INFECTION AND IMMUNITY, Dec. 1989, p. 3901–3906
0019-9567/89/123901-06$02.00/0

Vol. 57, No. 12

Structures and Sugar Compositions of Lipopolysaccharides Isolated from Seven *Actinobacillus pleuropneumoniae* Serotypes

WYATT BYRD AND SOLOMON KADIS*

Department of Medical Microbiology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602

Received 31 August 1989/Accepted 7 September 1989

Highly purified lipopolysaccharide (LPS) preparations obtained from seven *Actinobacillus pleuropneumoniae* strains representative of seven different serotypes were used to determine the structure and monosaccharide composition of the polysaccharide components of each lipopolysaccharide. An indication of the structure of each LPS was obtained by procedures that included sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by silver staining and gel chromatographic fractionation of acetic acid-hydrolyzed LPS. The polysaccharide components of the LPSs were analyzed by gas-liquid chromatography. The LPSs of the strains of serotypes 2, 4, and 7 were of the smooth type, and those of the strains of serotypes 3 and 6 were of the rough type; the LPSs of the strains of serotypes 1 and 5 could be considered semirough. Rhamnose was present only in the O polysaccharide of the smooth-type and semirough-type LPSs, whereas galactose was present only in the O polysaccharide of the smooth-type LPS and in the core oligosaccharides of the rough-type and semirough-type LPSs. Glucoheptose and mannoheptose were present in the core oligosaccharides of all the LPSs except for the strain of serotype 3, in which only mannoheptose was detected. *N*-Acetylglucosamine was detected only in the O polysaccharides of the strains of serotypes 1 and 5.

Actinobacillus (*Haemophilus*) *pleuropneumoniae* is the causative agent of a severe, contagious, and often fatal respiratory disease of swine that can be referred to as porcine *Actinobacillus* pleuropneumonia. This disease, which can occur in a peracute, acute, subacute, or chronic form, is characterized by fibrinous pleuritis and hemorrhagic, necrotizing pneumonia (16).

Although the pathogenesis of porcine *Actinobacillus* pleuropneumonia has by no means been completely elucidated, the lipopolysaccharide (LPS) synthesized by *A. pleuropneumoniae* has been implicated as a virulence factor (3, 4, 13, 18). The *A. pleuropneumoniae* LPS has been obtained in highly purified form essentially free (less than 0.1%) of contaminating protein, nucleic acid, cellular phospholipid, and capsular polysaccharide (11). Lesions similar to those observed in pigs or mice, which die during the acute course of the infection, can be reproduced in both of these species by intratracheal or intranasal administration, respectively, of purified *A. pleuropneumoniae* LPS (3, 18). However, the two main features of the lung lesions observed following natural or experimental infection (i.e., hemorrhage and extensive tissue necrosis) were not elicited following LPS administration. These manifestations of the lung lesions were probably induced by a heat-labile protein toxic factor produced by *A. pleuropneumoniae* (18). Thus, it appears that LPS is not by itself responsible for the full spectrum of characteristic lesions observed in the acute form of the disease but acts in conjunction with one or more other toxic factors liberated by the causative agent.

The lung lesions in pigs elicited by the smooth-type *A. pleuropneumoniae* LPS have been reported to be similar to those elicited by the rough-type LPS isolated from the same strain of *A. pleuropneumoniae* belonging to serotype 5 (3). However, much higher doses of the smooth-type LPS were required, and the onset was delayed. In addition, the clinical signs and the hematological alterations typical of endotoxic shock induced by the rough-type LPS were more severe and observed at lower doses than similar alterations induced by the smooth-type LPS. Structures of LPSs of various *A. pleuropneumoniae* strains of different serotypes have not been compared. This would be useful in comparing the ability of these LPSs to produce lung lesions in pigs. The purpose of this study was to obtain a better understanding of the role that *A. pleuropneumoniae* LPS plays in the pathogenesis of porcine *Actinobacillus* pleuropneumonia by evaluating the LPSs isolated and purified from seven different strains of *A. pleuropneumoniae* representative of seven different serotypes in terms of (i) the LPS structure ascertained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and gel chromatography and (ii) the LPS monosaccharide composition determined by gas-liquid chromatographic (GLC) analysis.

