JOURNAL OF BACTERIOLOGY, June 1994, p. 3631–3637
0021-9193/94/$04.00+0

Vol. 176, No. 12

Genetic and Biochemical Analysis of the Flagellar Hook of *Treponema phagedenis*

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Received 18 January 1994/Accepted 11 April 1994

The periplasmic flagellum of *Treponema phagedenis* consists of the flagellar filament and hook-basal body. We report here a characterization of the hook gene and flagellar hook of *T. phagedenis*, and in the process of this analysis we found evidence that the hook polypeptide is likely cross-linked in situ. A *T. phagedenis* genomic library was screened with a *Treponema pallidum* antiserum, and the DNA segments from several positive plaques were subcloned and sequenced. DNA sequencing of two overlapping segments revealed a 1,389-nucleotide (nt) open reading frame (ORF) with a deduced amino acid sequence that was 36% identical to that of FlgE, the hook polypeptide of *Salmonella typhimurium*. This gene was designated *T. phagedenis flgE*. Beginning at 312 nt downstream from *flgE* was a partial ORF of 486 nt with a deduced amino acid sequence that was 33% identical to that of MotA of *Bacillus subtilis*, a polypeptide that enables flagellar rotation. Upstream of *flgE*, separated by 39 nt, was a partial (291-nt) ORF with a deduced amino acid sequence that was homologous to that of ORF8, a polypeptide of unknown function located in an operon encoding polypeptides involved in motility of *B. subtilis*. The *T. phagedenis flgE* gene was cloned into an *Escherichia coli* protein expression plasmid, and the purified recombinant protein was used to prepare a FlgE antiserum. Western blots (immunoblots) of whole-cell lysates probed with this antiserum revealed a 55-kDa polypeptide and a ladder of polypeptide bands with increasing molecular masses. *T. phagedenis* hooks were then isolated and purified, and electron microscopic analysis revealed that the morphology of the hooks resembled that in other bacteria. The hooks were slightly curved and had an average length of 69 ± 8 nm and a diameter of 23 ± 1 nm. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blots of purified hook preparations using the FlgE antiserum also revealed a polypeptide ladder, suggesting that the hooks are composed of a covalently cross-linked polypeptide.

Treponema phagedenis is an anaerobic, host-associated spirochete that is used as a model for studying spirochete motility. The general structure of *T. phagedenis* resembles that of other spirochetes, including *Treponema pallidum* and *Borrelia burgdorferi* (5, 7, 8, 15, 39), whose motility plays an important role in the pathogenesis of both syphilis and Lyme disease (22, 41). Therefore, our initial studies have focused on the periplasmic flagella (PFs) of *T. phagedenis*, which are essential structures for motility (23).

The PFs of *T. phagedenis* consist of the filament and the hook-basal body (HBB) apparatus (18, 19). *T. phagedenis* has between four and six PFs that are inserted at each end of the cell cylinder and extend backwards along the cell body between the inner and outer membranes. Occasionally, the filaments of *T. phagedenis* may protrude from the cell but remain enclosed within membranous material (6). Extensive studies of the filament ultrastructure and molecular composition have revealed a complex structure consisting of multiple protein components comprising the filament core and sheath (for reviews, see references 32 and 40).

In contrast to the filament, little is known about the HBB of *T. phagedenis*. The filament is connected to the hook; however, hook-associated proteins which link the filament to the hook in other bacteria have not yet been identified in the spirochetes. The hook is connected via the rod to the basal disk, which is embedded in the inner cell membrane (19). It is not clear whether the basal disk of *T. phagedenis* consists of a single ring structure or two closely spaced ring structures. Although the structure of other spirochete PFs resembles that of *T. phagedenis*, there are variations in the number of filaments, the protein composition of filaments, and the number of rings making up the basal body apparatus of various spirochetes (4, 31).

We were interested in extending our previous molecular characterization of treponemal PF proteins to include the HBB apparatus of *T. phagedenis*. By analogy with other bacteria, the HBB apparatus is likely to play an essential role in the motility of spirochetes. We now report the purification and unique composition of *T. phagedenis* hooks and the identification and DNA sequence of the *T. phagedenis* hook gene.

