Isolation and Characterization of a Common Antigen in *Campylobacter jejuni* and *Campylobacter coli*

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Flagellin protein was isolated and purified from two serotype reference strains of *Campylobacter jejuni*, Pen 1 and Pen 3. Each preparation was shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to consist of a diffuse band with a molecular mass of approximately 62 kilodaltons. Antisera were prepared against flagellin from Pen 1, and specific antibody was isolated by affinity chromatography with flagellin protein covalently bound to cyanogen bromide-activated Sepharose. The high-affinity antibody was used to immune blot purified flagellin from Pen 1 and Pen 3, as well as whole-cell preparations and acid-glycine extracts from the 60 reference strains of the thermostable antigen serotyping system. From each of the 60 strains, a protein with a molecular mass of approximately 62 kilodaltons was identified which shared a common antigenic determinant. When the affinity-purified antibody was used in a coagglutination assay, washed whole cells were not agglutinable unless they had been pretreated with an acid buffer (glycine-hydrochloride [pH 2.0]). This indicated that the antigenic determinant common to strains of both *C. jejuni* and *Campylobacter coli* may not be exposed in the native state.

At present, there are several important areas of research in the *Campylobacter* field. Among these are biochemical studies focusing on the components responsible for the heterogeneity of thermolabile and thermostable antigens used as markers in serotyping schemes and serological studies involving the identification of common antigens that may be used in diagnostic tests to determine the presence of organisms in stool samples and the presence of antibodies in the serum of infected patients.

In recent investigations of the antigenic components of the thermostable antigen serotyping system, we showed that for at least five serogroups, the molecules involved are lipopolysaccharides (13). Other groups have investigated the thermolabile antigen serotyping systems for the same purpose. Hébert et al. (5) have used fluorescence-labeled antibody to implicate both flagella and cell surface antigens as determinants in their serotyping system. Another thermolabile antigen serotyping system is that of Lior et al. (7), and for at least three of the serogroups, the loss of flagella in aflagellated mutants or variants rendered the organism nonagglutinable in typing antisera (W. H. Wenman, D. E. Taylor, and H. Lior, Third International Workshop on Campylobacter Infections, abstract no. 128, 1985), suggesting that flagella are antigenically diverse. A 92-kilodalton (kDa) protein has also been implicated as a contributing factor in the serological heterogeneity of the thermolabile antigen scheme (9; Wenman et al., Third International Workshop on Campylobacter Infections).

Although these studies indicate the possible antigenic heterogeneity of flagella, other reports have implied that the flagellin was homogeneous. Immune blots with rabbit or human immune sera reacted strongly with the 62-kDa flagellin protein from a number of serologically distinct organisms that included *Campylobacter jejuni*, *C. coli*, *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, and *C. laridis* (2, 9, 12, 14, 21). To further our understanding of these

apparently contradictory results, we have prepared purified flagellins and antisera against them and examined their specificity in immune blot and coagglutination experiments.

MATERIALS AND METHODS

Bacterial strains. The 60 strains of *C. jejuni* and *C. coli* used in this study are the serotype reference strains for the thermostable antigen serotyping system of Penner and coworkers (16, 17).

Stock cultures were maintained at −70°C in 15% glycerol–1% proteose peptone no. 3 (Difco Laboratories, Detroit, Mich.). When required, strains were thawed slowly, plated on Brucella agar (Difco), and incubated for 24 to 48 h in a CO_2 incubator.

Isolation and purification of flagellin. Cells of *C. jejuni* Pen 1 and Pen 3 were grown overnight on Brucella agar, harvested in 10 mM sodium phosphate buffer (pH 7.0), and washed once by centrifugation (8,000 × g, 10 min, 4°C). Flagella were separated from the bacteria by sonication with three short pulses (15 s) followed by centrifugation (10,000 × g, 20 min, 4°C) to pellet whole cells. The supernatant was saved while the pellet was suspended, and the sonication and centrifugation steps were repeated. The two supernatants were pooled and centrifuged at 100,000 × g for 1 h (4°C) to pellet the flagella. The pellet was suspended in distilled water (pH 2.0), placed on ice for 20 min, and centrifuged at 100,000 × g for 1 h (4°C). The supernatant was brought to pH 7.0 by addition of 1 N NaOH and dialyzed overnight against distilled water, after which a precipitate appeared. The dialysate was then concentrated by lyophilization. To remove contaminating low-molecular-weight proteins from the preparation, gel filtration chromatography with Sephadex G-100 (Pharmacia, Uppsala, Sweden) was performed. The sample (1.5 mg of protein in 1.0 ml of 10 mM phosphate buffer [pH 7.0]) was heated at 100°C for 5 min to facilitate disaggregation, immediately applied to the Sephadex column (60 by 2.5 cm), and eluted at a flow rate of 0.5 ml/min. Protein was monitored by optical density at 280 nm, and

