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Electrophoretic and Chemical Characterization of Lipopolysaccharides of *Vibrio parahaemolyticus*†

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Lipopolysaccharides (LPSs) isolated from three Kanagawa-positive and three negative strains of *Vibrio parahaemolyticus* were characterized by using electrophoretic, immunochemical, and chemical methods. The results of this study indicated that the LPSs of all six strains of *V. parahaemolyticus* examined did not have an O-specific side chain. These *V. parahaemolyticus* LPSs appeared to have molecular weights similar to that of the rough-type (Ra) LPS of *Salmonella typhimurium* TV-119 and might just contain lipid A and a core region. However, the microheterogeneity of *V. parahaemolyticus* LPS observed was greater than that of *S. typhimurium* LPS. The profile of *V. parahaemolyticus* LPS consisted of closely spaced triplet or quadruplet bands, but that of *S. typhimurium* consisted of doublet bands. Slower-moving bands appeared on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels only when large amounts of *V. parahaemolyticus* LPS were loaded. These bands were proven to be the aggregates of the fastest-moving low-molecular-weight bands by re-electrophoresis. The banding pattern of *V. parahaemolyticus* LPSs produced on nitrocellulose membranes by immunoblotting indicated that the *V. parahaemolyticus* LPSs did not have an O-specific side chain. The low ratio of total carbohydrate to lipid A of *V. parahaemolyticus* LPSs also suggested that they were like rough-type LPS. The mobility and profile of *V. parahaemolyticus* LPS on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and its chemical composition were closely related to the serotype of a specific strain but not with the Kanagawa phenomenon.

Vibrio parahaemolyticus is a gram-negative pathogen to humans and other creatures (47). Since it is an inhabitant of estuarine and coastal waters of the ocean (28), fresh seafood is often contaminated with this pathogen (41). Most gastroenteritis outbreaks resulting from seafood consumption are caused by *V. parahaemolyticus* (22). As seafood consumption is increasing in this country, it is becoming more and more important to understand this pathogen and its virulence factors. However, the virulence factors of pathogenic *V. parahaemolyticus* are still unknown and not all *V. parahaemolyticus* strains are pathogenic (23). Most (96.5%) of the pathogenic strains isolated from patients are able to lyse erythrocytes on blood agar medium containing 7% NaCl (44), i.e., they are Kanagawa positive (K^+), but 99% of the strains isolated from natural environments do not have such hemolytic activity, i.e., they are Kanagawa negative (K^-), and are usually nonpathogenic (23). Although the thermostable direct hemolysin which accounts for K^+ activity has fluid-accumulating activity in the rabbit ileal loop (46), identification of thermostable direct hemolysin as the virulence factor is still unclear owing to its relatively low activity (21). Other biologically active toxins have been reported as possible virulence factors (21, 36, 40).

Lipopolysaccharide (LPS) may have an enhancing effect on the pathogenic process of gram-negative infection by generating inflammatory mediators through interaction with host defense systems (35). LPS and lipid A isolated from *V. parahaemolyticus* have been found to have macrophage-stimulating and antitumor activities (3–5).

Since the pathogenicity of *V. parahaemolyticus* is closely associated with its Kanagawa phenomenon, Pace and Chai (37) have conducted an extensive investigation of the composition of the *V. parahaemolyticus* cell envelope in regard to the Kanagawa phenomenon and the responses to different growth conditions. They have found differences between K^+ and K^- strains in terms of growth curves, morphology, membrane proteins, phosphate content, and LPS content under different growth conditions.

The purpose of this study was to further characterize LPSs isolated from both K^+ and K^- strains of *V. parahaemolyticus* by using gel electrophoretic, immunochemical, and chemical methods.

MATERIALS AND METHODS

Bacterial strains. Six strains of *V. parahaemolyticus* were used. Their serotypes and Kanagawa phenomenons are listed in Table 1. Cultures were grown in broth culture containing 1% Proteose Peptone, 0.2% beef extract, and 2.5% NaCl at 35°C in a rotary shaker. Cells were harvested at an optical density at 660 nm of 1.0 to 1.2 and then lyophilized.