MATERIALS AND METHODS

Strains, plasmids, and reagents. The culture conditions for *T. phagedenis* Kazan 5 have been described previously (23). *T. pallidum* subsp. *pallidum* cells were kindly provided by K. Wicher (Wadsworth Center for Laboratories and Research [WCLR], Albany, N.Y.), and DNA was isolated by standard methods (28). *Escherichia coli* ER1578 has been described elsewhere and was grown in L broth containing 50 μg of ampicillin per ml (26). *E. coli* JM101 and JM109 have been described elsewhere and were grown in L broth (29, 43). The culture and manipulation of bacteriophages M13mp18 and M13mp19 have been described previously (26, 43). Restriction enzymes, T4 DNA ligase, and materials for cloning and

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expression of antigens in the maltose-binding-protein (MBP) vectors by using the Protein Fusion and Purification System were obtained from New England BioLabs (Beverly, Mass.) Isotopes were purchased from Amersham Corporation (Arlington Heights, Ill.). The Erase-a-Base system for generating exonuclease III deletions was obtained from Promega Corp. (Madison, Wis.). *Taq* polymerase was purchased from PE Express (Norwalk, Conn.). DNA manipulations were done by standard methods (28).

Identification, subcloning, and DNA sequencing of the *T. phagedenis* hook gene. Construction of the *T. phagedenis* genomic library in λ gt11 has been described previously (26). The library was screened by standard methods (38), using a *T. pallidum* antiserum obtained from rabbits that were infected intratesticularly with viable *T. pallidum* cells (kindly provided by K. Wicher, WCLR). DNA was purified from positive recombinant plaques (26), digested with *Eco*RI to liberate the insert DNA, and then subcloned into M13mp18 and M13mp19 for sequencing. DNA sequencing was done on both single- and double-stranded DNA by the dideoxy method (37) with Sequenase (U.S. Biochemical Corp., Cleveland, Ohio) or Amplitaq (PE Express). Sequencing of the cloned DNA was accomplished by generating deletions with either exonuclease III (14) or the appropriate restriction endonucleases. Remaining gaps in the DNA sequence were filled by using synthetic oligonucleotide primers prepared at the Molecular Genetics Core Facility of the WCLR. Sequences were analyzed by using programs available through the University of Wisconsin Genetics Computer Group (10).

Expression and purification of *T. phagedenis* hook protein in *E. coli*. The Protein Fusion and Purification System (New England BioLabs [11]) was used to express and purify *T. phagedenis* hook protein in *E. coli*. The hook gene was synthesized in vitro by a PCR-based approach (36). The primers DAM1 (5′-TTTAGAATTCACCACAGGTTTTAAGCGCG GC-3′ [nucleotide {nt} 424 to 444, Fig. 1]) and KC1 (5′-GT GCGCATACTTTGATACCG-3′ [anneals at nt 2205 to 2227, Fig. 1]) were used to amplify the hook gene from *T. phagedenis* genomic DNA by PCR. To facilitate cloning, primer DAM1 contains a synthetic *Eco*RI restriction endonuclease site. There is a native *Nsi*I site in the DNA sequence downstream from the 3′ end of the hook gene at nt 1992 to 1997. After amplification, the DNA product was purified by agarose gel electrophoresis and digested with *Eco*RI and *Nsi*I. The DNA was further purified by using a Spin Bind column (FMC Corp., Rockland, Maine) and then ligated into the plasmid p770R and transformed into *E. coli* JM109. The plasmid p770R is similar to the pMAL-c plasmid available from New England BioLabs. Plasmid DNA was isolated from transformed cells and sequenced adjacent to the cloning site to confirm that the *T. phagedenis* hook gene was properly linked to the gene encoding the *E. coli* MBP. Plasmids containing the proper fusion were transformed into *E. coli* JM101 for protein expression.

Recombinant hook-MBP fusion protein was expressed and purified from *E. coli* JM101 harboring the recombinant plasmid by previously described methods (11). Briefly, overnight cultures were diluted 1:100 in L broth with ampicillin and grown for 2 h at 37°C prior to induction with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 1 h. Cultures were washed, sonicated, and poured over a column of amylose resin. After extensive washing of the column with 10 mM Tris (pH 7.2)–0.5 M NaCl, the fusion protein was eluted with 10 mM maltose and concentrated with a Centricon 30 (Amicon, Danvers, Mass.). Purified proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie blue R-250 (23) or silver (Bio-Rad Laboratories, Richmond, Calif.).

