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Isolation and Characterization of *Campylobacter* Flagellins

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Sequential acid pH dissociation, differential ultracentrifugation, and neutral pH reassociation were used to partially purify serotypically distinct flagella from three strains of *Campylobacter jejuni* and the two antigenic phases of flagella of *Campylobacter coli* VC167. Each *C. jejuni* flagellin and *C. coli* VC167 antigenic phase 1 flagellin were purified to homogeneity by reverse-phase high-performance liquid chromatography with a C8 Spheri-10 column. *C. coli* VC167 antigenic phase 2 was purified to homogeneity by ion-exchange chromatography with a Mono-Q column. Amino acid compositional analysis put the *C. jejuni* flagellin molecular weight in the range 63,200 to 63,800 and the *C. coli* antigenic phase 1 and 2 flagellins at 61,500 and 59,500, respectively. The amino acid compositions of the *C. jejuni* flagellins were similar to each other and to the *C. coli* VC167 antigenic phase 1 and phase 2 flagellins. One-dimensional peptide mapping of the *C. jejuni* flagellins by partial digestion with trypsin or chymotrypsin confirmed the structural similarities of the *C. jejuni* flagellins and the *C. coli* VC167 antigenic phase 1 flagellin and showed that *C. coli* VC167 antigenic phase 2 flagellin was structurally distinct from the phase 1 flagellin. The antigenic phase 2 flagellin was especially sensitive to digestion by chymotrypsin. Amino-terminal sequence analysis showed that the 20 N-terminal amino acids of the *Campylobacter* flagellins were highly conserved. The *Campylobacter* flagellins also shared limited sequence homology with the N-terminal sequences reported for *Salmonella* and *Bacillus* flagellins.

Flagella are best known for their role in bacterial motility (15). In certain pathogenic bacteria, flagella also appear to play an important role in the interaction of the parasite with its animal host. This is well illustrated in *Pseudomonas aeruginosa*; studies with isogenic mutants deficient in chemotaxis and motility indicate that nonflagellated derivatives are avirulent in the burned-mouse infection model (28; D. Drake, G. Shaw, and T. C. Montie, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, B155, p. 43). Flagella also appear to be important virulence factors in certain enteropathogenic bacteria. In the case of *Vibrio cholerae*, the polar flagellum enhances virulence by facilitating the initial colonization of the small intestine, imparting the motility necessary for mucus colonization, and perhaps functioning as an adhesin to attach the vibrio to target gastrointestinal cells (1, 10). In the case of *Salmonella typhimurium*, studies in mice infected either orally or parenterally have established that flagella are virulence factors independent of chemotaxis or motility, apparently either protecting *S. typhimurium* from the intracellular killing mechanisms of murine macrophages or enhancing the ability of *S. typhimurium* to multiply within the murine macrophages (5, 37).

The genus *Campylobacter* is comprised of gram-negative, polar flagellated spiral bacteria which form a previously undescribed eubacterial group which is related to the other major groups only by very deep branching (32). Over the past few years the thermophilic campylobacters, especially *Campylobacter jejuni* and *Campylobacter coli*, have become recognized as important bacterial enteric pathogens (3, 6, 33, 35). Indeed recent surveys consistently rank *Campylobacter* as one of the three most important genera of bacterial enteric pathogens (6, 35). These organisms initiate disease by colonizing the mucus lining the gastrointestinal tract (21), and motility appears to play a major role in this mucus colonization (21, 29, 30). Flagella must therefore be regarded as virulence factors of campylobacters, since they are essential for this motility and they are also the immunodominant protein antigen during infection in humans (38).

The immunogenicity of *Campylobacter* flagella also makes them important antigens in serotyping schemes based on heat-labile antigens, including the widely used serotyping scheme of Lior et al. (24, 25). The antigenic diversity of *Campylobacter* flagella is well documented (2, 23, 38, 39), and in several of the LIO serotypes the use of nonflagellated derivatives has shown that the flagella can carry the serotype-specific determinant on their surface (38, 39). However, in other cases the surface-exposed *Campylobacter* flagellum epitopes, although they still display strain-specific serospecificity, do not appear to be the epitopes responsible for LIO serogroup specificity (11). Western blotting (immunoblotting) experiments have also shown that *Campylobacter* flagella possess cross-reactive non-surface-exposed epitopes (22, 23, 27), which are shared with other spiral organisms that colonize gastrointestinal mucus but which are not shared with the flagellins in members of the family *Enterobacteriaceae* (20). In addition, both phase and antigenic variation of *Campylobacter* flagella have recently been demonstrated (4, 11), adding to the antigenic complexity conferred upon the campylobacters by their polar flagellum filaments.