Extraction of LPS. LPS was extracted from lyophilized cells by using the phenol-water method (45). This method had been demonstrated to be better than the phenol-chloroform-petroleum ether method (13) for extraction of LPS from the strains examined in this laboratory. Deneke and Colwell (8) have also reported that *V. parahaemolyticus* LPS was better extracted by the phenol-water method. LPS was purified by repeated high-speed centrifugation (10,000 × *g*, 30 min) and ultracentrifugation (105,000 × *g*, 3 h) (17). The smooth-type LPS of *Salmonella typhimurium* LT2 and the rough-type (Ra) LPS of *S. typhimurium* TV-119 were provided by V. Jimenez (National Institutes of Health).

Gel electrophoresis of LPS. LPS was characterized by

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TABLE 1. Strains of *V. parahaemolyticus* used in this study

Strain	Kanagawa phenomenon	Serotype	Isolation source	Donor
38C1	+	O4:K11	Blue crab hemolymph	R. R. Colwell
P7	+	O4:K4	Stool	A. Delisle
P26	+	O2:K3	Stool	A. Delisle
38C6	−	O3:K30	Hemolymph	R. R. Colwell
P68	−	O4:K34	Fish	A. Delisle
P6	−	O2:K30	Blue crab	A. Delisle

using the sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) system developed by Laemmli (27). The separating gel contained 12.5% acrylamide and 4 M urea. The sample buffer contained 4 mM EDTA in addition. The acrylamide concentration of the stacking gel was 4.5%. SDS-PAGE was carried out under constant voltages of 110 V for the stacking gel and 250 V for the separating gel. The dimensions of the slab gel were 180 by 160 by 0.75 mm. The gel was visualized by using a modified silver staining procedure described by Fomsgaard et al. (12).

Gradient SDS-PAGE of LPS. The microheterogeneity of LPS was characterized by gradient SDS-PAGE. The acrylamide concentration of the gradient separating gel ranged from 10 to 15%. Urea was omitted from the separating gel system. The amount of ammonium persulfate added to initiate gel polymerization was reduced by half. The dimensions of the slab gel were 180 by 320 by 0.75 mm. The running conditions were the same as those described above for regular SDS-PAGE. The gradient gel was stained by using the silver staining procedure described by Tsai and Frasch (43).

Isolation of LPS bands on preparative gel. The system used for preparative gel electrophoresis was the same as the regular SDS-PAGE system described above, except that the dimensions of the gel were 180 by 160 by 1.5 mm, which is twice as thick as the analytical gel, and 0.5 mg of strain 38C1 LPS was loaded for each gel. To locate the bands, a notched strip cut off from each side of the gel was stained by using the silver staining procedure described by Tsai and Frasch (43). The remaining gel was sectioned between bands by using a stainless steel blade. The corresponding sections from two gels were pooled and then homogenized by a Teflon tissue grinder. The homogenate was centrifuged at 10,000 × *g* for 15 min to remove polyacrylamide. The supernatant was dialyzed against deionized water containing 0.02% sodium azide for 48 h at room temperature to remove SDS and then lyophilized. The dried LPS bands were dispersed in water at a concentration of 10 mg/ml and subjected to re-electrophoresis as described above.

Antiserum preparation. Rabbit antisera were produced by intradermal injection of purified LPS of *V. parahaemolyticus* P26 or P68. The primary immunization dose consisted of a 0.2-ml suspension containing 200 μg of LPS emulsified with an equal volume of complete Freund's adjuvant (Difco). Three additional boosters were administered. Each booster consisted of 100 μg of LPS emulsified in incomplete Freund's adjuvant (Difco) as described by Meagher and Ornston (33).