Preparation of antiserum and Western blotting. Antiserum was generated against purified MBP (control) and MBP-hook fusion protein by immunization of 5-week-old BALB/c mice. The mice were injected with 50 μg of protein and Ribi adjuvant (Ribi Immunochemicals, Hamilton, Mont.); this was followed by a second injection 2 weeks later. Four weeks after the first injection, the antiserum was collected and tested for reactivity to the recombinant fusion protein. Western blots (immunoblots) using this antiserum were performed as previously described (23) and developed with alkaline phosphatase-conjugated anti-mouse antibody (Bio-Rad), using 5-bromo-1-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (Promega Corp.). Lysates of *T. phagedenis* and *T. pallidum* cells were prepared after multiple washes with Tris-buffered saline. *T. phagedenis* hook structures were purified for Western blotting as described below.

Isolation of hook structures of *T. phagedenis*. Hooks were isolated from *T. phagedenis* by a procedure similar to those used to isolate hooks from other bacteria (1, 4). Briefly, the outer cell membrane was removed and the PFs were sheared from the cells by using a blender and then purified by cesium chloride isopycnic gradient ultracentrifugation in the presence of N-lauroyl sarcosine as described by Isaacs et al. (20). The band containing PFs was dialyzed overnight in water, and the filaments were then dissociated by adjusting the pH to 2.2 with 0.2 N HCl (4) or glycine–Triton X-100 (pH 2.2) (1). After a 60-min incubation at 25°C, the hooks were overlaid on a 60% sucrose cushion and recovered by centrifugation at 100,000 × *g* for 2 h. The pellet was resuspended in the sucrose cushion and then extensively dialyzed against 10 mM Tris, pH 7.5, prior to concentration with polyethylene glycol.

Electron microscopy. For negative staining, purified hook preparations (10 μl) were applied to Formvar- and carbon-coated copper grids. After 3 min, the sample was removed, the grid was washed with 10 mM ammonium acetate to remove residual salts, and the sample was immediately stained with a 1% aqueous solution of uranyl acetate.

Nucleotide sequence accession number. The GenBank nucleotide sequence accession number for *T. phagedenis flgE* and flanking DNA is U04619.

RESULTS

DNA and deduced amino acid sequences of the *T. phagedenis* hook gene. To identify the gene encoding the hook structure of *T. phagedenis*, we reasoned that many of the motility proteins of *T. phagedenis* and *T. pallidum* were well conserved and were therefore antigenically similar. Screening of a *T. phagedenis* genomic library in λ gt11 with an anti-*T. pallidum* serum should result in the selection of recombinant clones expressing related conserved antigens, some of which should be related to motility. The initial screening of approximately 30,000 plaques resulted in 27 positive plaques. The DNA from four of these recombinant phages was subcloned into M13 and sequenced. The sequence from two clones revealed overlapping DNA sequences, which were assembled and are shown in Fig. 1. One complete open reading frame (ORF), ORF2, was identified on this DNA segment, which was flanked by two truncated potential ORFs (ORF1 and ORF3).

ORF2 is 463 amino acids long, with a putative ribosome binding site and a predicted mass of 49 kDa (Fig. 1). A comparison of the deduced amino acid sequence of ORF2 with the sequences of other known polypeptides revealed a 36% identity to the hook polypeptide (FlgE) of *Salmonella typhi-*