As an essential step to understanding the expression and antigenic structure of *Campylobacter* flagella in molecular terms, we have isolated the flagellins from three strains of *C. jejuni* which produce antigenically distinct flagella and from a strain of *C. coli* which produces two antigenically distinct phases of flagella. Here we report on the purification procedure which allows subsequent N-terminal sequence analysis to be successfully performed, and we compare the *Campylobacter* flagellins with respect to their amino acid compositions, N-terminal amino acid sequences, and chymotryptic and tryptic cleavage maps.

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MATERIALS AND METHODS

Bacterial strains. The bacteria used in this study were *C. jejuni* VC74, VC91, and VC185 and *C. coli* VC167 (H. Lior, National Enteric Reference Centre, Ottawa, Ontario, Canada). Derivatives of *C. coli* VC167 producing either antigenic phase 1 (P1) or antigenic phase 2 (P2) flagella were selected by the antibody-mediated immobilization technique of Harris et al. (11). All *Campylobacter* strains were isolated from human feces. Stock cultures were maintained at −70°C in 15% (vol/vol) glycerol–Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). Cultures were grown at 37°C in anaerobic jars on Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.). An atmosphere containing 5% oxygen and 10% CO_2 was produced with a gas-generating kit for campylobacters (Oxoid Ltd., Basingstoke, United Kingdom).

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a mini-slab gel apparatus (Hoeffer Scientific Instruments, San Francisco, Calif.) by the method of Laemmli (19). Proteins or peptides solubilized in sample buffer were stacked in 4.5% acrylamide (100 V, constant voltage) and separated with 7.5, 12.5, or 15% acrylamide (200 V, constant voltage). Protein was stained with Coomassie blue or by the silver staining procedure of Wray et al. (40).

Purification of flagellin. Flagella were isolated from 24-h cultures by homogenization, and flagellin was partially purified by the sequential acid pH dissociation, ultracentrifugation, and neutral pH association procedure previously described (22, 23). A Beckman high-performance liquid chromatography (HPLC) system with a Brownlee RP8-Spheri 10 analytical column (4.6 by 250 mm) was used to separate flagellin from minor contaminants. Chromatography conditions were as follows. Solvent A was 0.1% trifluoroacetic acid in water, and solvent B was 100% acetonitrile. The flow rate was 1 ml/min, and chromatography was performed at room temperature. Elution of flagellin was achieved by using a discontinuous gradient of 0 to 40% solvent B in the first 5 min, followed by an increase from 40 to 60% solvent B over 20 min and finally to 100% solvent B in 10 min (Fig. 1).

To purify *C. coli* VC167 phase 2 flagellin, ion-exchange chromatography was also used. The Beckman HPLC system previously employed was used in this separation, along with a Mono-Q column (Pharmacia Fine Chemicals, Uppsala, Sweden). The flagellin sample was centrifuged at 12,000 × *g* to remove insoluble material. A 500-μl sample of the supernatant containing approximately 250 μg of protein was injected into the HPLC apparatus and eluted as follows. The column was run isocratically for 10 min in 10 mM Tris (pH 4.0), and a gradient from 0 to 0.1 M NaCl in 10 mM Tris (pH 4.0) was then applied to the column for 5 min. The column was then run isocratically until the protein was eluted. The absorbance of the effluent from the RP8-Spheri 10 and Mono-Q columns was monitored at 230 and 280 nm. Importantly, lyophilization to reduce the volume of samples and freezing of flagellin samples were avoided at all times during the purification, since these procedures caused solubility problems with *Campylobacter* flagellins.

Amino acid composition and sequence analysis. Flagellin (3 nmol) was hydrolyzed for 18 h in 6 N HCl and dried by lyophilization. Amino acid composition was determined on a Beckman 119CL amino acid analyzer. The method used was as described by the manufacturer for a 90-min, single-column procedure. Cysteine was analyzed as cysteic acid, and tryptophane was determined by the method of Penke et al. (31). Sequencing was performed on a Applied Biosystems 470A gas-phase sequenator. The initial yield from 400 pmol was 270 pmol.

Peptide mapping. For trypsin digestion, purified flagellin (10 μg) was dissolved in 10 mM Tris (pH 7.8) and incubated for 1 h at 25°C with 1 μg of trypsin. Digestion was stopped by addition of 2 volumes of SDS-PAGE solubilization buffer to samples, which were then heated to 100°C for 5 min. Tryptic peptides were then separated on a 12.5% acrylamide gel and by HPLC with a reverse-phase C8 Spheri-10 column with the same flow rate and solvents A and B as described above. The chromatography conditions employed were a discontinuous gradient of 0 to 50% solvent B in the first 60 min, followed by an increase from 50 to 100% solvent B over 5 min. Chymotryptic digestion of flagellin was performed by the method of Cleveland et al. (7). Flagellin was dissolved in sample buffer (0.125 M Tris [pH 6.8], 0.5% SDS, 10% glycerol) and heated to 100°C for 2 min. Digestion was then carried out at 37°C for 30 min after the addition of 2 μg of chymotrypsin. SDS was then added to a final concentration of 10%, and proteolysis was stopped by boiling the samples for 5 min. Chymotryptic peptides were then separated on a 15% acrylamide gel.