Western immunoblotting of LPS. The conditions used for gel electrophoresis of LPS for Western immunoblotting were the same as those described earlier, except that SDS and urea were omitted from the separating gel. No differences in LPS mobility and pattern were observed between systems with and without these additives. Pyle and Schill (39) have reported that transfer efficiency was reduced 10-fold by SDS in the gel. The LPS immunoblotting conditions described by Preston and Penner (38) were used with a minor modification. After electrophoresis, the gel was equilibrated in transfer buffer containing 25 mM Tris and 192 mM glycine for 30 min. LPS bands were transferred from gel to nitrocellulose (NC) membranes (0.45-μm pore size; Bio-Rad) in the transfer buffer by electroblotting, which was performed at 40 V (initial current, 75 mA) for 18 h at 3.5°C. After transfer, the NC membranes were rinsed in Tris-HCl-buffered saline (TBS) containing 20 mM Tris and 0.5 M NaCl, pH 7.5, for 10 min. Then the NC membranes were blocked in TBS containing 3% gelatin (G-8150; Sigma) for 30 min. After blocking, the NC membranes were incubated with rabbit antisera diluted 1:100 in TBS containing 1% gelatin overnight at room temperature. The NC membranes were rinsed in deionized water once for 10 min and in TBS containing 0.05% Tween 20 twice for 10 min each time and then incubated with goat anti-rabbit immunoglobulin G-horseradish peroxidase conjugate (Sigma) diluted 1:1,000 in TBS containing 1% gelatin for 1 h. The NC membranes were rinsed in water and TBS-Tween 20 as described previously and then incubated in a substrate solution which was prepared by adding 40 ml of cold methanol containing 120 mg of 4-chloro-1-naphthol (Sigma) to 200 ml of TBS containing 120 μl of cold hydrogen peroxide (30%). Color development was stopped by rinsing the NC membranes in deionized water after maximal color development. The developed NC membranes were air dried.

Chemical analysis. Total carbohydrate was determined by the phenol-sulfuric acid method (10) using glucose as the standard. Total phosphorus was measured by the method described by Ames and Dubin (1).

RESULTS AND DISCUSSION

LPSs isolated from all six strains of *V. parahaemolyticus* by the phenol-water method used in this study contained 0.1 to 0.5% protein as determined by a modification of Lowry's method (32). No significant A_{260} was observed in any of the LPS preparations (data not shown). The yields of purified LPS from all six strains ranged from 1.5 to 1.9% of the freeze-dried cell weight. This yield is within the range reported by Hisatsune et al. (18), whose LPS yields were 1.5 to 3.4% of the acetone-dried cell weight from strains of 12 different serotypes.

When 20 μg of LPS was loaded for SDS-PAGE analysis, three major bands appeared on the gel for each strain of *V. parahaemolyticus* (Fig. 1). The rough-type (Ra) LPS of *S. typhimurium* TV-119 also appeared to have an extra band which was above the major doublet band and was not expected. It may have resulted from aggregation of rough-type LPS. However, the smooth-type LPS of *S. typhimurium* LT2 had a ladderlike banding pattern of doublet bands, as expected. The SDS-PAGE banding pattern of the LPSs of all six *V. parahaemolyticus* strains indicated that these LPS molecules possibly did not have the O-specific side chain of repeated oligosaccharide units.

The lack of O-specific oligosaccharide units in *V. parahaemolyticus* LPS was supported by the staining method used in this study. Fomsgaard et al. (12) have found that LPS molecules containing a low number of fatty acids were washed out of the gel during overnight fixation in the silver staining procedure described by Tsai and Frasch (43). Ladderlike LPS bands were observed in *Pseudomonas aeruginosa* LPS by a modified silver staining method which omitted overnight fixation and included a periodate oxidation step prolonged from 5 to 20 min (12). Otherwise, *P. aerug-*

