

Structural and Antigenic Properties of Lipopolysaccharides from Serotype Reference Strains of *Campylobacter jejuni*

MARTIN A. PRESTON AND J. L. PENNER*

Department of Microbiology, University of Toronto, Toronto, Ontario, Canada M5G 1L5

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To investigate the molecular basis for heat-stable antigenic diversity in *Campylobacter jejuni*, lipopolysaccharides (LPSs) from serotype reference strains and serotyped isolates were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis coupled with silver staining and immunoblotting. By silver staining, only low- M_r components, consisting of one major band and as many as three minor bands ranging in M_r from 4,500 to 5,000, were detected. However, by immunoblotting with homologous antisera, 10 of 34 strains were shown to have a series of high- M_r LPS components characteristic of molecules with O side chains of various lengths. Isolates of the same serotype as the reference strain that had high- M_r LPS molecules were also found to have high- M_r LPS and in one case of cross-reacting strains it was found that the cross-reaction was associated with antibodies against high- M_r LPS. The reactions of LPSs with homologous and heterologous antisera indicated that both high- and low- M_r -type LPSs were strain-specific antigens, but in some cases cross-reactions were noted. Evidently, all *C. jejuni* strains possess low- M_r LPS that is readily detectable by silver staining, but some serotypes also possess high- M_r LPS components that can be visualized by immunoblotting.

Lipopolysaccharide (LPS) is a major component of the outer membrane of gram-negative bacteria (14, 15). Members of the family *Enterobacteriaceae* have LPS consisting of three distinct regions: lipid A, core oligosaccharide, and O side chain or O antigen. The innermost region, lipid A, is embedded in the lipid bilayer of the outer membrane, and this portion of the LPS molecule is responsible for the endotoxic effects of LPS on higher organisms. The O side chain, covalently attached to lipid A via the core oligosaccharide, is a linear polymer of an oligosaccharide repeat unit. Extensive variation in the chemical composition and conformation of the repeat unit provides the basis for the classification of the *Enterobacteriaceae* into hundreds of heat-stable O serogroups (22). The O-side-chain region also protects the bacterium against phagocytosis (29). In contrast, the LPSs or lipooligosaccharides of nonenteric, gram-negative pathogens such as *Haemophilus influenzae*, *Neisseria meningitidis*, and *Bordetella pertussis* are devoid of O side chains, and the limited LPS serological diversity demonstrated by these organisms is believed to be provided by variation in the composition of the oligosaccharide (7, 8, 25, 31).

During the last decade, *Campylobacter jejuni* has come to be recognized as a major cause of human bacterial enteritis (2, 28), and recent investigations have shed light on the properties of the LPS from these pathogens. By using gel filtration, Naess and Hofstad (19, 20) found that these macromolecules could be divided into two structural types. The LPS from some strains appeared to consist of lipid A and core oligosaccharide, but in other strains it appeared to have short O side chains attached to the core oligosaccharide. In contrast, analyses of the migration patterns of *C. jejuni* LPSs determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining indicated that these LPSs are of low M_r and devoid of O side chains (1, 13, 18, 26). Mattsby-Baltzer et al. (17) demonstrated that the lipid A region is similar to that produced by other gram-negative bacteria.

Serological studies have shown that *C. jejuni* strains are antigenically diverse, and at least 42 serotypes are recognized in a serotyping system based on heat-stable O antigens (23, 24). The results of recent investigations suggest that these antigens are based on LPSs. Naess and Hofstad (21) demonstrated that antisera prepared against several *C. jejuni* strains have serological activities against the homologous LPSs. Jones et al. (9) and Mills et al. (18) showed that LPSs isolated from serotype reference strains possess the same serological specificities as heated cell extracts.

The relationships between *C. jejuni* LPS structure and O serotype diversity are of considerable interest. By using SDS-PAGE and immunoblotting, Logan and Trust (13) found that low- M_r LPS components conferred serological specificities to the parent strains. In contrast, Perez Perez et al. (27) have shown that low- M_r LPS structures cross-react with antisera prepared against several different strains and they have suggested the presence of LPS with O side chains in homologous reactions. To resolve this contradiction and to provide further insight into the serological relationships among the LPSs of this important pathogen, we examined the structural and antigenic properties of LPSs from a large number of serotype reference strains and serotyped isolates.