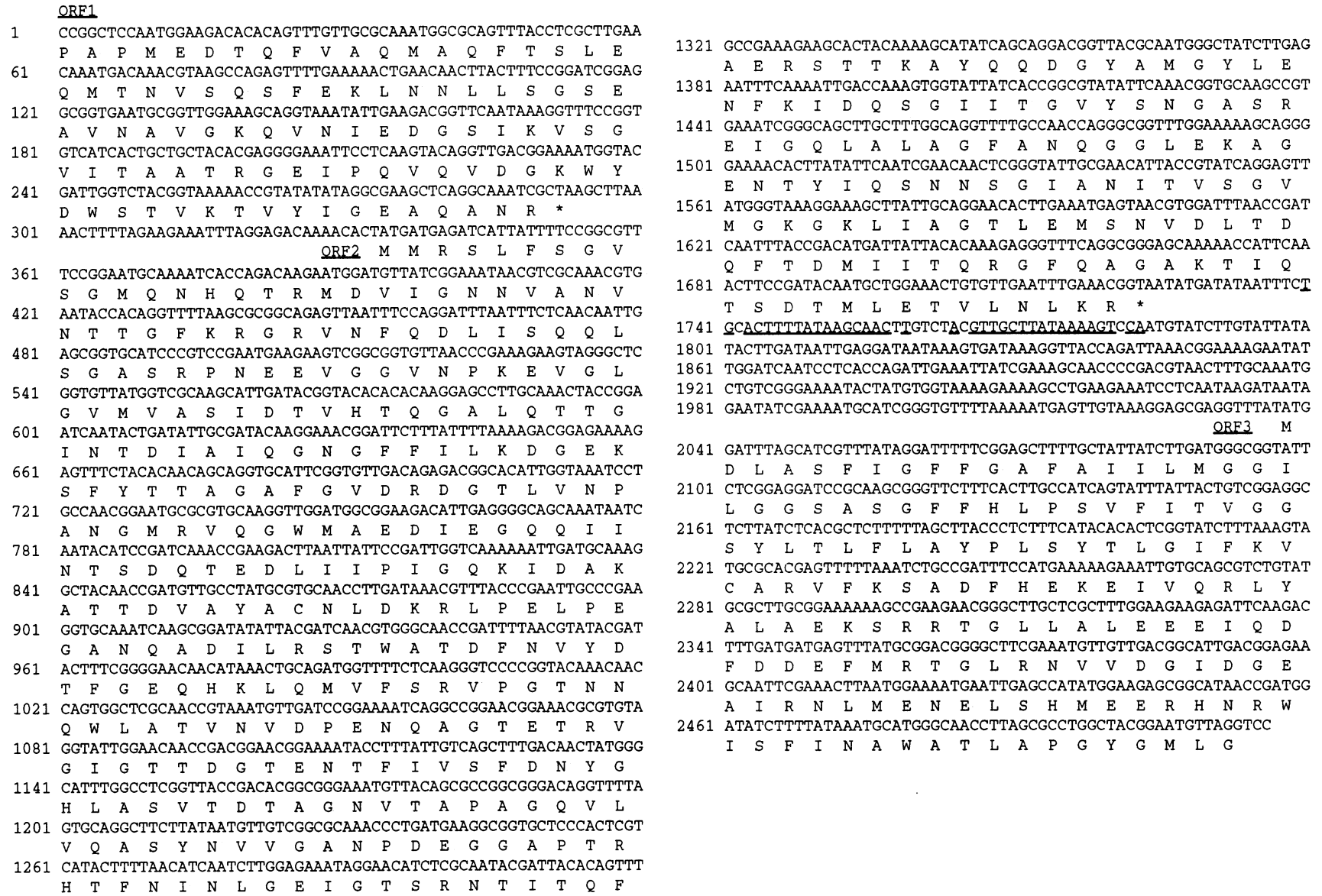

FIG. 1. DNA and deduced amino acid sequences of one complete ORF (ORF2 or *flgE*) and two partial ORFs (ORF1 and ORF3) from *T. phagedenis*. An inverted repeat (underlined at nt 1740 to 1783) may be involved in regulation of transcription. ORF3 is homologous to *motA* of *B. subtilis*. The function of ORF1 is unknown.

FIG. 2. (A) Comparison of the deduced amino acid sequences of FlgE of *T. phagedenis* and *S. typhimurium*. Identical matches (|) and strongly (:) and weakly (.) conserved amino acid sequences are indicated. The sequences corresponding with the conserved SGL, GNNIAN, and ELVNMI sequences of *S. typhimurium* FlgE are underlined. (B) Comparison of the known N-terminal amino acid sequence of the *C. coli* hook polypeptide (34) with the deduced amino acid sequence of the corresponding region of *T. phagedenis* FlgE.