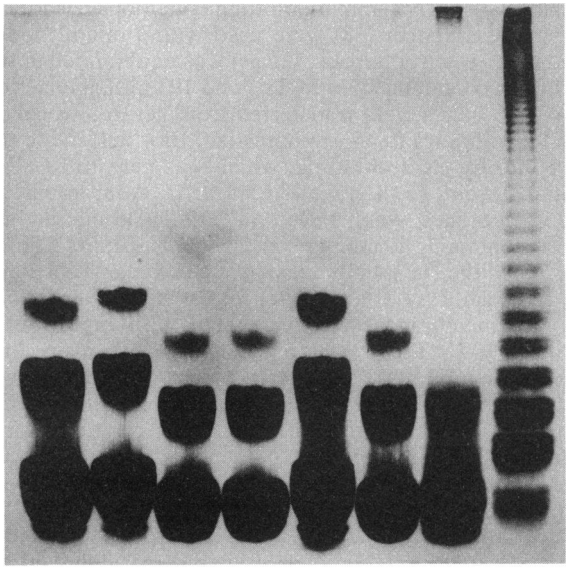

FIG. 1. Silver-stained SDS-PAGE banding patterns of LPSs. Lanes A through F contained LPSs from six strains of *V. parahaemolyticus* as follows: A, 38C1; B, P7; C, P26; D, 38C6; E, P68; F, P6. Lanes G and H contained LPSs from the Ra mutant of *S. typhimurium* TV-119 and smooth-type *S. typhimurium* LT2. The amounts of LPS loaded were 20 μg for all six strains of *V. parahaemolyticus* and the Ra mutant of *S. typhimurium* TV-119 and 10 μg for smooth-type *S. typhimurium* LT2.

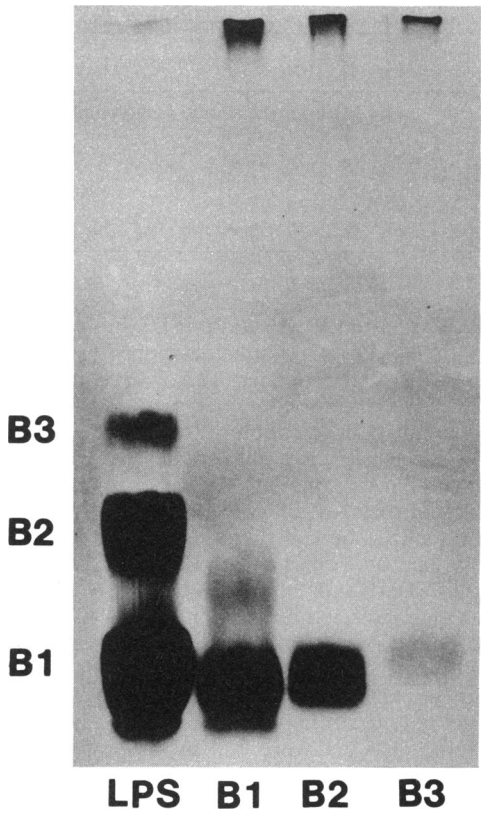

FIG. 2. Silver-stained SDS-PAGE banding pattern of the LPS and LPS bands from *V. parahaemolyticus* 38C1. Twenty micrograms of LPS and 20 μl of LPS band preparations were loaded for analysis.

inosa LPS appeared to be like rough-type LPS on SDS-PAGE gel stained by the traditional silver staining method described by Tsai and Frasch (43). The gel shown in Fig. 1 was visualized by the modified silver staining procedure (12), and no ladderlike LPS bands were observed. Kido et al. (25) reported that LPS containing acidic O-specific polysaccharides was better stained by ethidium bromide than by silver staining. Since uronic acids were found in *V. parahaemolyticus* LPS (19), ethidium bromide was used to stain the SDS-PAGE gel of the LPSs prepared from the six strains of *V. parahaemolyticus*. However, no high-molecular-weight ladderlike LPS was found (data not shown).