MATERIALS AND METHODS

Bacteria and growth conditions. Reference strains of a serotyping scheme based on thermostable antigens (23, 24) and serotyped isolates of *C. jejuni* were used in this study. Bacteria were grown routinely on blood agar (Columbia agar base [Oxoid Ltd., London, England], 7% horse blood) in an atmosphere containing CO₂ (7%) at 37°C for 48 h. To investigate the effect of growth conditions on LPS components, bacteria were grown on two sets of media, each set composed of blood agar, brucella agar (BBL Microbiology Systems, Cockeysville, Md.), and Mueller-Hinton agar (Oxoid). One set was incubated in an atmosphere containing CO₂ (7%) at 37°C for 48 h, and the other was incubated in an anaerobic jar containing a CampyPak gas generator envelope (BBL) at 37°C for 48 h. The Ra mutant strain of *Salmonella typhimurium* (kindly supplied by K. Sanderson, Department

* Corresponding author.

of Biology, University of Calgary, Calgary, Alberta, Canada) and *Escherichia coli* O111:B4 were grown on Trypticase soy agar (BBL) at 37°C for 24 h.

Antisera. Antisera used in this study were prepared by intravenous injections of rabbits with bacterial saline suspensions of reference strains for *C. jejuni* serotypes as described previously (23).

LPS preparation. (i) Proteinase K treatment of cell lysates. LPS components were isolated by enzymatic digestion of whole-cell lysates using a procedure modified from that of Hitchcock and Brown (6). Bacterial growth from a single agar plate was harvested in 5 ml of cold phosphate-buffered saline (pH 7.0), and this cell suspension was diluted in phosphate-buffered saline to an A_{600} of 0.3. A portion (1.5 ml) of the diluted suspension was centrifuged in a microcentrifuge for 1.5 min. The supernatant was discarded, and the cell pellet was solubilized in 200 μ l of lysing buffer containing glycerol (20%), 2-mercaptoethanol (5%), sodium dodecyl sulfate (4.6%), 0.125 M Tris hydrochloride (pH 6.8), and bromphenol blue (0.004%). The lysate was heated at 100°C for 10 min and cooled to room temperature before adding 40 μ l of lysing buffer containing proteinase K (Boehringer Mannheim Canada, Dorval, Quebec, Canada) at a concentration of 2.5 mg/ml. The enzyme-treated cell lysates were incubated at 60°C for 1 h and then boiled for 5 min before electrophoresis.

(ii) LPS extraction by the phenol-water method. LPS was also extracted from three *C. jejuni* reference strains by using the phenol-water technique described by Westphal and Jann (33). Bacteria grown on blood agar plates were harvested in saline containing formaldehyde (0.3%) and collected by centrifugation (4,000 $\times g$, 20 min). The cells were washed in saline once and stored at -20°C. After thawing, the bacteria were transferred to a solution containing equal volumes of water and phenol (90%) and stirred for 20 min at 65°C. The suspension was cooled to 4°C on ice and centrifuged (12,000 $\times g$, 30 min). The upper (aqueous) layer was dialyzed for 48 h against several changes of deionized water and then lyophilized. This material was solubilized in deionized water and purified by ultracentrifugation (105,000 $\times g$, 4 h) (4). The gel-like pellet was suspended in deionized water and lyophilized. The protein content of *C. jejuni* LPS averaged less than 3% as measured by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Mississauga, Ontario, Canada), and no bands were visualized by SDS-PAGE when stained with Coomassie brilliant blue.

SDS-PAGE. LPSs were fractionated by SDS-PAGE with the discontinuous buffer system described by Laemmli (12). To avoid formation of aggregates during electrophoresis, proteinase K-treated cell lysates were applied to the gels in minimal concentrations. The stacking gel contained 5% acrylamide. Preliminary experiments were performed to determine the electrophoretic conditions which provide for optimum resolution of low- M_r LPS components. The results showed that resolution was influenced markedly by the concentration of acrylamide used in the separation gel and by the level of current applied during electrophoresis. On the basis of these findings, electrophoresis was performed routinely with a constant current of 35 mA and a separation gel containing 12.5% acrylamide. LPS samples were electrophoresed until the tracking dye was within 1 cm of the bottom of the gel.