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peaks were pooled, concentrated with polyethylene glycol, dialyzed against buffer (as above), and frozen at −20°C.

Glycine extracts and whole-cell protein preparations. Acid-glycine extracts were prepared from the 60 serostrains of the thermostable antigen serotyping system (16, 17) by the method of McCoy et al. (11). Cultures were grown overnight on Brucella agar, harvested in distilled water, and washed twice. The final pellet was suspended in 0.2 M glycine-hydrochloride (pH 2.0) (4 g of cells per 100 ml) for 15 min and centrifuged at 12,000 × g for 15 min. The supernatant was neutralized with 1.0 N NaOH, dialyzed against distilled water, and lyophilized.

Whole-cell preparations consisted of cells which had been cultured on Brucella agar, harvested with distilled water, washed twice, and suspended in solubilizing buffer containing 1% sodium dodecyl sulfate (SDS) and 1.4 M β-mercaptoethanol in 0.05 M Tris hydrochloride buffer (pH 6.8).

Immune sera. Pure flagellin (250 μg) from Pen 1 was emulsified in Freund complete adjuvant and injected intramuscularly into New Zealand White rabbits (ca. 5 kg) at 1, 7, and 21 days. Thereafter, the rabbits were bled weekly, and the serum was stored at −20°C. The rise in antiflagellin antibodies was monitored by a dot blot enzyme immunoassay (Immun-Blot GAR-HRP kit; Bio-Rad Laboratories, Richmond, Calif.). Nitrocellulose disks were placed in the flat bottoms of the wells in a microtiter tray, and 0.5 μg of flagellin was spotted onto each disk. After blocking overnight with 1% gelatin in 10 mM Tris hydrochloride–0.9% NaCl (Tris-saline), serum (1:20 in Tris-saline) was added to the first well, and twofold dilutions were made.

Fractionation of serum. Antiserum against flagellin from Pen 1 was diluted 1:1 (vol/vol) with saturated ammonium sulfate, stirred gently for 1.5 h at room temperature, and centrifuged at 6,000 × g for 5 min (8). The resulting pellet was suspended in 10 mM sodium phosphate buffer (pH 6.8) and dialyzed extensively against the phosphate buffer to remove traces of ammonium sulfate.

Pure immunoglobulin G (IgG) was isolated from this material by anion-exchange chromatography (8). A column (30 by 5 cm) containing 500 ml of DEAE-Sephacel (Pharmacia) was equilibrated with 4 liters of 10 mM sodium phosphate buffer (pH 6.8). The sample (10 ml) was applied to the column and the void volume containing IgG was collected at a flow rate of 0.5 ml/min. A_{280} was monitored, and fractions containing protein were pooled, concentrated with polyethylene glycol, and frozen at −20°C until required. The column was regenerated by washing with 10 mM sodium phosphate buffer (pH 6.8) in 0.5 M NaCl until no A_{280} was observed.

Affinity chromatography. CNBr-activated Sepharose 4B (Pharmacia) was prepared as described in the instructions of the manufacturer. Pure flagellin (1.0 mg in 2 ml of buffer [0.1 M Na_2HCO_3 in 0.5 M NaCl]) was coupled to 1 ml of CNBr-activated Sepharose, washed in the appropriate buffers, and dispensed into a 1.5-ml glass column. The column was equilibrated with 10 mM sodium phosphate buffer (pH 6.8), the sample was applied (60 mg of protein in 2 ml of buffer), and the eluate was collected at a flow rate of 0.2 ml/min until the A_{280} was negligible. Elution of specific antibody was carried out in the reverse direction to that of sample application (8). Two different antibody preparations were obtained (19). A low-affinity antibody was eluted with 0.1 M acetate buffer (pH 4.0), and a high-affinity antibody was eluted with 0.2 M acetate buffer (pH 2.3). As the protein-containing fractions were collected, the pH was