The three major bands that appeared on an SDS-PAGE gel of the LPSs of all six strains of *V. parahaemolyticus* were farther apart away from each other than those in the smooth-type LPS of *S. typhimurium* LT2 (Fig. 1). Even the rough-type (Ra) LPS of *S. typhimurium* TV-119 appeared to have a slower-moving band above the fastest-moving band. A similar banding pattern had been observed with the Ra LPS of *Salmonella minnesota* (20). The slower-moving band had been proved to be an aggregate of the LPS molecules in the fastest-moving band by extensive gel electrophoresis studies (20).

Multiple (more than two) widely separated LPS bands on SDS-PAGE gel have been reported for other gram-negative bacteria (2, 9, 14, 29, 42). These widely separated bands may start from the bottom or middle of the gel. But the common characteristics of these LPS bands are (i) the decrease in the distance between bands and (ii) the decrease in the intensity of each band as the apparent molecular weight of each band increases. This type of banding pattern of *Campylobacter jejuni* VC74 and *Aeromonas hydrophila* TF7 LPSs have been demonstrated to be caused by aggregation by using the re-electrophoresis method (9, 29). Gillespie et al. (14) proved the aggregation of *Wolinella recta* ATCC 33238 LPS on SDS-PAGE gel by analyzing the same LPS using deoxycholate-PAGE, which apparently prevented aggregation of the LPS. Although this type of banding pattern found in *Vibrio cholerae* and *Aeromonas aquamarinus* LPS was considered to be a smooth type or ladderlike (2, 42), it very likely resulted from aggregation of low-molecular-weight LPS molecules. The three major bands of *V. parahaemolyticus* 38C1 LPS on SDS-PAGE gel were isolated by using preparative SDS-PAGE and then re-electrophoresed by SDS-PAGE along with the original LPS. All three bands had similar mobilities (Fig. 2), which indicated that bands B2 and B3 of *V. parahaemolyticus* 38C1 LPS were aggregates of band B1. Aggregate formation during SDS-PAGE is a typical characteristic of a rough-type LPS (29). The molecular sizes of *V. parahaemolyticus* LPS were like that of the rough-type (Ra) LPS of *S. typhimurium* TV-119.

The chemical composition of the LPS of *V. parahaemolyticus* also suggested that *V. parahaemolyticus* LPS might be like rough-type LPS. The total carbohydrate contents of the LPSs of all six strains of *V. parahaemolyticus*, measured by the phenol-sulfuric acid method, ranged from 15.2 to 18.9% (Table 2). This range was lower than that of the smooth-type LPS of enteric bacteria. The total carbohydrate found in wild-type *Escherichia coli* JM103 was reported to be 24.6% (7), but the amount of lipid A prepared from *V. parahaemolyticus* LPS was significantly higher than that from enteric bacteria. The LPSs of these six strains of *V.*

TABLE 2. Total carbohydrate, total phosphorus, and lipid A in LPSs of *V. parahaemolyticus*[a]

Strain	TC (%)[b]	TP (%)[b]	LA (%)[c]	TC/LA ratio
38C1	17.8 ± 1.0	4.8 ± 0.2	41.0	0.43
P7	17.3 ± 1.3	4.8 ± 0.1	41.8	0.41
P26	15.3 ± 0.7	5.3 ± 0.1	46.3	0.33
38C6	15.2 ± 0.0	4.8 ± 0.0	45.0	0.34
P68	18.9 ± 1.0	5.0 ± 0.0	39.5	0.48
P6	15.8 ± 0.7	4.7 ± 0.1	44.5	0.36

[a] Abbreviations: TC, total carbohydrate; TP, total phosphorus; LA, lipid A.
[b] These data are means ± standard deviations of three experiments done in duplicate.
[c] These data are from reference 16.

parahaemolyticus contained 39.5 to 46.3% lipid A (Table 2) (16). Bramanti et al. (7) reported that lipid A accounted for 23.7% of the LPS of *E. coli* JM103. Thus, *V. parahaemolyticus* LPS had a low total carbohydrate-to-lipid A ratio (Table 2), which is typical for rough-type LPS (14, 29, 30). The total carbohydrate-to-lipid A ratio of a smooth-type LPS is usually greater than 1 (7, 30).