Silver staining. After SDS-PAGE, the gels were fixed and stained for LPS by the method of Tsai and Frasch (32). Briefly, the gels were immersed in a fixative solution containing ethanol (40%) and glacial acetic acid (5%) for 18 h and

then transferred to an oxidizing solution consisting of periodic acid (0.7% wt/vol) in ethanol (40%)–acetic acid (5%) for 5 min. The gels were rinsed three times in deionized water (500 ml) for 15 min each and then immersed in ammoniacal silver nitrate staining solution containing 2 ml of concentrated ammonium hydroxide, 28 ml of sodium hydroxide (0.1 N), 5 ml of silver nitrate (20% [wt/vol]), and 115 ml of deionized water. The gels were rinsed three times in deionized water for 10 min each time and then incubated in developing solution consisting of 0.5 ml of formaldehyde (37%) and 50 mg of citric acid per liter. The development of the gels was halted at the first sign of discoloration of the gel background by rinsing the gels in several changes of water.

Detection of LPSs by immunoblotting. LPS components separated by SDS-PAGE were transferred to nitrocellulose sheets (0.45 μ m; Bio-Rad) by electrophoretic blotting with the transfer buffer described by Towbin et al. (30). After SDS-PAGE, the gels were equilibrated in transfer buffer containing 25 mM Tris, 192 mM glycine, and 20% (vol/vol) methanol (pH 8.3) for 30 min and then applied to nitrocellulose sheets. Blotting was performed for 18 h at 30 V (0.1 A). After transfer, the nitrocellulose sheets were immersed in Tris hydrochloride-buffered saline (TBS) (20 mM Tris, 500 mM NaCl, pH 7.5) containing 3% gelatin for 30 min and then incubated for 2 h in TBS containing 1% gelatin and rabbit antiserum diluted 1:100. The sheets were rinsed in deionized water once for 10 min and in TBS containing Tween 20 (0.05%) twice for 10 min each time and then incubated with goat anti-rabbit immunoglobulin G-horseradish peroxidase conjugate (Bio-Rad) diluted 1:1,000 in TBS containing gelatin (1%) for 1 h. The sheets were rinsed in water and TBS-Tween 20 as described previously and then immersed for 15 min in a substrate solution prepared by adding 60 mg of 4-chloro-1-naphthol (Bio-Rad) dissolved in 20 ml of cold methanol to 100 ml of TBS containing 60 μ l of cold hydrogen peroxide (30%). The color development was halted by rinsing the sheets in deionized water. Negative reactions were observed between the LPS preparations and normal rabbit serum diluted 1:100.

RESULTS

Examination for effects of cultural conditions on LPS synthesis. To determine whether different growth conditions could affect the production of LPS components, the reference strain for serotype 4 was seeded onto two sets of media, each set consisting of blood, brucella, and Mueller-Hinton agar. One set of plates was incubated in an atmosphere provided by a carbon dioxide incubator (7% CO₂), and the other set was incubated in an atmosphere produced by a CampyPak gas generator envelope. After incubation, the bacterial growth from each plate was harvested, cell lysate was prepared, and LPS was isolated by proteinase K digestion. The silver-stained electrophoretic profiles of the six *C. jejuni* LPSs were compared with that of smooth-type LPS from *E. coli* O111:B4 (Fig. 1). The profile of smooth-type LPS consists of a fast-migrating, low- M_r band and an orderly series of higher- M_r bands which form a characteristic ladder-like pattern. The fastest-migrating component is believed to represent the lipid A plus core oligosaccharide portion of the LPS, and the slower-migrating components represent LPS molecules with increasing numbers of O side chain repeat units (3). The LPS profiles of the *C. jejuni* serotype 4 strain consisted of a major low- M_r band and three closely spaced, faster-migrating minor bands. The major band was stained intensively and migrated almost as far as the lowest- M_r band

of *E. coli* LPS. Not seen in the *C. jejuni* LPS was the series of slower-migrating bands indicative of LPS with O side chains that were present in the *E. coli* LPS. However, the migration patterns of the six *C. jejuni* LPS preparations were virtually identical, indicating, that synthesis of LPS by this strain was not detectably altered by the different growth conditions. In subsequent work, therefore, *C. jejuni* strains were grown on blood agar in a carbon dioxide incubator (7% CO₂). The migration pattern of LPS extracted from the serotype 4 reference strain by the phenol-water technique was identical to those of the enzymatically isolated LPSs (data not shown).