murium (16) (Fig. 2A) and a 73% identity to the known N-terminal amino acid sequence of the *Campylobacter coli* hook (34) (Fig. 2B). ORF2 also shared a 38% identity with FlgG, the distal rod protein of *S. typhimurium*, though FlgG is only 260 amino acids long, which resulted in numerous extensive gaps in the alignment, primarily in the central region of ORF2 (17). The amino acid sequences and composition of ORF2 were further compared with those of FlgE and FlgG. The regions most conserved with both *Salmonella* polypeptides were located near the N and C termini, including hydrophobic residues located at every seventh residue near the termini. However, *T. phagedenis* ORF2 contains a sequence that is poorly conserved with the ELVNMI sequence found at the C termini of FlgG and FlgE of *S. typhimurium*. In contrast, as shown in Fig. 2, the amino terminus of *T. phagedenis* ORF2 does contain the amino acid sequences SGM and GNNVAN, which are similar to the conserved SGL and GNNIAN sequences found in the amino terminus of *S. typhimurium* FlgE (16) and the TGL and ANNLAN sequences in the amino terminus of FlgG (17). As with the deduced *Salmonella* polypeptide sequences, no typical signal sequences were noted at the N terminus. Only one cysteine was noted at amino acid 178, and prolines were located only in the central region of the polypeptide in a manner resembling the composition of the hook polypeptide of *S. typhimurium*. On the basis of the similar features and strong homology to the entire length of *S. typhimurium flgE*, we have designated ORF2 *flgE*, the gene that likely encodes the polypeptide comprising the hook of *T. phagedenis*. Additional evidence indicating that this gene encodes the hook protein was obtained by Western blots of purified hook preparations (see Western blot results below).

Sequence analysis of DNA flanking *flgE* of *T. phagedenis*. The DNA and deduced amino acid sequences flanking *T. phagedenis flgE* were examined in greater detail. Downstream of *flgE* is a noncoding region of 312 nt followed by ORF3. In this intergenic region, there is a sequence of DNA 14 nt downstream of *flgE* that may form considerable secondary structure in the RNA transcript (Fig. 1), but there is no string of U's characteristic of prokaryotic transcription terminators (35). Following this region lies the ORF3-encoded polypeptide which is at least 162 amino acids long, but the cloned DNA segment ends before encountering a stop codon. The predicted amino acid sequence of ORF3 was 33% identical to that of MotA of *Bacillus subtilis*, which encodes one of the genes enabling flagellar rotation (9, 30).

Examination of the DNA sequence upstream of *flgE* revealed no obvious consensus promoter sequences (3, 13). Rather, there is the putative ORF (ORF1) separated from *flgE* by 39 nt of noncoding DNA, suggesting that *flgE* is part of a larger operon. ORF1, which consists of at least 97 amino acids, was 30% identical to ORF8 of the *flaA* locus of *B. subtilis* (2). ORF8 of *B. subtilis* is part of a locus encoding motility proteins but has not been assigned any function because there is no homolog in *S. typhimurium*. Therefore, we are unable to assign a function to ORF1 of *T. phagedenis* on the basis of amino acid sequence identity.

Expression and purification of *T. phagedenis* hook protein in *E. coli*. To express and purify large quantities of *T. phagedenis* FlgE, *flgE* DNA was amplified and cloned into a plasmid in *E. coli* and expressed as a fusion with MBP. The initial attempts to express FlgE in *E. coli* were unsuccessful, possibly because of protease cleavage near the amino terminus of FlgE, which would separate the recombinant protein from the MBP protein and destroy the one-step purification capability of the amylose column. To avoid the problems associated with protein processing, *flgE* was cloned into the MBP vector by using a 5′ oligonucleotide primer (DAM1) that annealed about 90 nt downstream from the ATG initiation codon. A 1-liter culture of *E. coli* containing this plasmid yielded approximately 1 mg of fusion protein with an approximate mass of 88 kDa on SDS-PAGE (42 kDa for MBP and 46 kDa for this truncated FlgE) (Fig. 3). The 42-kDa polypeptide noted on the gel is MBP that is cleaved in vivo from the fusion protein but copurifies on the affinity column. This preparation of protein was used to immunize mice to produce antibodies for Western blotting analysis (see the description below).