immediately raised by the addition of solid Tris base (Sigma Chemical Co., St. Louis, Mo.). To ensure that there was complete removal of antibody, the void fractions were reapplied to the column, and any remaining antibody was eluted with the low-pH buffers. The column was regenerated immediately after use with 10 mM phosphate buffer (pH 6.8). Protein peaks were pooled, concentrated with polyethylene glycol, dialyzed against buffer, and frozen at −20°C. To monitor increases in specific IgG titer, samples were saved after each step in the purification procedure, diluted to equal protein concentrations, and subjected to the dot blot enzyme immunoassay.

SDS-PAGE and immune blotting. Electrophoresis was conducted with an SDS-polyacrylamide gel electrophoresis (SDS-PAGE) system by the method of Laemmli (6) with a stacking gel of 4% and a separating gel of 9%. Approximately 2 μg of flagellin protein or 30 μg of protein from glycine or whole-cell extracts was applied per lane, and electrophoresis was carried out under a constant current (20 mA). The molecular size markers included lysozyme (14 kDa), soybean trypsin inhibitor (21.5 kDa), carbonic anhydrase (31 kDa), ovalbumin (45 kDa), bovine serum albumin (66.2 kDa), phosphorylase b (92.5 kDa), β-galactosidase (116.25 kDa), and myosin (250 kDa). Gels were stained with Coomassie brilliant blue R dye (CBB; Sigma) and destained in several changes of 20% methanol–10% acetic acid.

Electrophoretic transfers were performed by the technique of Towbin et al. (20). The blot was blocked overnight in 1% gelatin in Tris-saline and then incubated for 1 h in buffer containing high-affinity antibody (1 μg/ml). The remainder of the procedure was as described in the instructions for the immune blotting kit produced by Bio-Rad Laboratories. In this technique, a peroxidase-labeled goat anti-rabbit IgG was used to detect the presence of rabbit antibody specific to flagellin.

Coagglutination assay. Protein A-bearing staphylococcal cells were prepared by the method of Edwards and Hilderbrand (4). A 10% suspension (wt/vol) of staphylococcal cells (500 μl) was sensitized with either preimmune serum (100 μl), whole antiserum (100 μl), high-affinity antibody (2.5 μg of protein), low-affinity antibody (5 μg of protein), or the voided-volume fraction from the affinity column (10 μg of protein) for 3 h at room temperature. The suspension was washed once in phosphate-buffered saline and reconstituted to 1.0%.

The campylobacters used in the coagglutination assays (Pen 1 to Pen 12) were harvested in saline (0.8% NaCl) after 24 h of incubation on Brucella agar plates and washed once. The cells were diluted to a final concentration of 10^8 CFU/ml in either glycine-hydrochloride (pH 2.0) or saline (0.8% NaCl), and after 20 min, the acidic solution was neutralized with 1 N NaOH.

For the coagglutination test, 10 μl of the 1.0% staphylococcal preparation was added to 20 μl of whole cells (Pen 1 to Pen 12), 20 μl of acid-treated whole cells, 10 μl of pure flagellin (2.5 μg of protein added per sample), 20 μl of acid-glycine extract (Pen 1 to Pen 12, 20 μg of protein added per sample), or 20 μl of saline (0.8% NaCl). Slides were gently rocked, and the results were scored after 2 min.

Chemical analysis. Protein was analyzed by the technique of Lowry (10) with lysozome as the standard.