RESULTS AND DISCUSSION

Previous studies have employed the acid pH disaggregation-differential ultracentrifugation-neutral pH reassociation procedure to isolate *Campylobacter* flagellins (22, 23). However the purity provided by this relatively simple procedure is not sufficient to allow for successful N-terminal amino acid sequence determination. To provide flagellin of sufficiently high purity for N-terminal amino acid analysis and amino acid compositional analysis, an additional purification step

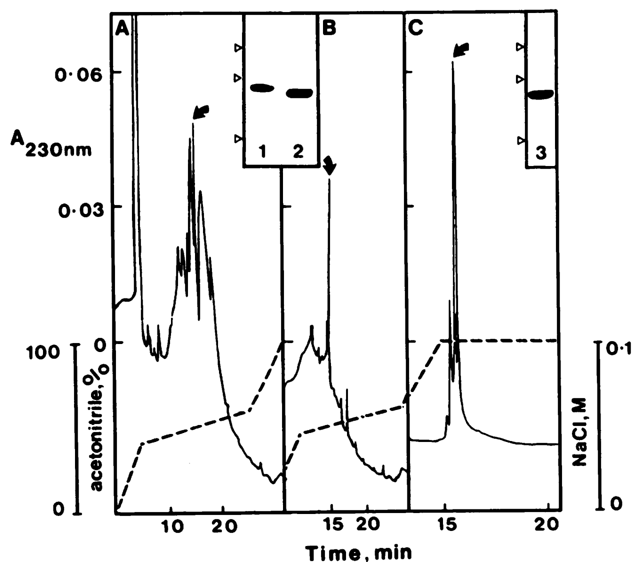

FIG. 1. Purification of *C. coli* VC167 antigenic P1 and P2 flagellins. HPLC elution profile of P1 (A) and P2 (B) flagellins, and Mono-Q ion-exchange elution profile of P2 flagellin (C). Solid arrows indicate flagellin peaks. Insets show SDS-PAGE (7.5% polyacrylamide) of M_r 61,500 P1 flagellin-containing peak fraction (lane 1), and M_r 59,500 P2 flagellin-containing peak fractions (lanes 2 and 3) stained by Coomassie blue. Arrows on left indicate M_rs 66,200 (top), 45,000, and 31,000.

TABLE 1. Characteristics of flagellins

Flagellin source	No. of residues/flagellin subunit																		No. of residues/mol	M_r (×1,000)	% Hydropholoic residues
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Cys	Try			
C. coli VC167																					
P1	86	40	73	53	0	68	72	29	14	50	37	8	19	4	27	18	0	0	598	61.5	37
P2	81	38	68	53	0	69	68	29	14	48	36	8	18	4	27	17	0	0	578	59.5	37
C. jejuni																					
VC74	85	43	70	65	0	101	66	32	11	35	40	10	18	2	34	18	0	0	630	63.8	33
VC91	91	47	74	60	0	79	82	29	13	37	41	8	15	0	32	16	0	0	624	63.2	35
VC185	82	46	70	50	0	91	74	32	11	49	48	10	16	1	41	12	0	0	633	63.8	36
81116[a]	90	55	71	48	0	71	68	34	11	48	39	9	19	1	33	16	0	0	613	62.9	36
S. typhimurium[b]	79	55	40	47	4	39	61	31	3	27	40	13	6	1	29	14	0	0	489	51.2	35
B. subtilis 168[c]	49	18	24	42	2	19	40	14	8	24	29	1	5	4	16	15	0	0	310	33.3	39

[a] Strain 81116 data from Newell et al. (30).
[b] *S. typhimurium* data from Joys (18), calculated based on the DNA sequence of the *H-1[i]* gene.
[c] *B. subtilis* data from Delange et al. (8).