MATERIALS AND METHODS

Bacteria and growth conditions. Seven different strains representing seven different serotypes of *A. pleuropneumoniae* were stored as lyophilized powders. The seven strains used were Shope 1 (4074), 1536, 12864, M62, K17, Femo, and 53, representative of serotypes 1 through 7, respectively. Each of the lyophilized materials was reconstituted in 50 ml of tryptic soy broth containing 0.6% yeast extract and 0.01% NAD and incubated at 37°C with shaking for 24 h. This culture (500 μl) was streaked onto solid medium (tryptic soy agar) of the same composition and allowed to grow at 37°C for 24 h. A heavy inoculum of the bacterial growth was placed into 100 ml of fresh liquid cultivation medium and allowed to incubate at 37°C with shaking for 12 h. Then, 20 ml of this culture was inoculated into 1 liter of fresh cultivation medium and allowed to incubate at 37°C with shaking for 24 h. The cells were harvested by centrifugation at 13,000 × *g* for 20 min at 4°C and washed twice with deionized water. The final pellet was suspended in deionized water and lyophilized. These lyophilized cells were used for the isolation and purification of the LPS.

LPS isolation and purification. The procedure of Darveau and Hancock (1) was used to isolate and purify LPS from

* Corresponding author.

each of the seven *A. pleuropneumoniae* strains used for this study. Disrupted *A. pleuropneumoniae* cells were treated with DNase, RNase, pronase, and SDS, and the LPS extract was subjected to magnesium chloride precipitation and high-speed centrifugation. All LPS preparations were essentially free (less than 0.1%) of contaminating protein, nucleic acid, cellular phospholipid, and capsular polysaccharide when analyzed by procedures previously described (11).

Analysis of structure of LPS by SDS-PAGE and gel chromatography. The LPS derived from each of the seven *A. pleuropneumoniae* strains was subjected to SDS-PAGE. The procedure involved the use of the Laemmli buffer system (10) and separating and stacking gels of 15 and 4% acrylamide-bisacrylamide, respectively. Gels were stained by the Morrissey silver staining procedure (12). The LPS derived from each of the seven *A. pleuropneumoniae* strains was also subjected to gel chromatography. To remove the lipid A from the polysaccharide portion of the LPS, each *A. pleuropneumoniae* LPS preparation was partially hydrolyzed with 1% acetic acid at 100°C for 1.5 h, neutralized with sodium hydroxide, and centrifuged at 10,000 × *g* for 30 min to remove the lipid A in the pellet (8). The pellet was washed twice with warm deionized water followed by centrifugation at 10,000 × *g* for 30 min to remove residual polysaccharide. All of the supernatant fluids containing the polysaccharide portion of the LPS were pooled and concentrated by evaporation under vacuum (lyophilization). The lyophilized polysaccharide was dissolved in buffer and fractionated on a Sephadex G-50 column (1.5 by 100 cm) at a flow rate of 6 ml/h. The void volume was determined by the use of dextran blue. The effluent was collected in 3-ml fractions, which were analyzed for neutral sugars by the anthrone method (15).

Analysis of sugar composition of LPS by GLC. GLC analysis was performed on the O-polysaccharide and core oligosaccharide fractions of the LPS derived from each of the seven strains of *A. pleuropneumoniae*. The GLC procedure of York et al. (21), involving alditol acetate derivatives of the O-polysaccharide and core oligosaccharide components, was used to determine the monosaccharide composition of each of these preparations. The polysaccharide samples (500 μg) were heated at 100°C for 1 h in 1% acetic acid. The acetic acid was removed by air flow at 40°C, and any residual acetic acid was removed with toluene (two 200-μl washes). The sample was dissolved in 200 μl of water containing 10 mg of NaB_2H_4 per ml and treated at 20°C for 1 h. Excess NaB_2H_4 was destroyed by the addition of 50 μl of glacial acetic acid, and solvents were removed by air flow. The residue was treated with methanol containing 10% acetic acid (three 200-μl washes) and then methanol (three 200-μl washes). The solvents were removed by air flow. The dry residue was heated at 121°C for 1 h with 200 μl of trifluoroacetic acid (containing 100 μg of inositol per ml as an internal standard). The trifluoroacetic acid was removed by air flow, and any residual trifluoroacetic acid was removed by the addition of isopropanol (two 200-μl washes). The residue was dissolved in 200 μl of water containing 10 mg of NaB_2H_4 per ml and treated at 20°C for 1 h. The NaB_2H_4 was removed as before. To the dry residue, 50 μl of acetic anhydride and 50 μl of pyridine were added, and the sample was treated at 121°C for 20 min. The sample was cooled, toluene was added (two 200-μl portions), and the solvent was removed by air flow. To the residue, 500 μl of water and 500 μl of dichloromethane were added, and the phases were separated by centrifugation. The organic phase was transferred to a clean tube and concentrated to dryness by air flow. The residue was dissolved in 100 μl of acetone prior to analysis by GLC.