Electrophoretic profiles of LPSs from different serotypes detected by silver staining. The LPS components from seven serotype reference strains of *C. jejuni* and the Ra mutant strain of *S. typhimurium* were compared by SDS-PAGE and silver staining (Fig. 2). The rough-type LPS of *S. typhimurium* Ra consists of lipid A plus a complete core oligosaccharide and has a molecular weight of approximately 4,700 (32). The banding patterns of the *C. jejuni* LPSs consisted of a major low-*M_r* band and, for some LPSs, one or more minor bands closely spaced to the major band, but considerable variation in the LPS profiles of different strains was noted. The major band migrated at rates similar to or slightly slower than that of the major band of the *S. typhimurium* LPS. Although there were observable differences in the molecular weights of these *C. jejuni* components among the different serotypes, they were all estimated to fall within a range of 4,500 to 5,000.

Detection of high-*M_r* LPS by immunoblotting. To investigate the antigenic properties of *C. jejuni* LPS components, cell lysates of 34 serotype reference strains were digested with proteinase K and then fractionated by SDS-PAGE. Gels were run in duplicate to permit examination of LPS from each strain by both silver staining and immunoblotting

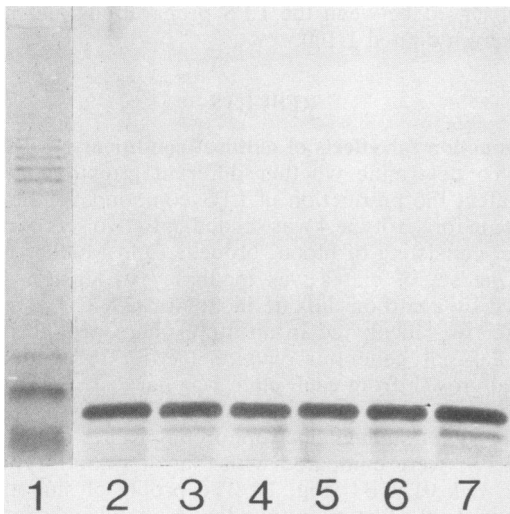


FIG. 1. Silver-stained electrophoretic profiles of LPSs isolated by proteinase K digestion from the *C. jejuni* reference strain for serotype 4 grown under various conditions and from *E. coli* O111:B4. A 15- μ l volume of LPS sample was applied to each lane. Lane 1, *E. coli* O111:B4. Lanes 2 through 7 contained *C. jejuni* serotype 4 LPS obtained from organisms grown under one of six growth conditions (lanes); 2, blood agar with 7% CO₂; 3, brucella agar with 7% CO₂; 4, Mueller-Hinton agar with 7% CO₂; 5, blood agar with CampyPak; 6, brucella agar with CampyPak; 7, Mueller-Hinton agar with CampyPak.

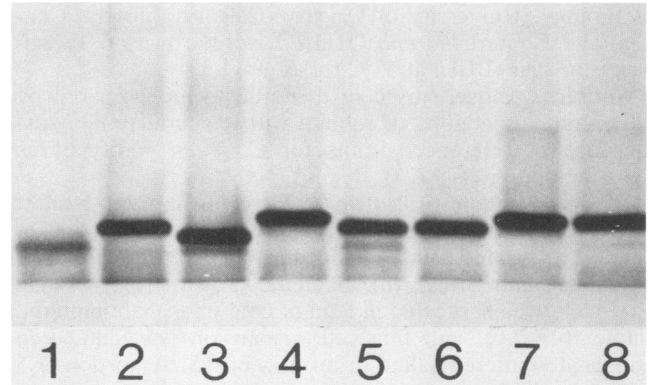


FIG. 2. Silver-stained electrophoretic profiles of LPSs isolated by proteinase K digestion from seven serotype reference strains of *C. jejuni* and the *S. typhimurium* Ra mutant. A 15- μ l LPS sample was applied to each lane. Lanes: 1, *S. typhimurium* Ra mutant; 2, *C. jejuni* O:1; 3, *C. jejuni* O:2; 4, *C. jejuni* O:3; 5, *C. jejuni* O:4; 6, *C. jejuni* O:5; 7, *C. jejuni* O:6; 8, *C. jejuni* O:7.

with serotyping antisera that had been prepared previously against whole cells of the homologous strains. LPSs from 24 strains were found by both procedures to consist of only fast-migrating, low-*M_r* molecules. The profiles of reference strains for serotypes 17, 44, and 52 are shown as representative examples of these results (Fig. 3, lanes 1, 2, and 3).