RESULTS

Isolation and purification of flagellin. SDS-PAGE and CBB staining of the flagellin preparation isolated from Pen 1
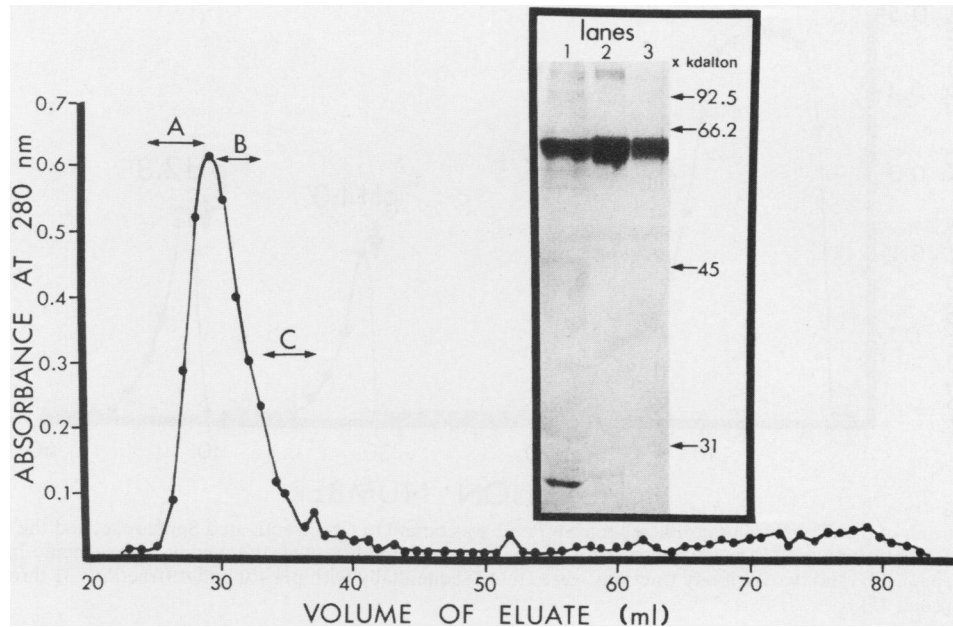
FIG. 1. Chromatograph of Sephadex G-100 gel filtration of flagellin from Pen 1. Fractions were collected, and pools A, B, and C were concentrated and electrophoresed by SDS-PAGE. The CBB-stained gel is shown in the insert. Pools A, B, and C are shown in lanes 1, 2, and 3, respectively.

revealed two bands, a flagellin with a molecular mass of 62 kDa and another smaller protein of 27 kDa. The 27-kDa protein could not be removed by a second acid disaggregation-aggregation step nor by gel filtration on Sephadex G-100 or G-150. However, when the preparation was heated at 100°C for 5 min to facilitate disaggregation and applied immediately to a Sephadex G-100 column, the two proteins could be separated. The elution profile (Fig. 1) shows that only one major protein peak came off the column. However, when separate fractions were pooled (Fig. 1A, B, and C) and electrophoresed (Fig. 1, lanes 1 through 3), it was shown that the first few fractions obtained close to the voided volume (eluted volume/voided volume ratio, 1) consisted of both proteins (Fig. 1, lane 1), while later fractions (eluted volume/voided volume ratio, 1.0 to 1.5) contained only the 62-kDa protein (Fig. 1, lanes 2 and 3). The 27-kDa protein appeared after two voided volumes had passed through the column. By using this protocol, preparations were obtained from Pen 1 and Pen 3, and each was shown by SDS-PAGE and CBB staining to consist of one protein with a molecular mass of 62 kDa (see Fig. 3, lanes 2 and 3). It should be noted that when gels were silver stained, no other bands could be detected, but flagellin did not stain very well.

Fractionation of antisera. Flagellin isolated from Pen 1 was injected into a New Zealand White rabbit, and the development of antiflagellin antibodies was monitored by an enzyme immunoassay. The IgG antibody titers reached over 1:40,000 approximately 4 weeks after the last injection, while the titer of the preimmune serum, obtained before the first rabbit injection, was less than 1:40. When the hyperimmune sera were used in immune blots in an attempt to detect flagellin in whole-cell preparations, many bands, including molecular mass markers, became visible, indicating a large degree of nonspecificity. For this reason, the high-titered antisera were pooled and affinity purified before use in immune blotting.

Throughout the purification procedure, the increase in antiflagellin IgG was monitored in each serum fraction by the

enzyme immunoassay described previously. The results (Table 1) indicate that, after each step in the purification procedure, there was a substantial increase in the antiflagellin antibody titer.