The LPSs of these six strains of *V. parahaemolyticus* were very similar to that of *C. jejuni* in several respects. *C. jejuni* LPS contains approximately 16% total carbohydrate by the phenol-sulfuric acid method and 48% lipid A (29). Thus, *C. jejuni* has a low total carbohydrate-to-lipid A ratio of 0.33, which is typical for the rough-type LPS of enteric bacteria (30). The banding pattern of *C. jejuni* LPS on SDS-PAGE gel is also like that of *V. parahaemolyticus*. When large amounts of LPS were loaded for SDS-PAGE, slower-moving aggregates containing dimers, trimers, or tetramers of the fastest-moving low-molecular-weight LPS appeared on the gel. However, when *C. jejuni* LPS was analyzed by SDS-PAGE and visualized by the immunoblotting method, the LPSs of some strains appeared to have a long O-specific side chain forming high-molecular-weight ladderlike bands which could not be visualized by silver staining (38). The immunoblotting method has been found to be superior to traditional silver staining for visualization of LPS profiles on SDS-PAGE gel (12, 15, 24, 31, 38).

The LPSs of all six strains of *V. parahaemolyticus* were further characterized by the immunoblotting method. The results indicated that none of the LPSs of these six strains had an O-specific side chain (Fig. 3 and 4). Relatively large amounts (10 μg) of LPS were loaded for PAGE, and aggregates of LPS were revealed on the gel by silver staining (Fig. 3). However, no ladderlike bands were revealed on the NC membranes by the immunoblotting method in any of the LPSs of these six strains of *V. parahaemolyticus* (Fig. 4). SDS was intentionally omitted from the gel system, since it reduces transfer efficiency during electroblotting (39). The LPS pattern on PAGE gel appeared to be the same as that on SDS-PAGE gel. Antiserum prepared from P68 LPS reacted relatively strongly with all serotype O4 LPSs, i.e., 38C1, P7, and P68 LPSs, although the reaction with 38C1 LPS was slightly weaker than that of the other two LPSs. It also had some weak cross-reaction with P26 LPS, which is serotype O2. P26 LPS antiserum not only reacted with serotype O2 P26 and P6 LPSs but also cross-reacted as strongly with serotype O3 38C6 LPS. There was no cross-reaction between P26 LPS antiserum and any O4 serotype LPS (Fig. 4). It was interesting that the immunochemical properties of the LPSs of these six strains were more correlated with mobility on PAGE gel than with serotype.

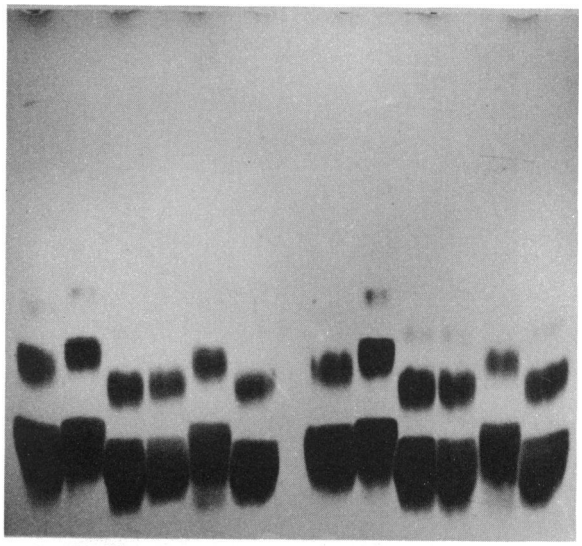

FIG. 3. Silver-stained PAGE banding patterns of the LPSs from six strains of *V. parahaemolyticus*. Two sets of LPS are displayed on this gel. Lanes: A, 38C1; B, P7; C, P26; D, 38C6; E, P68; F, P6. Ten micrograms of LPS from each strain was loaded for analysis.