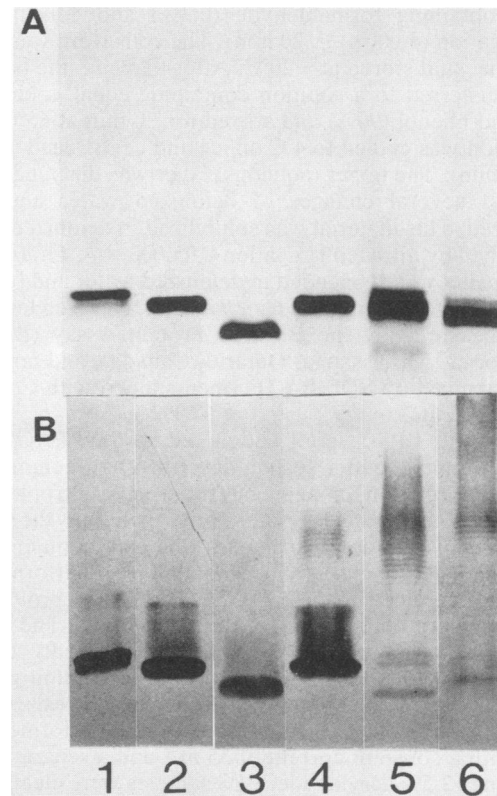


FIG. 3. Silver-stained electrophoretic profiles (A) and parallel immunoblots (B) of LPSs isolated by proteinase K digestion from six serotype reference strains of *C. jejuni*. A 10- μ l LPS sample was applied to each lane. The dilution of the homologous antisera was 1:100. Lanes: 1, *C. jejuni* O:17; 2, *C. jejuni* O:44; 3, *C. jejuni* O:52; 4, *C. jejuni* O:12; 5, *C. jejuni* O:21; 6, *C. jejuni* O:33.

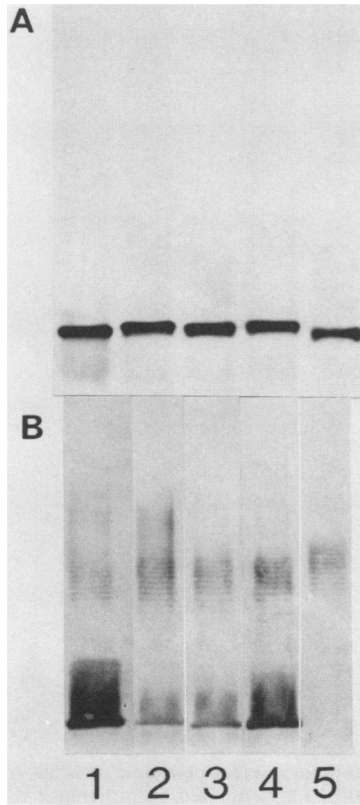


FIG. 4. Silver-stained electrophoretic profiles (A) and parallel immunoblots (B) of LPSs isolated by proteinase K digestion from the reference strain and four isolates of serotype 12. A 10- μ l LPS sample was applied to each lane, and the dilution of homologous antiserum was 1:100. Lanes: 1, *C. jejuni* O:12; 2, SH 57475; 3, 36662; 4, SH 42; 5, RO 237.

For 10 other strains, the results were strikingly different. Like the 24 strains described above, each was found to possess LPS consisting of low- M_r material with a pronounced major component that stained strongly with the silver reagent, but in addition, each strain was also found by immunoblotting to have a series of slow-migrating bands characteristic of enterobacterial LPS molecules with O side chains. The profiles of serotypes 12, 21, and 33 are representative examples (Fig. 3, lanes 4, 5, and 6). There were strain-to-strain differences in the ladderlike banding patterns of the high- M_r LPS components, and in some cases, antisera recognized the series of high- M_r LPS components but not the silver-stainable components from homologous strains (see Fig. 5). These results provide evidence suggesting that approximately one-third of the serotype reference strains of *C. jejuni* are capable of synthesizing high- M_r components characteristic of LPS molecules with O side chains.