Ultrastructure of the *T. phagedenis* hook. Hooks were isolated and purified from *T. phagedenis* by a combination of cell shearing in a blender, isopycnic density gradient ultracentrifugation, and dissociation of flagellar filaments at pH 2.2. After CsCl centrifugation, two sharp bands were detected on the

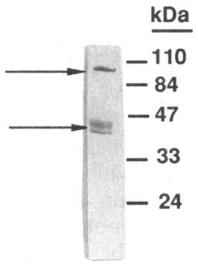

FIG. 3. SDS-PAGE of a purified MBP-FlgE fusion protein sample isolated from *E. coli* containing the cloned *flgE* gene. Arrows indicate the MBP (42 kDa) and MBP-FlgE (88 kDa) polypeptides that are visible after staining with Coomassie blue. The numbers at the right indicate the molecular mass standards.

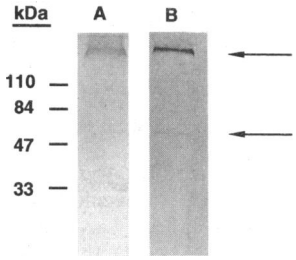

FIG. 5. SDS-PAGE (12% gel) of purified hooks of *T. phagedenis* after silver staining (lane A) and a Western blot probed with FlgE antiserum (lane B). The arrows indicate the weakly reactive 55-kDa band in lane B and the compressed polypeptide ladder that does not migrate significantly into the gel. The numbers indicate the molecular masses of protein size markers.

gradient, though on occasion only one band was noted. The granular upper band was composed primarily of PFs, whereas the viscous lower band contained some PFs along with unidentified material. Occasionally, the positions of these bands were reversed. After dissociation of the filaments at pH 2.2, the material from the upper band was composed primarily of hooks, with an occasional basal body (Fig. 4A). The lower band contained considerable unidentifiable debris along with hooks and some HBBs (not shown). The rather harsh nature of the isolation procedure would likely result in detachment of the basal body while leaving the hook structure intact. The morphologies of the hooks in both preparations were similar. The hooks were slightly curved, and measurement of 50 hooks indicated an average length of 69 ± 8 nm and a diameter of 23 ± 1 nm. We noted a sharp indentation at one end and a small knob at the other end where the hook is connected to the rod, similar to the morphologies of *Spirochaeta aurantia* and *Campylobacter* hook structures (4, 34) (Fig. 4B).

Western blot and SDS-PAGE analysis of *T. phagedenis* hooks. The following evidence suggests that ORF2 encodes the polypeptide that comprises the hook structure of *T. phagedenis* and that the hook polypeptide is likely covalently cross-linked. SDS-PAGE of the purified hooks revealed multiple protein bands near the top of the resolving gel (Fig. 5A, lane A). The antiserum generated against the recombinant *T. phagedenis* FlgE was reacted with purified hook preparations by Western blotting. Western blots revealed a similar reactive area at the top of the gel (Fig. 5A, lane B). In addition to this intensely reactive area, there was usually one additional minor band at a molecular mass of approximately 55 kDa. Heating of hooks at 100°C in SDS-PAGE sample buffer for up to 1 h slightly increased the amount of 55-kDa protein that was visible (data not shown). Hooks that were not heated prior to SDS-PAGE showed patterns similar to those of hooks that were heated for the standard 3 min (data not shown). Treatment of the hooks with 8 M urea, 8 M guanidine, 1 M NaOH, or an excess of β-mercaptoethanol or dithiothreitol failed to result in a significant migration of the high-molecular-mass polypeptides into the gel (not shown). Additional Western blots were done at longer SDS-PAGE run times on a 4 to 15% gradient gel and revealed a ladder of protein bands, suggesting that *T. phagedenis* hooks consist of a high-molecular-weight ladder of cross-linked polypeptides (Fig. 6, lane A). These bands had approximate masses of 55, 155, 220, and 280 kDa, and there were additional polypeptides of greater mass not measurable on these gels. A weak band was often noted at approximately 102 kDa as well (data not shown).