Affinity chromatography with purified flagellin as the substrate was used to select for specific IgG antibody in the voided-volume fraction from the DEAE-Sephacel column. Two antibody fractions were obtained by sequential elution at pH 4.0 and then at pH 2.3 (Fig. 2). When the two pools were diluted to equal protein concentrations and subjected to an enzyme immunoassay to obtain antibody titers, the pH 4.0 fractions had a lower titer (1:64,000) and therefore a lower affinity than did the pH 2.3 fractions (1:256,000) (Table

TABLE 1. Dot blot enzyme immunoassay obtained during the purification of anti-flagellin antibody[a]

Step in the purification protocol	Enzyme immunoassay titer[b]	Fold increase in titer relative to whole serum
Preimmune whole sera[c]	10	
Immune whole sera[d]	640	
Ammonium sulfate precipitate	10,240	16
Ammonium sulfate supernatant	40	
DEAE column voided-volume pool	40,690	64
DEAE column high-salt eluate pool	10	
Affinity column		
Voided-volume pool	<10	
pH 4.0 pool	64,000	100
pH 2.3 pool	256,000	400

[a] Antigen (0.5 µg of flagellin) was spotted onto nitrocellulose disks placed in the wells of a microtiter tray. Each antibody preparation was diluted to an equal concentration and added to the first well in a row, and twofold dilutions were made.

[b] Titers are expressed as reciprocals.

[c] Sera obtained before the immunization schedule was started.

[d] Sera obtained 6 weeks after the final immunization and used for the isolation of affinity-purified antibody.
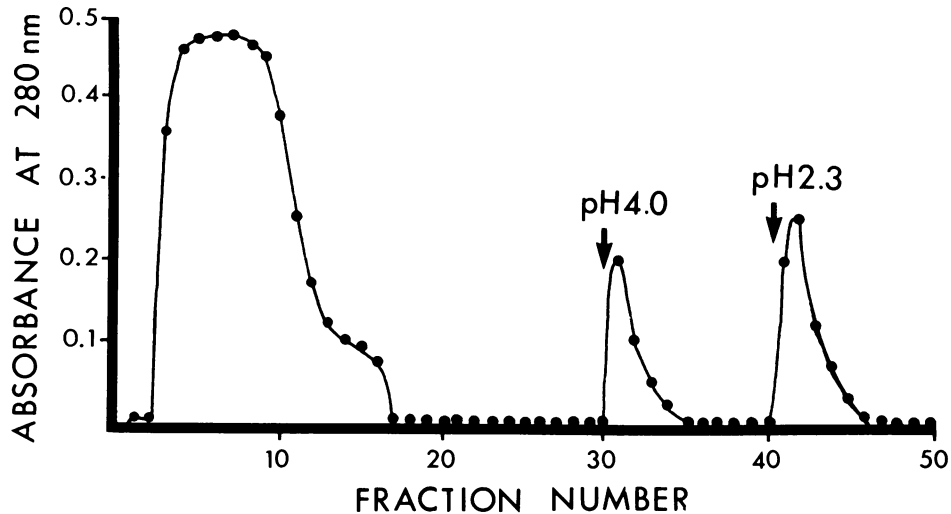
FIG. 2. Chromatograph of the affinity column. Flagellin from Pen 1 was bound to CNBr-activated Sepharose, and the rabbit IgG obtained after ammonium sulfate precipitation and ion-exchange chromatography was eluted through the column. Nonspecific IgG eluted at voided volume (fractions 1 through 17), and two antibody fractions were eluted sequentially with pH 4.0 buffer (fractions 31 through 34) and pH 2.3 buffer (fractions 41 through 45).

1). Although a titer could not be obtained with the voided-volume fraction (Table 1), it was applied to the column a second time to ensure removal of all antiflagellin antibody. As expected, no protein was detected when the column was eluted with either of the low-pH buffers.

Immune blots. When the high-affinity antibody (pH 2.3 eluate) was used in immune blot experiments with purified flagellin from Pen 1 and Pen 3, the antibody reacted with the 62-kDa proteins from both strains (Fig. 3). In addition, when the antibody was reacted to whole-cell extracts from the 60 reference strains for the thermostable antigen serotyping system, a positive reaction was observed for all strains (Fig. 4). In most of the strains, the antigenic protein had a molecular mass of approximately 62 kDa, but two strains, Pen 55 and Pen 56, had bands corresponding to 60 kDa (results not shown). Faintly visible bands corresponding to 120 and 180 kDa seen frequently in these immune blots were interpreted to be dimers and trimers of the flagellin. Evidently, electrophoretic conditions did not result in complete dissociation into 62-kDa monomers.