was required. Antigenically distinct flagellins partially purified by acid disaggregation from *C. jejuni* VC74, VC91, and VC185 and *C. coli* VC167 antigenic P1 and P2 variants was subjected to HPLC with a reverse-phase C8 Spheri-10 column and a discontinuous gradient of acetonitrile. Typical elution profiles with this gradient when applied to the antigenically distinct P1 and P2 flagellins of *C. coli* VC167 are shown in Fig. 1A and B. SDS-PAGE analysis of the eluted HPLC fractions showed that the flagellins from the two flagellum antigenic phase variants of *C. coli* VC 167 and the antigenically distinct flagellins from all three strains of *C. jejuni* eluted as a single peak at 50% acetonitrile. All flagellins were assessed as pure by the presence of a single band when SDS-PAGE gels were stained by either Coomassie blue or silver. The three *C. jejuni* flagellins exhibited an M_r of 63,500 by SDS-PAGE (7.5% polyacrylamide; data not shown). The single polypeptide bands of the HPLC-purified P1 and P2 flagellins of *C. coli* VC167 in SDS-PAGE (7.5% polyacrylamide) gels are shown in insets in Fig. 1A and B, with an apparent M_r for the P1 flagellin of 61,500 and an apparent M_r for the P2 flagellin of 59,500.

Initial N-terminal amino acid sequencing of the HPLC-purified *C. coli* P2 flagellin indicated that the protein had not been purified to homogeneity, even though SDS-PAGE had shown only a single polypeptide band. This flagellin was therefore subjected to ion-exchange chromatography with a Mono-Q column. With this ion-exchange column, the P2 flagellin was eluted with 0.1 M NaCl. The separation profile is shown in Fig. 1C, and the purity of the flagellin-containing fraction was indicated by the presence of a single polypeptide of apparent M_r 59,500 in SDS-PAGE gels stained by Coomassie blue (inset, Fig. 1C). When this material was subjected to N-terminal amino acid sequence analysis, purity to homogeneity was confirmed.

The amino acid compositions of the purified *Campylobacter* flagellins are shown in Table 1. The predicted M_rs of the *C. coli* VC167 P1 and P2 flagellins were 61,500 and 59,500, respectively, whereas the predicted M_rs for the three *C. jejuni* flagellins purified in this study ranged from 63,200 to 63,800. The calculated total number of amino acid residues per *Campylobacter* flagellin molecule was between 578 (*C. coli* VC167 P2) and 633 (*C. jejuni* VC185), compared with the 489 residues of *S. typhimurium* flagellin (17) and the 310 residues of *Bacillus subtilis* flagellin (8). When considered on the basis of the various amino acid classes, the amino acid compositions of the *Campylobacter* flagellins were similar to each other; although the *Campylobacter* flagellins were higher in M_r than the flagellins of *S. typhimurium* and *B. subtilis*, overall similarities with these two flagellin compositions were obvious (8, 17, 30). For example, the 33 to 37% hydrophobic amino acid content of the *Campylobacter* flagellins was comparable to the 38 to 39% hydrophobic content calculated for *Salmonella* and *Bacillus* flagellins, and cysteine and tryptophane were absent from all flagellins. Proline was also absent from all *Campylobacter* flagellins, and histidine content was minimal. However the 1.3 to 1.7% tyrosine content of the *Campylobacter* flagellins was significantly higher than the 0.3% tyrosine content of *B. subtilis* flagellin, and the 1.8 to 2.7% methionine content of the *Campylobacter* flagellins was significantly higher than the 0.6% methionine content of *S. typhimurium* flagellin. This relatively high methionine content allowed the *C. jejuni* flagellins to be hydrolytically cleaved by CNBr in a previous study, producing peptides with a range of M_rs, including major peptides of M_r 22,400 and 11,000 that carried antigenically cross-reactive, non-surface-exposed linear epitopes (23). The M_r 22,400 peptide also carried linear epitopes for two monoclonal antibodies; one epitope was strain specific, whereas the other was shared by some but not all *C. jejuni* flagellins tested. Unfortunately, CNBr cleavage produced extensive damage to serospecific flagellin epitopes, but Western blot experiments did provide evidence that an M_r 4,000 CNBr fragment carried linear surface-exposed epitopes which contributed to flagellar filament serospecificity.

Although the overall amino acid compositions were similar, especially in the case of the P1 and P2 flagellins of *C. Coli* VC167, individual amino acid differences were seen among other antigenically distinct *Campylobacter* flagellins. This has also been demonstrated to be the case with antigenically distinct *Salmonella* flagellins (26). These differences were not, however, reflected in the 20-residue N-terminal amino acid sequence of the *Campylobacter* flagellins sequenced in this study (Table 2). Indeed, the *Campylobacter* flagellins displayed strong conservation in this region of the flagellin molecule. The sequences of the *C. jejuni* flagellins were identical for the first 20 residues. The *C. coli* sequences differed from the *C. jejuni* sequences by the substitution of serine for alanine at residue 17, a change which can result from a single base substitution.