The presence of high- M_r LPS components in *C. jejuni* was further investigated by comparing the LPS of the reference strain for serotype 12 with LPS from isolates belonging to the same serotype. When visualized by silver staining, the profile of the reference strain and the profile of each isolate consisted of a single low- M_r LPS component (FIG. 4A). However, by immunoblotting with the serotype 12 antiserum, an orderly series of high- M_r bands was observed for each of the LPS preparations (Fig. 4B). Although there were slight strain-to-strain differences in staining intensity, the ladderlike, high- M_r banding patterns were markedly similar.

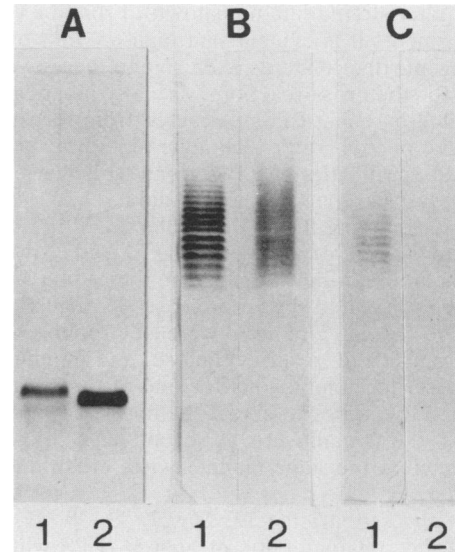


FIG. 5. Silver-stained electrophoretic profiles (A) and parallel immunoblots (B and C) of LPS isolated by proteinase K digestion from *C. jejuni* reference strains for serotypes 23 and 36. A 10- μ l LPS sample was applied to each lane, and the dilution of the antisera was 1:100. The gels were immunoblotted with antiserum against serotype 36 (panel B) or serotype 23 (panel C). Lanes: 1, *C. jejuni* O:23; 2, *C. jejuni* O:36.

Comparable results were obtained for the serotype 21 reference strain and four isolates of the same serotype (data not shown). These results indicate that high- M_r LPSs constitute serotype-specific antigens and that the presence of the high- M_r LPS is apparently restricted to particular serotypes.

Investigation of the cross-reaction between serotype reference strains 23 and 36. To examine the basis for the cross-reaction between strains known to cross-react in passive hemagglutination (PHA) titrations (Table 1), LPS was extracted from the reference strains for serotypes 23 and 36 by proteinase K digestion and subjected to analysis by SDS-PAGE, silver staining, and immunoblotting with homologous and heterologous antisera. The silver-stained LPS profiles consisted only of low- M_r bands, but by immunoblotting with homologous antisera an orderly series of higher- M_r bands was visualized for both strains (Fig. 5). In addition, antiserum to serostrain 36 reacted with a series of high- M_r bands from serostrain 23 LPS with the same migration pattern as that detected by the homologous antiserum. The staining intensity of this heterologous reaction was greater than that of the homologous reaction, an observation consistent with the higher heterologous PHA titer (Table 1). In a further examination of the antigenic specificity of this cross-reaction, antisera against 16 other *C. jejuni* reference strains were reacted with LPSs from serostrains 23 and 36 by immunoblotting. However, none of these antisera detected

TABLE 1. Titrations of *C. jejuni* O:23 and O:36 antisera against homologous and heterologous serotype reference strains

| Antigen | Titer in <i>C. jejuni</i> O antiserum ^a | | |
|-----------------|--|---------|---------------------|
| | O:23 | O:36 | 40 other O antisera |
| Serostrain O:23 | 1:1,280 | 1:2,560 | <1:40 |
| Serostrain O:36 | 1:80 | 1:5,120 | <1:40 |

^a Titrations by PHA were as described previously (23).