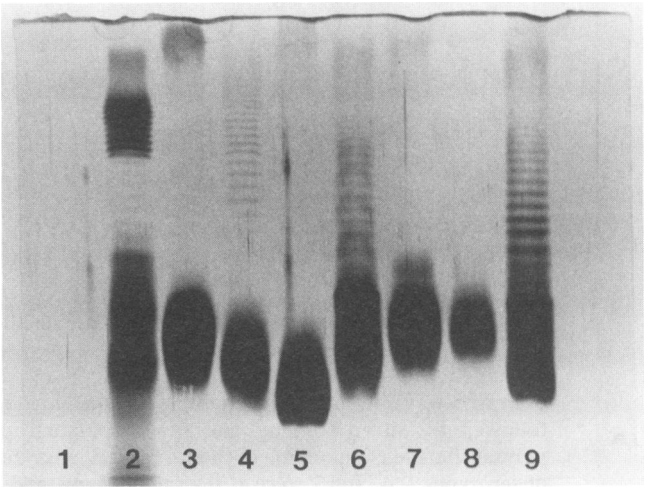

FIG. 1. Silver-stained SDS-polyacrylamide gels of the LPSs of *A. pleuropneumoniae* strains representative of serotypes 1 through 7 and *E. coli* O111:B4. Lane 1, Sample buffer; lane 2, *E. coli* O111:B4 LPS; lane 3, LPS of serotype 1; lane 4, LPS of serotype 2; lane 5, LPS of serotype 3; lane 6, LPS of serotype 4; lane 7, LPS of serotype 5; lane 8, LPS of serotype 6; lane 9, LPS of serotype 7. In lanes 2 through 9, 20 μg of LPS was added per lane.

The GLC analysis was done with a Hewlett-Packard 5970 chromatograph fitted with a hydrogen flame ionization detector (240°C) and a 5880A electronic integrator. The alditol acetates were analyzed by GLC in a SP2330 fused silica capillary column (15 m by 0.25 mm; Supelco) at 235°C, and samples (1 μl) were injected in the split mode (injection temperature, 240°C).

The quantities of monosaccharides present are stated in moles percent (mol%), defined as moles of carbohydrate recovered per 100 mol of total carbohydrate recovered.

RESULTS

SDS-PAGE of intact LPS and gel chromatography of partially hydrolyzed LPS. The SDS-PAGE profiles of silver-stained LPSs isolated and purified from seven different strains representative of seven different serotypes are shown in Fig. 1. The LPS profiles of strains representative of serotypes 2, 4, and 7 demonstrate the ladderlike arrangement characteristic of smooth-type LPS (6). The LPSs of strains of serotypes 1, 3, 5, and 6 lacked the ladderlike SDS-PAGE profile, but a large, deeply staining area representative of the core oligosaccharide and lipid A was detected just trailing the leading edge of the dye front. The strains of serotypes 3 and 6 showed only representative LPS profiles for rough-type LPS, whereas the LPS profiles of the strains of serotypes 1 and 5 displayed stained bands in addition to that of the core oligosaccharide and lipid A. The SDS-PAGE profile for the LPS of the strain of serotype 1 displayed a high-molecular-weight band at the top of the gel, and the SDS-PAGE profile for the LPS of the strain of serotype 5 displayed a low-molecular-weight band just above the deeply staining area representative of the core oligosaccharide and lipid A.