N-terminal conservation has also been shown in the case

TABLE 2. N-terminal amino acid sequence of *Campylobacter* flagellin and flagellins of other bacteria

Flagellin source (reference)	Residues[a]			
C. jejuni				
VC91	G F R I N T N V A A	L N A K A N A D L N	S K S L D A S L S S	L S S G L G I N
VC74	G · · · · · · · · ·	· · · · · · · · · ·		
VC185	G · · · · · · · · ·	· · · · · · · · · ·		
C. coli VC167				
P1	G · · · · · · · · ·	· · · · · · S · · ·	· R A · · Q · · · R	· · · · · X · ·
P2	G · · · · · · · · ·	· · · · · · S · · ·	· R A · · Q · · · R	· · X X X X · ·
S. typhimurium (18, 34, 36)[b]	A Q V · · · · S L S	· L T Q N · L N K S	Q S A · G T A I E R	· · · · · R · ·
B. subtilis (8)	M · · · H · I · ·	· · T L N R L S S ·	N S A S Q K N M E K	· · · · · R · ·
C. crescentus (9)[c]	A L S V · · · Q P ·	· I · L Q · L N R T	N D D M Q · V Q T R	I N T · E A · S

[a] Amino acid residues are designated by the single-letter nomenclature. Symbols: ·, residue homologous with *C. jejuni* VC91 sequence; X, not identified.
[b] Sequence derived from DNA sequence of *H-1[i]* gene.
[c] Sequence derived from DNA sequence of the M_r 28,500 flagellin gene.

of *Salmonella* flagellins (17, 36). Using nucleotide sequencing, Wei and Joys (36) showed that the first 100 amino acids in the sequences of P1 flagellin antigens *a*, *c*, *d*, and *i* were identical. The N-terminal sequence of the *Campylobacter* flagellins also showed homology with the *S. typhimurium* sequence and with the *B. subtilus* sequence. In the case of the *B. subtilus* flagellin, sequence alignment showed that 8 of the first 11 residues were identical to the *Campylobacter* sequence, with the conservative replacement of isoleucine for valine at residue 8 of the *Campylobacter* sequence possibly arising as a result of a single nucleotide coding difference. In the case of *S. typhimurium* and *Caulobacter crescentus* flagellins, sequence alignment showed that 5 of the first 11 residues were identical to the *Campylobacter* sequence. Replacement of the N-terminal glycine of *Campylobacter* flagellin by alanine in the *Salmonella* and *Caulobacter* sequence also represents a conservative change, as does the replacement of isoleucine with valine at residue 4 in *Caulobacter* species. For *C. jejuni* VC91 flagellin, sequence data were obtained to residue 38. Sequence alignment revealed a second length of pronounced sequence homology with the *B. subtilus* and *S. typhimurium* flagellins between residues 31 and 38 of the *Campylobacter* sequence, with 7 of the 8 residues being identical. Wei and Joys (36) have suggested that the structural conservation at this N-terminal end of the flagellin molecule may reflect the involvement of this area of the gene in regulatory functions. However, Homma et al. (12) have recently suggested that in the case of *S. typhimurium* the structural and genetic data suggest that the flagellin subunit may, roughly speaking, be a hairpin, with both the N terminus and the C terminus toward the interior of the native flagellum filament and implying a structural role in filament assembly. The Homma et al. (12) model has the central portion of the flagellin sequence forming a potential surface-exposed antigenic domain; this is in keeping with the demonstration by Joys (17) that a determinant involved in the *i* H-antigen serospecificity is located in the region of highest sequence diversity, between residues 182 and 299 of the 493-residue *Salmonella* flagellin molecule.

To further examine the structural relatedness of the antigenically distinct *Campylobacter* flagellins, limited tryptic and chymotryptic digests were prepared and analyzed by SDS-PAGE (Fig. 2A and B). The electrophoretic profiles of the tryptic peptide of *C. jejuni* flagellins displayed an overall similarity, with predominant peptides of M_r 26,000 and 25,000 and a shared minor peptide of approximate M_r 10,000. Both VC74 and VC185 flagellin digests also shared a peptide of M_r 13,000, whereas in VC91 flagellin a peptide of slightly higher M_r, 14,000, was present. The electrophoretic profiles of the *C. coli* VC167 P1 and P2 flagellins differed from that of the *C. jejuni* flagellins. The largest peptides of the *C. coli* flagellins had apparent M_rs of 24,500 and 23,000, and peptides of M_r 15,000 and 9,000 were also present in the tryptic digests. This M_r 9,000 peptide predominated in the tryptic digests of the P1 flagellin, whereas the M_r 23,000 peptide was predominant in tryptic digests of the P2 flagellin. A minor peptide of M_r 19,000 was also contained in the digest of P1 flagellin. The elution profiles of HPLC analysis of the tryptic digests of the two *C. coli* flagellins allowed many additional minor peptides to be demonstrated (Fig. 2C), in numbers consistent with the lysine and arginine contents determined in the amino acid composition analysis. Although peptide differences between the two flagellins were shown by this sensitive technique, the great majority of peptides in the two elution profiles molecules appeared to be the same, further emphasizing the overall structural relatedness of the P1 and P2 flagellins of *C. coli* VC167.