Acid-glycine extracts were prepared from the same 60 serotype reference strains, electrophoresed by SDS-PAGE, and stained with CBB (Fig. 4). Although there was some variability in the CBB-stained protein profiles between strains, most of the preparations contained bands with approximate molecular masses of 62, 44, 31, 27, 24, and 19 kDa (Fig. 4, lanes 12 through 16). When similar gels were immune blotted with high-affinity antibody, all 60 samples had a band around 62 kDa that reacted with the antiflagellin antibody (Fig. 4).

Coagglutination reactions. Protein A-bearing staphylococci were sensitized separately with the seven different whole sera and antibody-containing serum fractions listed in Table 2. Use of the appropriate controls allowed monitoring of nonspecific agglutination of either the sensitized staphylococcal reagent or the campylobacter cells, and autoagglutination of *C. jejuni* could be prevented by washing cells in saline and using them immediately. Although acid-treated whole cells tended to clump and settle to the bottom of Eppendorf tubes, the supernatant could be used in tests.

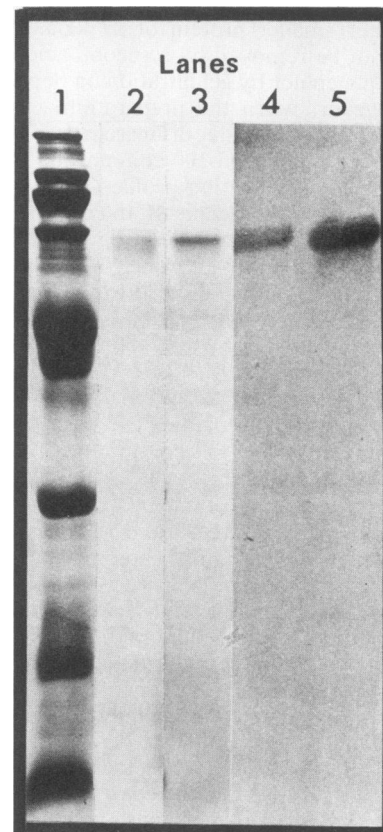Whole cells from strains Pen 1 to Pen 12 did not aggluti-