Gel chromatography of the partially hydrolyzed LPS obtained from each of the seven different strains was performed to obtain a more complete analysis of the structure of

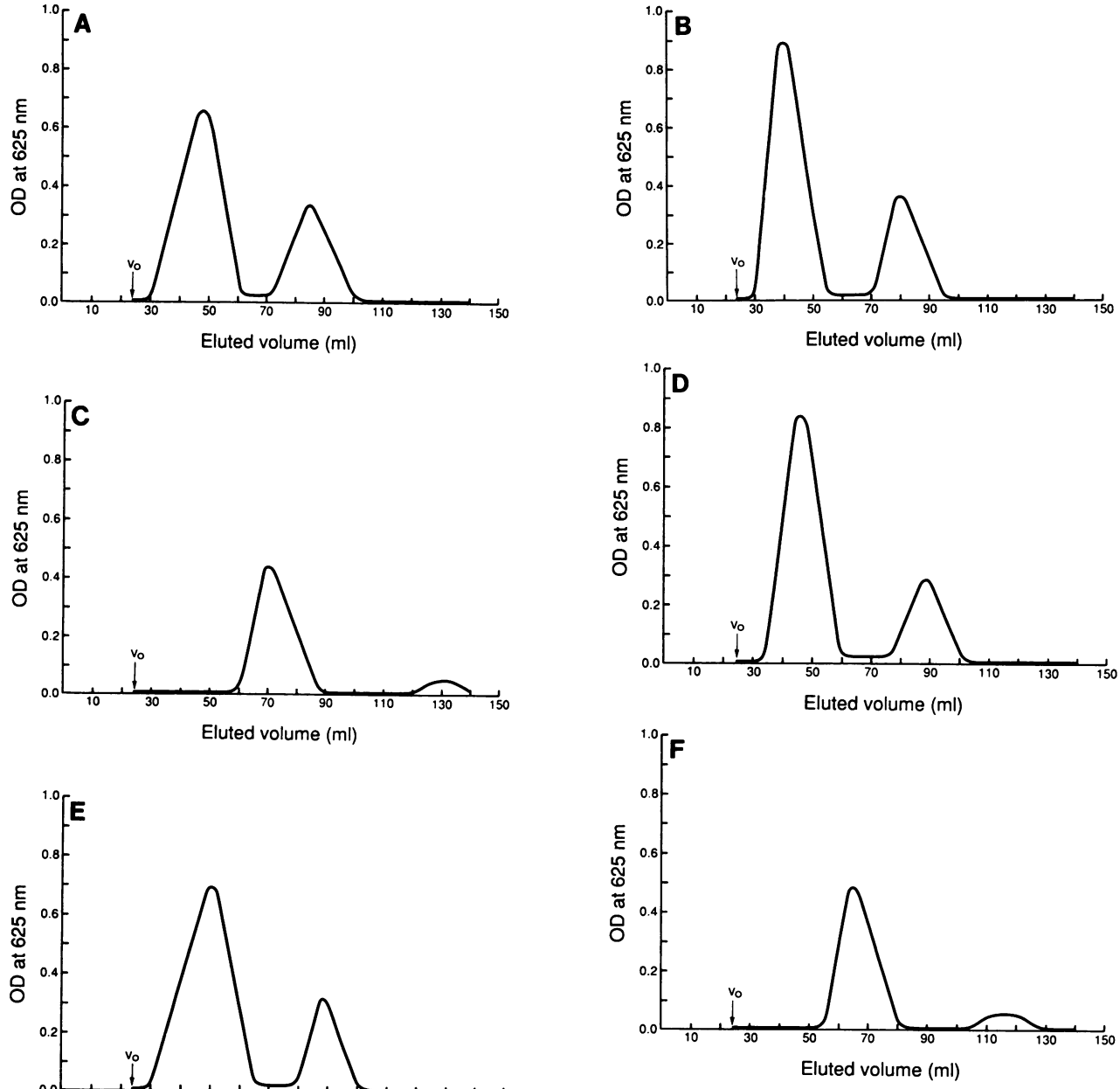

FIG. 2. Gel chromatographic analysis of acetic acid-hydrolyzed LPSs from *A. pleuropneumoniae* strains representative of serotypes 1 through 7. The first peak for the smooth-type (B, D, and G) and semirough (A and E) LPSs is representative of O-antigenic side chains, and the second peak is representative of the R core. The first peak for the rough-type (C and F) LPS is representative of the R core, and the second peak is representative of hydrolysis products of the R core. V_O, Void volume. OD, Optical density.