Limited chymotryptic digestion showed the structural

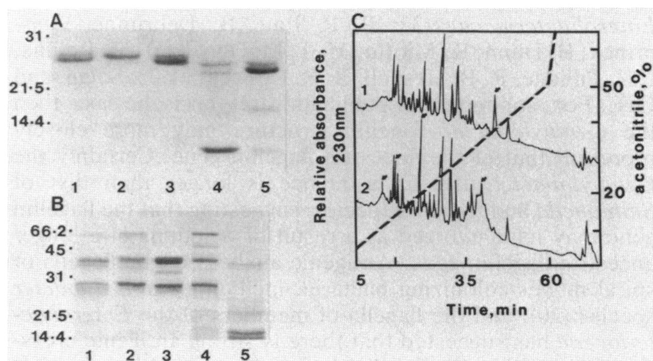

FIG. 2. SDS-PAGE of partial tryptic digests (A, 12.5% acrylamide), and partial chymotryptic digests (B, 15% acrylamide) of purified flagellins from *C. jejuni* VC74 (lane 1), VC91 (lane 2), and VC185 (lane 3) and *C. coli* VC167 antigenic P1 (lane 4) and P2 (lane 5) flagellins stained by Coomassie blue. M_rs (x1,000) are indicated on the left. (C) HPLC elution profile of partial trypsin digests of *C. coli* VC167 antigenic P1 (tracing 1) and P2 (tracing 2) flagellins with major peptide differences indicated by arrows.

relatedness of the *C. jejuni* flagellins with *C. coli* VC167 P1 flagellin (Fig. 2B), each with major peptides of M_r 42,000 and 30,000. A minor peptide of M_r 38,000 was present in digests of VC74, VC91, and VC167P1 flagellins, whereas in VC185 flagellin a minor peptide band of M_r 35,000 was present. *C. coli* VC167 P2 flagellin was much more sensitive to chymotryptic digestion than the VC167 P1 flagellin, however. Under identical cleavage conditions, less undigested flagellin was always seen in P2 digests compared with P1 digests in SDS-PAGE. However, cleavage of this P2 flagellin with chymotrypsin did yield peptides of M_r 40,000 and 28,000 which appear to be equivalent to the M_r 42,000 and 30,000 peptides of the other *Campylobacter* flagellins when the lower M_r of the P2 flagellin is taken into account. In the case of digests of P2 flagellin, both peptides were present at reduced levels. Another peptide which appeared to be shared by both *C. coli* flagellins had an M_r of 45,000 in P1 digests and an M_r of 44,000 in P2 digests, and both flagellins displayed M_r 22,500 peptides. The major chymotryptic peptide of P2 flagellin had an M_r 14,000 peptide. In addition, chymotryptic digests of P2 flagellin contained a significant amount of material close to the dye front which stained with Coomassie blue and presumably represented small peptide fragments unresolved by the gel system. Since the P2 flagellin does not have higher numbers of tyrosine and phenylalanine residues than the P1 flagellin, this increased number of small peptides in the chymotryptic digests of P2 flagellin perhaps reflects a different positioning of some of the aromatic residues in the P2 flagellin sequence. Such a change in amino acid sequence could contribute to the antigenic differences shown by the P1 and P2 flagellins and the different antigenic phases of flagellum filaments containing these flagellins (11). In this regard, the overall structural similarity of the *Campylobacter* flagellins as indicated by their major tryptic and chymotryptic peptides is consistent with immunochemical data, provided by a number of investigators using a variety of antisera, that show strong antigenic cross-reactivity between non-surface-exposed flagellin epitopes (2, 11, 20–22, 26, 38).