FIG. 3. Purified flagellin preparations from Pen 1 and Pen 3. Lanes 1 through 3, CBB-stained SDS-polyacrylamide gel of molecular mass markers (lane 1) and purified flagellin from Pen 1 (lane 2) and Pen 3 (lane 3); lanes 4 and 5, immune blot with the high-affinity antibody reacted against flagellin from Pen 1 (lane 4) and Pen 3 (lane 5).
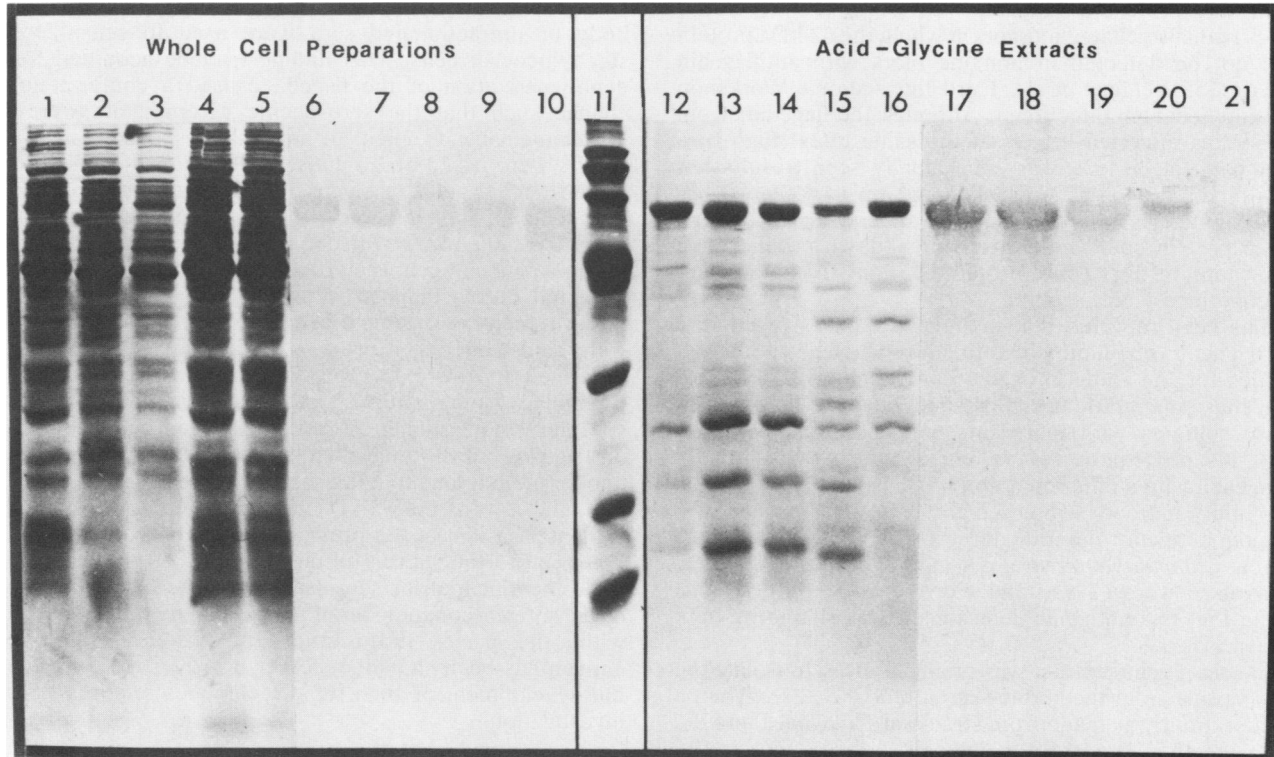
FIG. 4. CBB-stained SDS-polyacrylamide gels and immune blots of whole-cell preparations (lanes 1 through 10) and acid-glycine extracts (lanes 12 through 21) from various strains of *C. jejuni* and *C. coli*. Whole-cell preparations: lanes 1 through 5, CBB-stained gel of *C. jejuni* Pen 21, *C. jejuni* Pen 22, *C. jejuni* Pen 23, *C. coli* Pen 24, and *C. coli* Pen 25, respectively; lanes 6 through 10, immune blot with high affinity antibody reacted with Pen 21, Pen 22, Pen 23, Pen 24, and Pen 25, respectively. Acid-glycine extracts: lanes 12 through 16, CBB-stained gel of Pen 21, Pen 22, Pen 23, Pen 24, and Pen 25, respectively; lanes 17 through 21, immune blots with high affinity antibody reacted with Pen 21, Pen 22, Pen 23, Pen 24, and Pen 25, respectively. Molecular mass markers are shown in lane 11.

nate in the presence of staphylococci coated with any of the antibody preparations. However, when cells were suspended in an acid buffer (glycine-hydrochloride [pH 2.0]) followed by neutralization, coagglutination of all strains occurred when either the high- or low-affinity antibody was used as the sensitizing agent. Purified flagellin from both Pen 1 and Pen 3 reacted very strongly with either whole immune sera or affinity-purified antibody. All reactions with affinity-purified antibody were much faster and stronger than those observed with whole immune sera, as expected because of

the higher proportion of specific antibody present in the affinity-purified antibody fractions.

DISCUSSION

Purified proteins from two strains of *C. jejuni*, Pen 1 and Pen 3, had molecular masses of 62 kDa when electrophoresed by SDS-PAGE, which is consistent with results obtained by other groups (9, 15; Wenman et al., Third International Workshop on Campylobacter Infections). The iden-

TABLE 2. Coagglutination reactions of whole cells, acid-treated whole cells, and acid-glycine extracts with staphylococci sensitized with various antibody preparations[a]

	Coagglutination reactions[b]				
	Strains Pen 1 to Pen 12			Pure flagellin	
Antibody preparation	Whole cells		Glycine hydrochloride extract	Pen 1	Pen 3
	Saline	Glycine hydrochloride			
Pen 1 antiflagellin serum					
Unfractionated	−	−	− to +	+ +	+ +
High-affinity antibody	−	+ to + +	+ +	+ + +	+ + +
Low-affinity antibody	−	+ to + +	+ to + +	+ +	+ +
Voided-volume pool	−	−	−	−	−
Pen 3 antiflagellin serum (unfractionated)	−	− to +	+ to + +	+ +	+ +