each LPS; the results are shown in Fig. 2. The LPSs of the strains of serotypes 2, 4, and 7 (Fig. 2B, D, and G, respectively) showed two peaks. The first peak represents primarily the O polysaccharide, and the second peak represents primarily the core oligosaccharide. This gel chromatographic profile is indicative of a smooth-type LPS. The gel chromatographic analysis of the partially hydrolyzed LPSs of the strains of serotypes 3 and 6 (Fig. 2C and F, respectively) showed a single large peak representative of the core oligosaccharide. A second smaller peak was detected after the

TABLE 1. Sugar compositions of LPS components (O-antigenic side chain and R core) from *A. pleuropneumoniae* strains representative of seven different serotypes

Monosaccharide[a]	Amt (mol%) of monosaccharide in strain of indicated serotype													
	O-antigenic side chain							R core						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Rha	45.5	11.8		22.9	41.2		20.2							
Gal		20.9		33.6			73.9	17.8		6.1		16.7	29.6	
Glc	41.9	62.3		32.8	34.3		5.9	27.4	55.2	46.5	35.2	28.2	48.8	45.8
Glchep								12.1	18.2		21.3	15.1	6.0	5.6
Manhep								24.2	26.6	40.8	25.9	36.5	12.0	21.1
GlcNAc	9.1				9.9									
KDO								0.9	0.9	2.7	0.9	1.1	0.7	1.5

[a] Rha, Rhamose; Gal, galactose; Glc, glucose; Glchep, glucoheptose; Manhep, mannoheptose; GlcNAc, *N*-acetylglucosamine; KDO, 2-keto-3-deoxyoctulosonic acid.

R core had eluted from the column. This was believed to contain hydrolysis products such as 2-keto-3-deoxyoctulosonic acid (KDO), ethanolamine, and phosphorylated derivatives of the core oligosaccharide and not to be indicative of LPS (9). This gel chromatographic profile indicated that these LPSs are of the rough type. The gel chromatographic analysis of the partially hydrolyzed LPSs of the strains of serotypes 1 and 5 (Fig. 2A and E, respectively) revealed two peaks, indicative of the O polysaccharide and core oligosaccharide. Lack of a typical smooth-type SDS-PAGE profile coupled with the presence of two distinct polysaccharide components of the LPS suggests that the LPSs of these strains of serotypes 1 and 5 could be considered semirough.

Analysis of the sugar composition of the O-polysaccharide and core oligosaccharide components of partially hydrolyzed LPS by GLC. The monosaccharide contents of the O-polysaccharide and core oligosaccharide components of the seven different partially hydrolyzed LPS preparations as revealed by GLC are given in Table 1. Rhamnose was detected only in the O polysaccharides of the smooth-type and semirough LPSs of the strains of serotypes 2, 4, and 7 and serotypes 1 and 5, respectively, and not at all in their core oligosaccharides; also, strains of serotypes 3 and 6, which contain rough-type LPSs, displayed no rhamnose peak during GLC analysis. Galactose was detected only in the O polysaccharides of strains with smooth-type LPSs (i.e., strains of serotypes 2, 4, and 7) and not at all in their core oligosaccharides or in the core oligosaccharides of the rough-type and semirough LPSs (i.e., strains of serotypes 3 and 6 and serotypes 1 and 5, respectively). In the semirough LPSs of the strains of serotypes 1 and 5, galactose was not detected in the O polysaccharide. The galactose content detected in the O polysaccharide of the LPS of the strain of serotype 7 was much higher than that detected in the other LPS preparations and represented 73.9 mol% of the total sugar content of the O polysaccharide of this LPS.