The strong conservation of the N-terminal region of the *Campylobacter* flagellin and its relatedness to the other flagellins are of considerable interest. Recent phylogenetic analysis by partial 16S rRNA sequencing has indicated that the campylobacters may be an older group of organisms in evolutionary terms than, for example, members of the family *Enterobacteriaceae* (32; P. P. Lau, B. Debrunner-Vossbrinck, B. Dunn, K. Miotto, M. T. McDonall, D. M. Rollins, C. J. Pillidge, R. B. Hespell, R. R. Colwell, M. L. Sogin, and G. C. Fox, submitted for publication). If this is the case, then the *Campylobacter* flagellin structure may more closely represent that of the ancestral flagellin gene. Certainly the *Campylobacter* flagellin sequence is longer than that of *Salmonella* and *Bacillus* species, suggesting that the flagellin gene may have evolved as a result of deletions of a longer ancestral flagellin gene. Antigenic analysis of the flagella of spiral mucus-colonizing bacteria, including *Campylobacter* species (20), and the flagella of members of the *Enterobacteriaceae* has suggested that there is strong antigenic cross-reactivity within the flagellins of each group of bacteria but little if any antigenic cross-reactivity between the flagellins of each group (13, 14, 20). This suggests that the evolution of flagellin may have provided several structural groups of flagellins, just as appears to have occurred in the case of the other filamentous protein appendage, the pilus (16). Further information on these possibilities, together with an explanation of the antigenic similarities and differences of different *Campylobacter* flagellins, should be provided when the nucleotide sequences of *Campylobacter* flagellin genes are obtained.

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LITERATURE CITED

1. **Attridge, S. R., and D. Rowley.** 1983. The role of flagellum in the adherence of *Vibrio cholerae*. J. Infect. Dis. **147**:864–872.
2. **Blaser, M. J., J. A. Hopkins, G. I. Perez-Perez, H. J. Cody, and D. G. Newell.** 1986. Antigenicity of *Campylobacter jejuni* flagella. Infect. Immun. **53**:47–52.
3. **Butzler, J. P., and M. B. Skirrow.** 1979. *Campylobacter* enteritis. Clin. Gastroenterol. **8**:737–765.
4. **Caldwell, M. B., P. Guerry, E. C. Lee, J. P. Burans, and R. I. Walker.** 1985. Reversible expression of flagella in *Campylobacter jejuni*. Infect. Immun. **50**:941–943.
5. **Carsiotis, M., D. L. Weinstein, H. Karch, I. A. Holder, and A. D. O'Brien.** 1984. Flagella of *Salmonella typhimurium* are a virulence factor in infected C57BL/6J mice. Infect. Immun. **46**:814–818.
6. **Chowdhury, M. N. H.** 1984. *Campylobacter jejuni* enteritis: a review. Trop. Geogr. Med. **36**:215–222.
7. **Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102–1106.
8. **Delange, R. J., J. Y. Chang, J. H. Shaper, R. J. Martinez, S. K. Komatsu, and A. N. Glazer.** 1973. On the amino-acid sequence of flagellin from *Bacillus subtilis* 168: comparison with other bacterial flagellins. Proc. Natl. Acad. Sci. USA **70**:3428–3431.
9. **Gill, P. R., and N. Agabian.** 1983. The nucleotide sequence of the Mr = 28,500 flagellin gene of *Caulobacter crescentus*. J. Biol. Chem. **258**:7395–7401.
10. **Guentzel, M. N., and L. J. Berry.** 1975. Motility as a virulence factor for *Vibrio cholerae*. Infect. Immun. **11**:890–897.
11. **Harris, L. A., S. M. Logan, P. Guerry, and T. S. Trust.** 1987. Antigenic variation of *Campylobacter* flagella. J. Bacteriol. **169**:5066–5071.
12. **Homma, M., H. Fujita, S. Yamaguchi, and T. Iino.** 1987. Regions of *Salmonella typhimurium* flagellin essential for its polymerization and excretion. J. Bacteriol. **169**:291–296.
13. **Ibrahim, G. F., G. H. Fleet, M. J. Lyons, and R. A. Walker.** 1985. Immunological relationship between *Salmonella* flagella and their potential application for Salmonellae detection by immunoassay. Med. Microbiol. Immunol. **174**:87–99.
14. **Ibrahim, G. F., G. H. Fleet, M. J. Lyons, and R. A. Walker.** 1985. Immunological relationship between *Salmonella* flagellins and between these and flagellins from other species of enterobacteriaceae. Med. Microbiol. Immunol. **174**:101–103.
15. **Iino, T.** 1977. Genetics of structure and function of bacterial flagella. Annu. Rev. Genet. **11**:161–182.
16. **Jones, G. W., and R. E. Isaacson.** 1984. Proteinaceous adhesins and their receptors. Crit. Rev. Microbiol. **10**:229–260.
17. **Joys, T. M.** 1976. Identification of an antibody binding site in the phase-1 flagella protein of *Salmonella typhimurium*. Microbios **15**:221–228.
18. **Joys, T. M.** 1986. The covalent structure of the phase-1 flagellar filament of *Salmonella typhimurium* and its comparison with other flagellins. J. Biol. Chem. **260**:15758–15761.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Lee, A., S. M. Logan, and T. J. Trust.** 1987. Demonstration of a flagella antigen shared by a diverse group of spiral-shaped bacteria that colonize intestinal mucus. Infect. Immun. **55**:

828–831.