Western blots of whole cells of *T. phagedenis* with the FlgE antiserum revealed a band at 55 kDa in addition to the high-molecular-weight protein ladder (Fig. 6, lane B). A minor

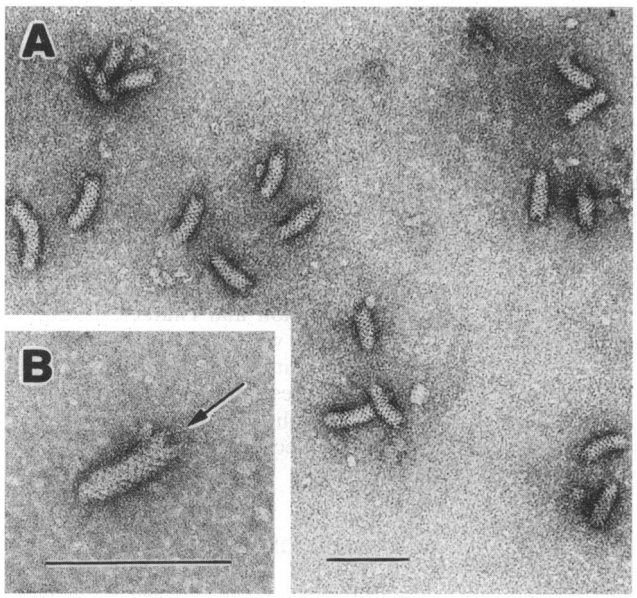

FIG. 4. Electron micrographs of hooks purified from *T. phagedenis*. (A) Typical hook sample from the upper granular band from a CsCl gradient. (B) Closer view of a hook, with the arrow indicating the V-shaped indentation where the hook presumably connects to the filament. Bar, 100 nm.

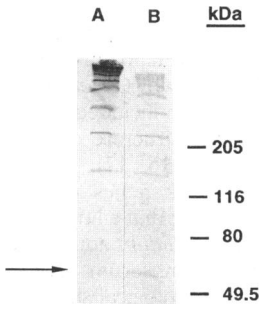

FIG. 6. Western blots using the FlgE antiserum and a 4 to 15% gradient gel that was run for an extended time to show the polypeptide ladder. Lane A, purified hooks; lane B, *T. phagedenis* cells. The numbers indicate the molecular masses of protein size markers. The arrow indicates the weakly reactive 55-kDa band in the purified hook sample.

polypeptide of 50 kDa was occasionally noted, although its significance is presently unclear (data not shown). Control blots using MBP antiserum did not react with *T. phagedenis*.

DISCUSSION

Analysis of the flagellar hook of *T. phagedenis* by electron microscopy and SDS-PAGE revealed unique features. Although the general morphology was quite similar to that of other bacterial hooks (1, 4, 27, 34), the novel feature noted for *T. phagedenis* was the unique protein ladder that was revealed after SDS-PAGE and Western blotting of purified hook samples. Measurements of the approximate masses of the polypeptides indicated a 55-kDa polypeptide monomer with additional polypeptide bands at increasing increments of about 45 to 65 kDa. Most of the hook polypeptide failed to significantly enter the resolving gel, even after we used several different methods to try to dissociate the polypeptide. Taken together, these SDS-PAGE results suggested that the *T. phagedenis* hooks consist of covalently cross-linked monomers similar to the cross-linked head proteins of mycobacteriophage L5 (12) and bacteriophage HK97 (33). For HK97, it is postulated that the cross-linking could be accomplished by the addition of a short peptide to a lysine residue of one monomer and the other half of the cross-link would come from a glutamine or asparagine residue. Although we have no direct evidence for a cross-linked hook structure in *T. phagedenis*, the similarities are intriguing.

Additional biochemical studies are needed to precisely measure the polypeptide masses and to reveal the nature of the putative cross-linking in *T. phagedenis*. This unusual composition of *T. phagedenis* hooks appears thus far to be unique among bacteria, although the purpose or evolutionary advantage is unclear. Similarly, there is no clear understanding of the function of cross-linking in the major head shell subunits of certain bacteriophages (12, 33). In *Halobacterium* spp., the flagellins are modified by sulfated glycoproteins, which results in multiple bands on SDS-PAGE gels (42); however, the pattern is rather different from the extensive incremental ladder seen in *T. phagedenis* hooks. However, we cannot rule out the possibility of either posttranslational modifications of the hook polypeptide or an unusual in vitro event that results in an artifact upon SDS-PAGE.