Glucose was detected in the O polysaccharides and core oligosaccharides of both the smooth-type LPSs of the strains of serotypes 2, 4, and 7 and the semirough LPSs of the strains of serotypes 1 and 5. Glucose also was found in the core oligosaccharides of the rough-type LPSs of strains of serotypes 3 and 6. The glucose content of the O polysaccharide of the smooth-type LPS of the strain of serotype 7 was surprisingly low, representing only 5.9 mol% of the total sugar present in the O polysaccharide of this LPS.

Two sugars, glucoheptose and mannoheptose, were detected in the core oligosaccharide of the LPS from each of the seven strains, except for the rough-type LPS of the strain of serotype 3, which contained only mannoheptose in its core oligosaccharide. When glucoheptose and mannoheptose were detected together, glucoheptose was the predominant heptose. The semirough LPSs of the strains of serotypes 1 and 5 contained *N*-acetylglucosamine in the O polysaccharide but not in the core oligosaccharide. *N*-Acetylglucosamine was not detected in the O-polysaccharide or core oligosaccharide components of the partially hydrolyzed LPSs of the other strains analyzed by GLC.

The semirough LPS of the strain of serotype 1 was very similar in its GLC profile to the LPS of the strain of serotype 5. The O polysaccharides and core oligosaccharides of both of these semirough LPS preparations contained the same sugars and in very similar amounts. The monosaccharide *N*-acetylglucosamine was present only in the O polysaccharide of each of these semirough LPS preparations.

The KDO contents detected in the LPS preparations from the seven strains tested appeared to be quite similar but rather low compared with the amounts of the other sugars in these preparations. The exact quantitation of KDO by the use of GLC analysis appears questionable because of the instability of KDO upon treatment with weak acid to remove the lipid from the polysaccharide portion of the LPS. Nevertheless, the quantity of KDO revealed by GLC analysis appeared to be quite similar to that obtained by colorimetric analysis for *A. pleuropneumoniae* serotype 2 LPS (11).

DISCUSSION

The accuracy of the findings stemming from the analyses of the structures and sugar compositions of the LPSs of the seven different *A. pleuropneumoniae* strains representative of seven different serotypes reported herein depends to a large extent on the purity of the LPS preparations. The method of Darveau and Hancock (1) was used for the isolation and purification of *A. pleuropneumoniae* LPS because it has been found eminently satisfactory for obtaining both smooth- and rough-type LPSs from a variety of bacterial species in a highly purified form. This was considered a primary attribute of this method because it is now well established that within a single bacterial species, size heterogeneity expresses itself such that organisms containing smooth-type LPS may also contain some LPS molecules lacking O-antigenic side chains, as well as molecules containing various numbers of covalently bound O-antigenic side chain units (1, 5, 11, 20). In fact, it has been demonstrated that 40 to 50% of the smooth-type LPS of *Escherichia coli* O111 contains fewer than five repeating units of the O-antigenic side chain (7). In contrast, the LPS preparations of selected strains of *Pseudomonas aeruginosa* contained

over 80% rough core oligosaccharides without any covalently linked O-antigenic side chains (20). A previous study involving the isolation, purification, and partial characterization of *A. pleuropneumoniae* 1536, serotype 2, revealed that the use of the method of Darveau and Hancock yielded a highly purified, smooth-type LPS in relatively high yields (53%) (11). Analyses of the seven LPS preparations used for this study showed that they were as highly purified as the serotype 2 preparation described previously.

