a compact conformation and the localization of these trypsin-sensitive amino acids away from the molecular surface.

The size similarity between the recombinant T6 protein and the form extracted with nonionic detergent from streptococci suggests that the latter represents the complete uncleaved protein rather than a digested fragment. This extraction method also indicates that like M protein (32), T protein is noncovalently linked to the streptococcal cell and may be bound to the cytoplasmic membrane through the hydrophobic C-terminal amino acids and charged tail deduced from the DNA sequence.

In *E. coli* containing the *tee*6 gene, the T6 protein is mainly localized within the membrane fraction. Although the inner and outer membranes were not separated, the C-terminal hydrophobic and charged sequence together with the appearance of small quantities of T protein in the periplasmic space suggests that the location of the molecule is probably the *E. coli* inner membrane. The occurrence of some T6 protein in the periplasmic space indicates that the TEE signal sequence is probably recognized and processed in *E. coli*, as it is for other streptococcal surface proteins (12). However, for the M protein, substantial quantities of the molecule are localized in the periplasm. This difference in cellular localization may be due either to differences in anchoring efficiency, peripheral membrane attachment (42), or the insertion of the T6 protein into the membrane by an uncleaved signal sequence. We consider the phage lysate preparations to be somewhat equivalent to the periplasmic space, since membranes were removed by a centrifugation-filtration step; this would account then for the appearance of a single band in these samples as found in the periplasmic space.

At the protein sequence level, the hydrophobic-terminal segment of T6 protein is homologous but not identical to the hydrophobic C-terminal region found in M protein (19), IgA-binding protein (14), fibronectin-binding protein (41), wall-associated protein A (8), and protein A (17); this homology is lost, however, at the level of the DNA sequence. A region that is highly homologous in all these proteins in both protein and DNA sequence is located 10 residues N terminal to the membrane anchor beginning at Leu-504 and

continuing to Ser-509, having the sequence LPSTGS. A nearly identical hexapeptide with the consensus sequence LPSTGE is conserved among proteins reported here (Fig. 6) and nearly all reported surface proteins from gram-positive cocci (V. Fischetti, V. Pancholi, and O. Schneewind, submitted for publication) and is involved in the release of M protein from isolated protoplasts (33). This hexapeptide is also homologous to the proposed cleavage and glycosylphosphatidylinositol attachment sequence found in glycosylphosphatidylinositol-anchored proteins of eucaryotes, particularly those associated with cell adhesion (i.e., neural cell adhesion molecule, N-CAM; lymphocyte function associated protein, LFA-3; and *Dictyostelium discoideum* contact site A molecule, CsA [7]). Whether, as suggested for M protein (33), this hexapeptide sequence is responsible for a posttranslational modification necessary for the proper anchoring of the T protein through a separate anchor complex remains to be elucidated. However, since the T6 protein appears as a triple band when extracted from *E. coli*, similar to that found in M proteins (12), a property attributed to the C-terminal half of the M molecule (11), it implies some form of modification within this region of the molecule.

Our data on the deduced amino acid sequence and composition of T6 protein are the first for a complete T molecule. The amino acid composition and size of the purified T1 (20) and T12 (27) proteins derived after extensive proteinase digestion differ from those reported here for the T6 molecule. These differences are likely due to the analysis of a fragment of the native T protein in the earlier studies. While more than 25 immunologically different T proteins have been described for *S. pyogenes*, the nature of this heterogeneity is unknown. Whether this characteristic along with the presence of cross-reactive epitopes is, like M protein, the result of specific variable and conserved domains is presently under study.

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