[a] The appropriate preimmune sera were used as negative controls.
[b] Results are expressed as follows: −, no agglutination after 2 min; +, slight agglutination visible at ×40 magnification; + +, agglutination visible by eye within 1 min; + + +, rapid agglutination with large granular precipitate.

tity of this protein as flagellin was suggested by earlier studies with aflagellated mutants in which the 62-kDa protein could not be detected in immune blots with antiflagellin antisera (15; Wenman et al., Third International Workshop on Campylobacter Infections). Although the flagellins from both strains migrated in gels at the same rates (Fig. 3), a minor band at 60 kDa was occasionally seen (results not shown). The reason for this phenomenon is not presently known, but it is not unreasonable to speculate that the 60-kDa protein could be either a breakdown product or a modification of the 62-kDa protein or simply a contaminant that copurified with flagellin.

It has been reported that immune responses result in a heterogeneous population of antibodies which have different affinities for the same antigenic component (19). Furthermore, these preparations can be subdivided by elution from affinity columns with increasing concentrations of acetic acid. This procedure was used to produce two antibody pools, each with a different affinity to flagellin (Table 1). The high-affinity antibody was used to show the presence of a common antigenic determinant on the flagellins from 60 different Campylobacter strains. Since 18 of the 60 reference strains used were C. coli and 42 were C. jejuni (17), it is evident that this antigenic determinant was shared by both species.

The acid-glycine extract was originally used to isolate the microcapsule from the surface of C. fetus (11, 22). When a similar extract was made from strains of C. jejuni, there did not appear to be a corresponding cell surface structure (9). Later studies, however, showed that these preparations contained a common antigen that could be used as the basis for serological tests to determine the presence of antibodies during or after infection with C. jejuni. When acid-glycine extracts were prepared from the 60 strains used in this study, many proteins were observed after electrophoresis, including a 62-kDa band which was thought to be flagellin. These results corresponded to those previously reported (1, 9). Use of the high-affinity antibody in immune blots with these preparations clearly demonstrated the presence of a common antigenic component. This finding signified that the common antigens in the whole-cell protein preparations and in the acid-glycine extracts were the same and were located on the 62-kDa flagellin. The common antigen in the acid extract was reported to be heat stable; that is, it was still able to produce an antibody response in rabbits after being heated for 1 h at 100°C (18). Although our preparation was heated for only 5 min to facilitate disaggregation before column chromatography, Buck et al. (3) have reported that the molecular weight of flagellin remains stable for prolonged periods at this temperature.

Although these results appear in sharp contrast to previous reports of serological heterogeneity for this protein, it must be stressed that our additional experiments with staphylococcal protein A-bearing cells sensitized with affinity-purified antibody (Table 2) indicated that, in the native whole-cell state, the antigenic determinant was cryptic and could not participate in coagglutination. However, the determinant evidently became exposed in cells that were treated with acid to depolymerize the flagella and in purified flagellin preparations. Serotyping systems based on agglutination of live bacteria would, therefore, not be expected to detect the presence of the common antigen.

The finding that antibody produced against purified flagellin was devoid of activity against serotype-specific determinants was unexpected. Reactions between such determinants and antiflagellin antibody would have been ex-

pected in the coagglutination assay when high-affinity antibody or unfractionated sera were used to sensitize the staphylococcal cells. This finding may be acounted for if depolymerization of the flagella caused a conformational change such that the serospecific determinants were no longer available to elicit an antibody response. Since our results were based on an antiserum produced against acid-dissociated flagella, the possibility that flagella possess thermolabile antigens that are degraded during the acid treatment cannot be discounted. The antigens of the thermolabile antigen typing schemes, to our knowledge, have not been examined for stability in glycine-hydrochloride. If the type-specific determinants were not available or were acid labile, then proteins purified by the procedures described in this paper are not a suitable material for producing typing antisera. Alternatively, the serological specificity in thermolabile serotyping systems may not reside on flagellin but on a flagellum-associated protein such as a hook protein or an unrelated surface protein. In this connection, the role of the 92-kDa protein needs to be critically evaluated, as suggested previously (9, 21).

Crucial to the successful development of procedures for rapid identification or serodiagnosis is the characterization of an antigen common to all or the majority of the strains within the species. In this study, the existence of one such antigen has been shown. Whether it proves to be useful in the development of such rapid techniques requires further investigation.

ACKNOWLEDGMENTS

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