LPS from either strain (data not shown). From the results of these experiments it is evident that high- M_r LPS expresses shared antigenic determinants exclusive to these two strains to provide for the cross-reaction. Virtually identical results were obtained by using LPSs extracted from serostrains 23 and 36 by the phenol-water technique (data not shown).

Serological specificities of LPSs from serostrains. Ten *C. jejuni* serotype reference strains that did not have significant cross-reactions among themselves (titers of <1:40 by PHA cross-titrations) were examined by SDS-PAGE, silver staining, and immunoblotting to determine their antigenic specificities. LPSs isolated by proteinase K digestion from serostrains 12, 17, 21, 27, and 33 were electrophoresed in the same gel, transferred to nitrocellulose, and immunoblotted with an antiserum homologous for one of the strains. This was repeated five times to detect the five LPS preparations with each of the five antisera. A second set of experiments was performed in the same manner with serostrains 38, 40, 42, 52, and 57.

Seven of the serostrains, 12, 21, 27, 33, 38, 42, and 57, displayed bands characteristic of high- M_r LPS with O side chains by immunoblotting, and the high- M_r LPSs reacted only with homologous antisera, an observation consistent with the lack of cross-reactions previously noted in PHA cross-titrations (23, 24). Only three antisera, those against serostrains 33, 42, and 57, did not react with homologous low- M_r LPS at the antiserum concentration used. The other seven antisera reacted with homologous low- M_r LPS components, and antisera 12, 27, and 38 reacted weakly with low- M_r LPSs from serostrains 17, 33, and 40, respectively. The latter heterologous reactions were unilateral (nonreciprocal) since antisera 17, 33, and 40 did not react with antigenic material from strains 12, 27, and 38. Reactions corresponding to these unilateral reactions were not seen in cross-reactions by PHA. It should be noted that the unilaterally reacting antisera, 12, 27, and 38, were against strains that possessed high- M_r components. In contrast, antisera against serostrains 17, 40, and 52, which possessed only low- M_r LPSs, reacted only with LPSs from the homologous strains (Fig. 6). Since the 40 heterologous immunoblot reactions failed to provide evidence for the occurrence of extensive cross-reactions, it is evident that low- M_r LPSs display unique serological specificities.

DISCUSSION

The results of our study showed that, in addition to the low- M_r LPS possessed by all *C. jejuni* strains, some strains also synthesize high- M_r molecules that are characteristic of LPS with O side chains. With the conditions of electrophoresis defined in this study and the silver staining procedure developed by Tsai and Frasch (32), the low- M_r LPSs from serotype reference strains were found to fall within a range of 4,500 to 5,000 and could be resolved into a single discrete band or, in some cases, into a major band with as many as three minor bands usually of lower M_r than the major band. The electrophoretic mobility of these LPS components appeared to be a stable property of the individual strain under different conditions of culture. The M_r of the major band varied slightly from one serotype to the next, and thus the correlation between structural heterogeneity of the low- M_r LPS and serological heterogeneity, as proposed earlier (13, 18), was confirmed.

With the application of the immunoblotting technique to gain further insight into the structural basis for serotypic specificity, it was found that in homologous reactions 10 of

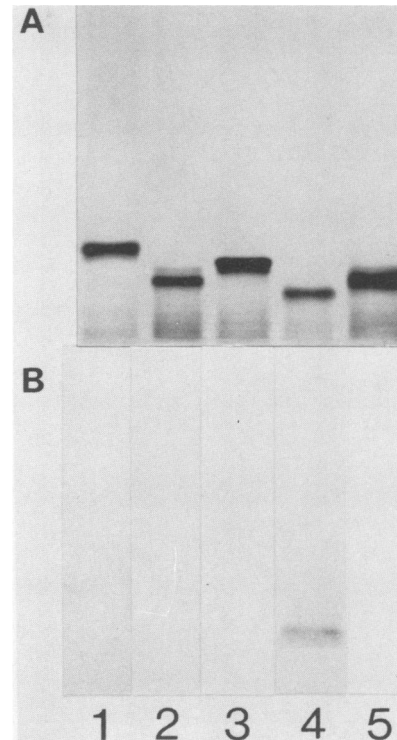


FIG. 6. Silver-stained electrophoretic profiles (A) and parallel immunoblots (B) of LPSs isolated by proteinase K digestion from five serotype reference strains of *C. jejuni*. A 10- μ l LPS sample was applied to each lane. Each LPS was immunoblotted with antiserum against serotype O:52 diluted 1:100. Lanes: 1, *C. jejuni* O:38; 2, *C. jejuni* O:40; 3, *C. jejuni* O:42; 4, *C. jejuni* O:52; 5, *C. jejuni* O:57.