21. **Lee, A., J. L. O'Rourke, P. J. Barrington, and T. J. Trust.** 1986. Mucus colonization by *Campylobacter jejuni*: a mouse cecal model. Infect. Immun. **51**:536–546.
22. **Logan, S. M., and T. J. Trust.** 1983. Molecular identification of surface protein antigens of *Campylobacter jejuni*. Infect. Immun. **42**:675–682.
23. **Logan, S. M., and T. J. Trust.** 1986. Location of epitopes on *Campylobacter jejuni* flagella. J. Bacteriol. **168**:739–745.
24. **Lior, H., and D. L. Woodward.** 1985. Serogrouping and biotyping of *Campylobacter* an update, p. 217–218. *In* A. D. Pearson, M. B. Skirrow, H. Lior, and B. Rowe (ed.), Campylobacter III. Public Health Laboratory Service, London.
25. **Lior, H., D. L. Woodward, J. A. Edgar, L. J. Laroche, and P. Gill.** 1983. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. J. Clin. Microbiol. **15**:761–768.
26. **McDonough, M. W.** 1965. Amino acid composition of antigenically distinct *Salmonella* flagellar proteins. J. Mol. Biol. **12**: 342–355.
27. **Mills, S. D., W. C. Bradbury, and J. L. Penner.** 1986. Isolation and characterization of a common antigen in *Campylobacter jejuni* and *Campylobacter coli*. Infect. Immun. **24**:69–75.
28. **Montie, T. C., D. Doyle, R. C. Craven, and I. A. Holder.** 1982. Loss of virulence associated with absence of flagellum in an isogenic mutant of *Pseudomonas aeruginosa* in the burned mouse model. Infect. Immun. **38**:1296–1298.
29. **Morooka, T., A. Umeda, and K. Amako.** 1985. Motility as an intestinal colonization factor for *Campylobacter jejuni*. J. Gen. Microbiol. **131**:1973–1980.
30. **Newell, D. G., H. McBride, and J. M. Dolby.** 1985. Investigations on the role of flagella in the colonization of infant mice with *Campylobacter jejuni* and attachment of *Campylobacter jejuni* to human epithelial cell lines. J. Hyg. **95**:217–227.
31. **Penke, B., R. Ternezi, and K. Kovass.** 1974. A new acid hydrolysis method for determining tryptophan in peptides and proteins. Anal. Biochem. **60**:45–50.
32. **Romaniuk, P. J., B. Zoltowska, T. J. Trust, D. J. Lane, N. R. Pace, and D. A. Stahl.** 1987. *Campylobacter pylori*: the spiral bacterium associated with human gastritis is not a true campylobacter. J. Bacteriol. **170**:2137–2141.
33. **Skirrow, M. B.** 1977. *Campylobacter* enteritis: a "new" disease. Br. Med. J. **2**:9–11.
34. **Szekely, E., and M. Simon.** 1983. DNA sequence adjacent to flagellar genes and evolution of flagellar-phase variation. J. Bacteriol. **155**:74–81.
35. **Walker, R. I., M. B. Caldwell, E. C. Lee, P. Guerry, T. J. Trust, and G. M. Ruiz-Palacios.** 1986. Pathophysiology of *Campylobacter* enteritis. Microbiol. Rev. **50**:81–94.
36. **Wei, L.-N., and T. M. Joys.** 1985. Covalent structure of three phase-1 flagellar filament proteins of *Salmonella*. J. Mol. Biol. **186**:791–783.
37. **Weinstein, D. L., M. Carsiotis, C. R. Lissner, and A. D. O'Brien.** 1984. Flagella help *Salmonella typhimurium* survive within murine macrophages. Infect. Immun. **46**:819–825.
38. **Wenman, W. M., J. Chai. T. J. Louie, C. Goudreau, H. Lior, D. G. Newell, A. Pearson, and D. E. Taylor.** 1985. Antigenic analysis of *Campylobacter* flagellar protein and other proteins. J. Clin. Microbiol. **21**:108–112.
39. **Wenman, W. M., D. E. Taylor, and H. Lior.** 1985. The flagella protein determines *Campylobacter* heat-labile serotype, p. 212. *In* A. D. Pearson, M. B. Skirrow, H. Lior, and B. Rowe (ed.), Campylobacter III. Public Health Laboratory Service, London.
40. **Wray, W., T. Boulikas, V. P. Wray, and R. E. W. Hancock.** 1981. Silver staining in polyacrylamide gels. Anal. Biochem. **118**:197–203.