The SDS-PAGE silver-stained LPS profiles of the strains of serotypes 2, 4, and 7 demonstrated the ladderlike arrangement that is characteristic of smooth-type LPS, whereas the LPSs of the strains of serotypes 3 and 6 gave a staining characteristic of rough-type LPS. The SDS-PAGE silver-stained gel of the LPSs of the strains of serotypes 1 and 5 gave a somewhat typical rough-type LPS profile characterized by a large, deeply staining R core and lipid A and a lack of the ladderlike arrangement seen for smooth-type LPS. However, the LPS profiles of these strains of serotypes 1 and 5 displayed stained bands in addition to that of the R core and lipid A. The SDS-PAGE silver-stained LPS profile for the strain of serotype 1 showed a high-molecular-weight band at the top of the gel, and the SDS-PAGE silver-stained LPS profile for the strain of serotype 5 showed a low-molecular-weight band just above the R core and lipid A stained area. The high-molecular-weight band observed in the SDS-PAGE LPS profile for the strain of serotype 1 is unexplained at the present time. The low-molecular-weight band observed in the SDS-PAGE LPS profile for the strain of serotype 5 is believed to represent one repeat unit of the O polysaccharide. This SDS-PAGE LPS profile is consistent with the LPS of this strain of serotype 5 being considered semirough. The bands that make up the ladderlike arrangement are believed to be composed of different-size O-antigenic side chain repeat subunits, with the larger side chain repeat units migrating at a slower rate in the polyacrylamide gel. Tsai and Frasch (17) and Morrissey (12) have developed highly sensitive staining procedures for bacterial LPS in polyacrylamide gels, which together with various modifications are being extensively used to detect and reveal the structure of LPS (1, 6, 14).

Gel chromatographic fractionation was performed on each LPS polysaccharide component obtained after acetic acid hydrolysis of the LPS isolated from each of the seven *A. pleuropneumoniae* strains. When the LPSs of the strains of serotypes 2, 4, and 7, which exhibited a smooth-type LPS profile on SDS-PAGE, were passed through a Sephadex G-50 column, two elution peaks indicative of O polysaccharide and core oligosaccharide were detected. GLC analysis of these two peaks revealed rhamnose and galactose occurring only in the first peak (indicative of the O-polysaccharide component of the LPS), whereas the sugars glucoheptose and mannoheptose were found to occur only in the second peak (indicative of the core oligosaccharide component of the LPS). This finding is consistent with rhamnose appearing in only the O polysaccharide of LPS and was expected since rhamnose is found in the O-antigenic side chains of the LPSs obtained from a variety of different gram-negative bacteria (2, 3, 19). Thus, it appears that the LPSs of the strains of serotypes 2, 3, and 7 are of the smooth type. When the LPSs of the strains of serotypes 3 and 6, which exhibited rough-type LPS profiles on SDS-PAGE, were passed through a Sephadex G-50 column, only one large elution peak (indicative of core oligosaccharide) was detected. GLC analysis of this peak revealed galactose but no rhamnose and also the presence of the sugars glucoheptose and/or mannoheptose, which are found only in core oligosaccharide. Consequently, it appears that the LPSs of the strains of serotypes 3 and 6 are of the rough type. On the other hand, gel chromatographic fractionation of the polysaccharides of the LPSs of the strains of serotypes 1 and 5, which exhibited somewhat typical rough-type LPSs on SDS-PAGE, yielded two elution peaks, indicative of O polysaccharide and core oligosaccharide. GLC analysis of the peaks revealed rhamnose and *N*-acetylglucosamine occurring only in the first peak (indicative of the O-polysaccharide component of the LPS), whereas GLC analysis of the second peak revealed the presence of galactose and also the sugars glucoheptose and mannoheptose, which are found only in the core oligosaccharide. This seems to indicate that these LPSs can be considered semirough. It appears that they contain only truncated O-antigenic side chains with reduced numbers of O-antigenic side chain units.

Thus, SDS-PAGE, gel chromatographic fractionation, and subsequent GLC analysis of the fractionated polysaccharide components of the *A. pleuropneumoniae* LPSs indicated that the LPSs of the strains of serotypes 2, 4, and 7 are of the smooth type, the LPSs of the strains of serotypes 3 and 6 are of the rough type, and the LPSs of the strains of serotypes 1 and 5 are of the semirough type.

ACKNOWLEDGMENTS

This work was supported by U.S. Department of Agriculture Special Animal Health Research grant 84-CRSR-2-2433, by grant 87-224 from the Veterinary Medical Experiment Station, University of Georgia, and by grant 87-206 from the Georgia Department of Agriculture Swine Disease Eradication Program.