the 34 antisera revealed an orderly series of high- M_r bands which formed a ladderlike pattern characteristic of LPS with O side chains. Furthermore, isolates of two serotypes displayed high- M_r LPS banding patterns indistinguishable from the corresponding reference strains, and high- M_r LPS components from two reference strains were found to provide the antigenic determinants associated with a serological cross-reaction. It needs to be emphasized that this high- M_r material could not be detected with the silver staining procedure and was thus not likely to consist of aggregated low- M_r LPS, which is readily stained (13, 18). In addition, high- M_r components with the same electrophoretic mobilities were detected in LPS samples prepared by enzymatic digestion or by phenol-water extraction.

To our knowledge, the occurrence of LPS with O side chains in *C. jejuni* has not been demonstrated previously but has been suggested (19, 27). Two reasons may be cited for our success in detecting these molecules. (i) Our study included larger numbers of strains, all of which were either serotype reference strains or isolates that had been serotyped. (ii) It has only recently been recognized that differences may exist between the electrophoretic profiles of LPSs detected by silver staining or immunoblotting. Karch et al. (10) found that the profile of LPS from *E. coli* O26 was detectable by immunoblotting with antiserum prepared to the homologous strain but not by a periodic acid-silver staining procedure. A series of higher- M_r antigenic components from the phase 1 LPS of *Coxiella burnetii* not visualized by LPS silver staining was visualized by immunoblotting with homologous antiserum (5). Mandrell et al. (16)

demonstrated that LPS components from some strains of *N. gonorrhoeae* were recognized by immunoblotting with monoclonal antibodies but not by periodic acid-silver staining. The results from the present study on *C. jejuni* lend support to the view that structural analysis of LPS by SDS-PAGE and silver staining alone may be inconclusive (11).

Our examination of LPSs from serotype reference strains indicates that these molecules behave like strain-specific antigens. Of 40 heterologous immunoblot reactions, only three unilateral cross-reactions were noted. Logan and Trust (13) also showed that LPSs from strains belonging to different heat-stable serotypes conferred serotypic specificities. In contrast, Perez Perez et al. (27) found that *C. jejuni* low- M_r LPS structures cross-reacted with antisera prepared against several different strains, although the serotypes of the strains used in the latter study were not specified.

C. jejuni strains with high- M_r LPS are similar to *Salmonella* and *E. coli* S (smooth) forms, and their serological specificities may be attributed to differences in composition of the repeat units of the O side chains. Other *C. jejuni* strains that possess LPS without O side chains are perceived to reflect enterobacterial SR (semirough) forms which consist of lipid A, core oligosaccharide, and a single repeat unit. Consistent with this perception are the analyses performed by Naess and Hofstad (19), which established that the low- M_r LPSs of *C. jejuni* and *C. coli* are composed of lipid A, 3-deoxy-D-manno-2-octulosonic acid, L-glycero-D-mannoheptose, glucose, galactose, and glucosamine and that there are 10 to 25 hexose units for every three residues of heptose. They suggested that the core oligosaccharide may be substituted by antigenic side chain repeat units but the numbers of units must be low. This evidence, in conjunction with our demonstration of bands characteristic of LPS with O side chains in at least some strains, precludes a strong case for the occurrence in *C. jejuni* of LPS molecules of the lipooligosaccharide type described for *N. meningitidis* (8), *H. influenzae* (7), and *B. pertussis* (25).

The earlier observation that *C. jejuni* cannot be reliably serotyped on the basis of thermostable antigens by slide agglutination because heated cell suspensions generally tended to autoagglutinate (23) is consistent with the lack of long O side chains in most of the strains. Why only few of the strains are in the S form remains an intriguing question. Evidently most of the strains do not possess a functional enzyme(s) required to polymerize the second and subsequent repeat units to produce long O side chains. The elucidation of the roles in pathogenesis of these two forms of LPS will no doubt be undertaken by investigators in future studies.

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