The gene designated *T. phagedenis flgE* likely encodes the hook component of the periplasmic flagellar apparatus. The predicted mass was 49 kDa, which is slightly smaller than the apparent molecular mass of the 55-kDa hook monomer. Similar discrepancies in predicted and observed mass have been noted for *T. phagedenis* periplasmic flagella (26) as well as components of the *Salmonella* basal body (21). The deduced amino acid sequence showed homology to that of the complete hook polypeptide of *S. typhimurium* and to the available N-terminal amino acid sequences of the hook protein of *C. coli*. Homology at the amino terminus of *Salmonella* FlgE included conserved sequences that may be important for the structure or targeting of proteins to specific cellular locations (16, 17). Because of the extensive homology among the hook genes of these distantly related bacteria, it was not surprising that a homologous *flgE* segment has also been identified in *T. pallidum* and *B. burgdorferi* DNAs (25). In addition, *T. pallidum* antiserum reacts with *T. phagedenis* hooks, indicating an immunological similarity (24). The remarkable homology of *T. phagedenis* FlgE with the N-terminal amino acid sequence of the *Campylobacter* hook polypeptide and with the entire *S. typhimurium* FlgE polypeptide strongly suggests that *flgE* encodes the hook polypeptide. Additional evidence was obtained by Western blotting, which revealed that antiserum prepared from the protein encoded by the cloned *flgE* gene reacted with the hook polypeptide of *T. phagedenis*. On the basis of the amino acid sequence homologies, Western blots, and SDS-PAGE, we conclude that *flgE* encodes the hook of *T. phagedenis*.

DNA sequence analysis has suggested that *T. phagedenis flgE* is part of an operon. ORF1, which is located upstream of *flgE*, is separated from *flgE* by a noncoding segment of only 39 bp. No obvious promoters were noted in this region, suggesting that ORF1 and *flgE* may be transcribed from the same promoter. At the 3′ end, *T. phagedenis flgE* is followed by a rather long noncoding region of 312 bp before the beginning of ORF3 (the MotA homolog). In this noncoding region lies a section of DNA with considerable potential secondary structure that may be involved in regulation of RNA transcription. This sequence may be involved in transcription termination, though it would be atypical in that there is no string of U's located distal to the stem. Alternatively, this region may function to stabilize RNA transcripts by protecting them from RNase III degradation or possibly may be involved in regulation of ORF3 transcription. Additional studies are aimed at confirming the proposed transcription pattern and operon organization.

Apparently, we have identified a locus that is involved in encoding polypeptides related to the motility of *T. phagedenis* but has a gene organization different from those of homologous genes of *B. subtilis* and *S. typhimurium*. The partial ORF1 located upstream of *T. phagedenis flgE* showed a 30% amino acid sequence identity with ORF8 of the *flaA* locus of *B. subtilis*, which encodes polypeptides involved in motility (2), but the function of ORF8 is unknown. ORF3, located downstream of *flgE*, showed a 33% amino acid sequence identity with MotA of *B. subtilis* and 19% identity with *E. coli* MotA, which are polypeptides important for enabling flagellar rotation. Therefore, we propose that the *T. phagedenis* genes are organized in the order ORF1, *flgE*, and *motA*. In *B. subtilis*, the gene organization at the *flaA* locus consists of ORFs 1 to 7 (Fli homologs) followed in order by ORF8, *flgG*, *fliL*, and *fliM* homologs. In contrast, *S. typhimurium flgE* is preceded by *flgD*, which is important for hook assembly, followed by *flgF*, a distal rod protein. *E. coli* and *S. typhimurium motA* are part of another operon located elsewhere on the chromosome. Thus, it is clear that despite the conserved amino acid sequences in the motility polypeptides of various bacteria, there is a unique gene organization present in *T. phagedenis* and additional DNA sequencing will be useful in determining whether additional motility genes are present at this locus.

On the basis of the clustering of many bacterial motility genes into functional operons (27), we expect that identification of additional genes flanking *flgE* will reveal a family of motility-related genes. Identification and characterization of these genes and their regulation will help to determine the structural and regulatory genes involved in the motility of spirochetes. The availability of these cloned genes and development of well-characterized motility mutants of *T. phagedenis* may enable one to dissect the mechanisms involved in spirochete motility and pathogenesis.

ACKNOWLEDGMENTS

We thank N. Charon, K. Derbyshire, L. Parsons, and M. Shayegani for their critical reviews of the manuscript; R. Biega, K. Cronin, C. Csiza, and R. Vinson for technical assistance; and K. Wicher for donation of the *T. pallidum* cells.

This work was supported in part by National Institutes of Health grant AI34354 and the New York State Department of Health.

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