LITERATURE CITED

1. **Darveau, R. P., and R. E. W. Hancock.** 1983. Procedure for isolation of bacterial lipopolysaccharides from both smooth and rough *Pseudomonas aeruginosa* and *Salmonella typhimurium* strains. J. Bacteriol. **155**:831–838.
2. **Dmitriev, B. A., Y. A. Knirel, N. K. Kochetkov, and I. L. Hofman.** 1976. Somatic antigens of *Shigella*. Structural investigation on the O specific polysaccharide chain of *Shigella dysenteriae* type 1 lipopolysaccharide. Eur. J. Biochem. **66**:559–566.
3. **Fenwick, B. W., B. I. Osburn, and H. J. Olander.** 1986. Isolation and biological characterization of two lipopolysaccharides and a capsular-enriched polysaccharide preparation from *Haemophilus pleuropneumoniae*. Am. J. Vet. Res. **47**:1433–1441.
4. **Fenwick, B. W., B. I. Osburn, and H. J. Olander.** 1986. Resistance of C3H/HeJ mice to the effects of *Haemophilus pleuropneumoniae*. Infect. Immun. **53**:474–479.
5. **Goldman, R. C., and L. Leive.** 1980. Heterogeneity of the antigenic-sidechain length in lipopolysaccharide from *Escherichia coli* O111 and *Salmonella typhimurium* LT2. Eur. J. Biochem. **107**:145–153.
6. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154**:269–277.
7. **Inzana, T. J., W. E. Seifert, and R. P. Williams.** 1985. Composition and antigenic activity of the oligosaccharide moiety of *Haemophilus influenzae* type b lipooligosaccharide. Infect. Immun. **48**:324–330.
8. **Kawaoka, Y., G. Wauters, K. Otsuku, and M. Tsukokura.** 1986. Identification of *Yersinia enterocolitica* O15 lipopolysaccharide as a rough antigen. J. Clin. Microbiol. **24**:272–274.
9. **Kropinski, A. M., J. Kuzio, B. L. Angus, and R. E. W. Hancock.** 1982. Chemical and chromatographic analysis of lipopolysaccharide from an antibiotic-supersusceptible mutant of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **21**:310–319.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the

assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Maudsley, J. R., S. Kadis, and W. R. Mayberry.** 1986. Isolation, purification, and partial characterization of a lipopolysaccharide from *Haemophilus pleuropneumoniae*. Infect. Immun. **51**:501–506.
12. **Morrissey, J. H.** 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. Anal. Biochem. **117**:307–310.
13. **Perfumo, C. J., C. Rehbinder, and K. Karlsson.** 1983. Swine pleuropneumonia produced by *Haemophilus pleuropneumoniae* III. An electron microscopic study. Zentralbl. Veterinaermed. **30**:678–684.
14. **Progulske, A., and S. C. Holt.** 1984. Isolation and characterization of the outer membrane and lipopolysaccharide from *Eikenella corrodens*. Infect. Immun. **43**:166–177.
15. **Scott, T. A., and E. H. Melvin.** 1983. Determination of dextran with anthrone. Anal. Chem. **25**:1656–1661.
16. **Sebunya, T. N. R., and J. R. Saunders.** 1983. *Haemophilus pleuropneumoniae* infection in swine. A review. J. Am. Vet. Med. Assoc. **182**:1331–1337.
17. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
18. **Udeze, F. A., K. S. Latimer, and S. Kadis.** 1987. Role of *Haemophilus pleuropneumoniae* lipopolysaccharide endotoxin in the pathogenesis of porcine *Haemophilus* pleuropneumonia. Am. J. Vet. Res. **48**:768–773.
19. **White, P. J., M. A. Gaston, and S. G. Wilkinson.** 1984. Composition of O-antigenic lipopolysaccharides from *Enterobacter cloacae*. Microbiol. Immunol. **28**:1169–1180.
20. **Wilkinson, S. G., and L. Galbraitit.** 1975. Studies of lipopolysaccharides from *Pseudomonas aeruginosa*. Eur. J. Biochem. **52**:331–343.
21. **York, W. S., A. G. Darvill, M. McNeil, and P. Albersheim.** 1985. 3-Deoxy-D-manno-L-octulosonic acid (KDO) is a component of rhamnogalacturonan II a pectic polysaccharide in the primary cell walls of plants. Carbohydr. Res. **138**:109–126.