Despite these findings indicating that the LPSs of these six strains of *V. parahaemolyticus* were rough-type LPSs, other characteristics suggested otherwise. The LPSs of all six strains had to be extracted by the phenol-water method, which is usually used for smooth-type LPS (45). The phenol-chloroform-petroleum ether method for rough-type LPS extraction was virtually useless for *V. parahaemolyticus*

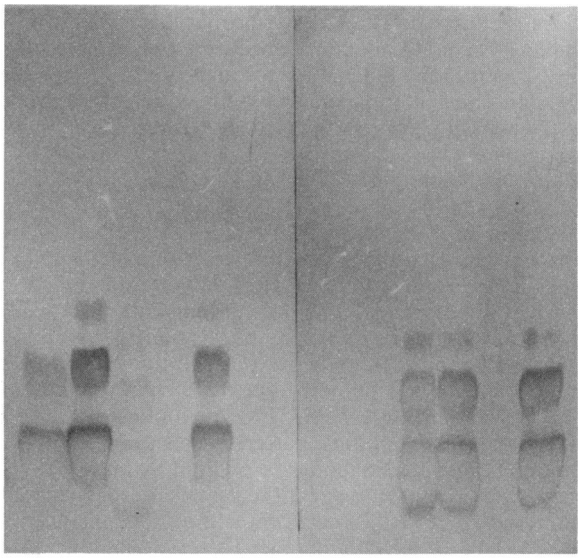

FIG. 4. Immunoblots of the LPSs of the six strains of *V. parahaemolyticus* in parallel with the silver-stained PAGE gel in Fig. 3. The lane assignments are the same as in Fig. 3. The left set was developed with P68 LPS rabbit antiserum, and the right set was developed with P26 LPS antiserum.

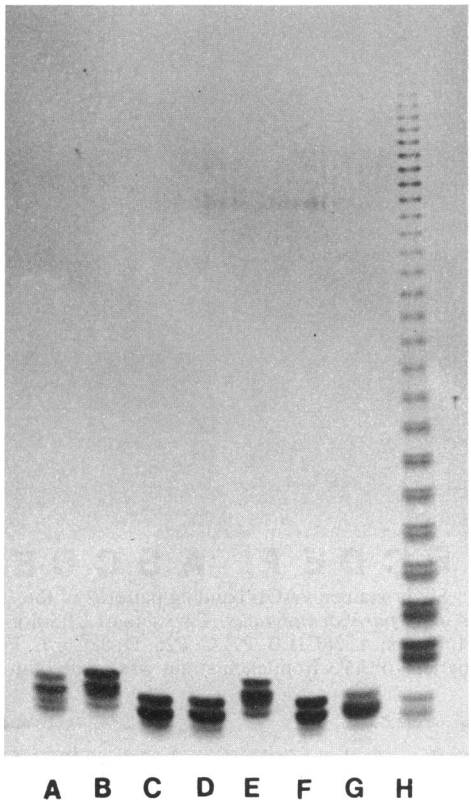

FIG. 5. Silver-stained gradient SDS-PAGE banding patterns of LPSs. The lane assignments are the same as in Fig. 1. The amounts of LPS loaded were 0.3 μg for all six strains of *V. parahaemolyticus* and the Ra mutant of *S. typhimurium* TV-119 and 2.5 μg for smooth-type *S. typhimurium* LT2.

LPS extraction (8). The colony morphology of *V. parahaemolyticus* on solid medium appeared to be smooth and entire. Lastly, it is unusual for a rough-type LPS lacking an O-specific side chain to have an extensive serotyping scheme (29). Twelve O serotypes have been identified for *V. parahaemolyticus* (34).

When small amounts (0.3 μg) of *V. parahaemolyticus* LPS were analyzed by gradient SDS-PAGE (10 to 15%), no aggregation of LPS was observed and the fastest-moving bands were resolved into either a triplet or a quadruplet unit (Fig. 5). This unit contained two major and one or two additional minor bands. The overall molecular size was like that of the rough-type (Ra) LPS of *S. typhimurium* TV-119, which had mobility similar to that of the fastest-moving doublet band of the smooth-type LPS of *S. typhimurium* LT2. The smooth-type LPS of *S. typhimurium* LT2 has a typical ladderlike banding pattern. This suggests that *V. parahaemolyticus* LPS consists of lipid A and a core oligosaccharide only. However, the microheterogeneity of *V. parahaemolyticus* LPS was greater than that of the rough-type (Ra) LPS of *S. typhimurium* TV-119 (triplet or quadruplet versus doublet). This indicates that *V. parahaemolyticus* LPS could have more diversified lipid A-core oligosaccharide molecules than does *S. typhimurium* LPS. Whether the diversity of lipid A-core oligosaccharide molecular structure is due to (i) variation of the lipid A or core oligosaccharide structure or (ii) the extent of phosphorylation is still unknown (29). The dark bands across the upper part of the gel resulted from the impurities in the mercaptoethanol added to the sample buffer (11).

The LPSs of these six strains of *V. parahaemolyticus* can be categorized into two groups based on mobility on SDS-PAGE gel. Strains 38C1, P7, and P68 had similar mobilities, which were slightly slower than those of strains P26, 38C6, and P6. The latter three strains had similar mobilities. This suggests that the LPSs of strains 38C1, P7, and P68 have larger molecular sizes than do those of strains P26, 38C6, and P6. This is supported by the ratio of total carbohydrate to lipid A of each strain (Table 2). Strains 38C1, P7, and P68 had slightly higher total carbohydrate/lipid A ratios than strains P26, 38C6, and P6. Also, strains 38C1, P7, and P68 all belong to serotype O4 but strains 38C1 and P7 are K^+ and strain P68 is K^-. Strains P26 and P6 are both serotype O2; however, P26 is K^+ and P6 is K^-. Strain 38C6 is serotype O3 and K^-. Therefore, the characteristics of *V. parahaemolyticus* LPS were dependent on the serotype of the particular strain and had no correlation with the Kanagawa phenomenon.

All six strains of *V. parahaemolyticus* had similar levels of total phosphorus in their LPSs, ranging from 4.7 to 5.3%, regardless of their serotypes or Kanagawa phenomenons (Table 2). This result is consistent with that of Hisatsune et al. (18). It is noteworthy that total phosphorus in *V. parahaemolyticus* LPS was much higher than that in enterobacterial LPS. The LPS of *E. coli* JM103 had only 0.72% total phosphorus (or 2.2% total phosphate) (7). A high level of phosphorus in *V. parahaemolyticus* LPS has also been reported by Deneke and Colwell (8). The 3-deoxyoctulosonic acid in the LPSs of *V. cholerae* and *V. parahaemolyticus* has been found to be virtually completely phosphorylated (6, 16). Highly phosphorylated LPS may be critical for survival of *V. parahaemolyticus* in natural aquatic environments (8).

In summary, the LPSs of all six strains of *V. parahaemolyticus* appeared to be like rough-type (Ra) enterobacterial LPS lacking an O-specific side chain when they were analyzed by SDS-PAGE with silver staining and by immunoblotting with PAGE gel. This was supported by their rather low ratio of total carbohydrate to lipid A and aggregate formation during gel electrophoresis. Kondo et al. (26) also found that the LPS of *V. parahaemolyticus* serotype O12 strain OP204 was of the rough type and lacked an O-specific side chain. However, *V. parahaemolyticus* LPS was extracted better by the phenol-water method, which was developed for smooth-type LPS (8, 45), and *V. parahaemolyticus* colonies appeared to be entire and smooth on solid medium. The results of both SDS-PAGE and the chemical methods used in this study showed that the characteristics of *V. parahaemolyticus* LPS are dependent on the serotype and not the Kanagawa phenomenon of the specific strain.

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