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Physical Properties of Short- and Long-O-Antigen-Containing Fractions of Lipopolysaccharide from *Escherichia coli* 0111:B4

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Aggregates of short- and long-chain O-antigen-containing fractions of lipopolysaccharide were analyzed by electron spin resonance probing to reveal differences in their physical properties. The fluidities of the lipid regions of the two fractions were quite similar, although the long-chain lipopolysaccharide aggregates appeared to be more hydrated as reflected by the polarity determined with a lipid probe. In contrast, the head-group region of the long-chain fraction was dramatically more mobile than that of the short-chain sample. The binding of polycations (e.g., polymyxin B, spermine) to lipopolysaccharide aggregates was measured by the partitioning of a cationic spin probe. Less probe was displaced from the long-chain fraction and unseparated lipopolysaccharide than from the short-chain fraction by the addition of cations, suggesting that the long O-antigen masks anionic sites on lipopolysaccharide. These results indicate that the aggregate shape and reactivity of lipopolysaccharide are affected by O-antigen length. Thus, the biological activity of lipopolysaccharide may be modulated directly by the presence of O-antigen and indirectly by the effects of O-antigen on the lipopolysaccharide aggregate structure.

Gram-negative bacteria possess an outer membrane which serves as a permeability barrier to toxic compounds. A key component in this barrier function is the lipopolysaccharide (LPS) which is a major part of the outer monolayer of the membrane. LPS forms a rigid, highly charged surface that prevents the diffusion of hydrophobic compounds across the bilayer (24). In addition, LPS can form blebs off the bacterium and is found in sera of patients suffering from septicemia. This LPS can cause adverse host responses, including fever, shock, and even death (22).

In its interactions within a host or on the surface of the bacteria, LPS does not act in monomeric form but as large aggregates. The size and shape of these aggregates depend on temperature (3), ion content (4, 11, 17), and pH (R. T. Coughlin, A. A. Peterson, A. Haug, H. T. Pownall, and E. J. McGroarty, Biochim. Biophys. Acta, in press), as well as the composition of the LPS (17). Variations in biological activities between preparations may then be caused by variations in the physical properties of different aggregate structures. The LPS isolated from *Rhodospirillum tenue* is moderately toxic, whereas the lipid A prepared from it is 70 to 140 times more toxic than the intact LPS (21). Differences in toxicity may thus result from covering the lipid A by the core O-antigen of *R. tenue* LPS or from differences in the physical state of the aggregates modulating whether the inner regions of LPS are exposed and able to bind to or react with target structures.

LPS, as isolated from bacteria, is a heterogeneous collection of molecules which can vary as to substitution of the core and lipid A (20) and in the length of the O-antigen polysaccharide chain (12, 16, 23, 25, 27). The presence of covalently bound phosphate and acidic sugars in the core and lipid A make LPS a highly anionic molecule that typically has a number of associated cations (5, 11). Polycationic antibiotics can bind to these anionic sites, altering the LPS aggregate structure (1, 9, 15, 26, 30) and increasing outer membrane permeability (14, 31). The affinity of cations for LPS appears to depend on the charges in the core lipid A region, but the effect of O-antigen length on cation binding is unknown.

We separated the LPS of *Escherichia coli* 0111:B4 into short- and long-O-antigen-containing fractions to examine their respective aggregate structures by physical techniques. It was found that the length of the O-antigen did indeed affect LPS aggregate structure and polycation binding.

MATERIALS AND METHODS

LPS samples. LPS from *E. coli* 0111:B4 (phenol extracted) was purchased from Sigma Chemical Co. (St. Louis, Mo.). Short- and long-chain LPS fractions were separated on a Sephadex G-200 column in 0.2 M NaCl–0.25% deoxycholate–1 mM EDTA–0.02% NaN_3–10 mM Tris (pH 8.0) as previously described (27). Samples were dialyzed extensively in column buffer without deoxycholate to remove detergent and then in distilled water to form the sodium salt (sodium LPS). Unseparated LPS samples were washed of soluble contaminants by centrifugation at 100,000 × *g* for 90 min and dialyzed extensively against 10 mM sodium EDTA (pH 7.2) followed by dialysis against distilled water to form sodium LPS. The magnesium salt of LPS (magnesium LPS) was formed by extensive dialysis of sodium LPS against 10 mM $MgCl_2$ followed by distilled water. Samples were lyophilized and weighed to quantitate LPS concentrations. Elemental analysis of LPS was done by inductively coupled plasma emission spectroscopy of wet ashed samples as described previously (5).

ESR. Electron spin resonance (ESR) spectroscopy was carried out with a Varian X-band spectrometer. Measurements of LPS head-group mobility were made by using the probe 4-dodecyl dimethylammonium-1-oxyl-2,2,6,6-tetramethylpiperidine bromide (CAT_{12}) as previously described (2, 4), and LPS lipid mobility was detected with the probe 5-doxyl stearate (5DS) purchased from Molecular Probes (Portland, Oreg.). Synthetic 1-palmitoyl-2-oleoylphosphatid-

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ylethanolamine was obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Titrations of magnesium LPS suspended at 10 mg/ml were performed by measuring the spectral parameters of CAT_{12} (added at an 18:1 molar ratio of LPS-CAT_{12}) after successive additions of cations to samples at 37°C in 50 mM KOH-HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.0). Sample temperature was measured by a thermocouple placed within the cuvette. Spectra were analyzed for $2T_{\parallel}$, the hyperfine splitting (an indicator of mobility); S, the order parameter (13); A_o, the isotropic hyperfine coupling constant (an indicator of polarity [13]); and Ψ_i, the partitioning of probe between the free aqueous environment and bound LPS (2).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out in the buffer system of Laemmli (19) with a 15% acrylamide running gel and a 7.5% stacking gel as previously described (27). LPS bands were visualized by using a silver stain (7).

Electron microscopy. LPS samples were suspended at 0.1 mM in 50 mM KOH-HEPES (pH 7.0) and negatively stained with 1% (wt/vol) potassium phosphotungstate (pH 6.6) or 1% (wt/vol) ammonium molybdate (pH 7.0) at 37°C. The samples were examined in a Philips EM 300 transmission electron microscope.

RESULTS

The LPS of *E. coli* 0111:B4 was separated by gel filtration into short- and long-chain fractions containing average lengths of 1 and 18 O-antigen repeats, respectively (Fig. 1). Both fractions and unseparated material were converted into uniform magnesium salts to minimize the effects of different ions on their physical properties. Elemental analysis of these samples indicated that each had a phosphate-to-LPS ratio of approximately 6:1 (mol/mol), suggesting similar levels of phosphorylation (Table 1). The long-chain LPS fraction, though, had a slightly higher amount of Mg^{2+} bound per phosphate, possibly reflecting a slight amount of ion binding to the long carbohydrate (29). Isolated O-capsule treated in a manner identical to the magnesium LPS fractions had small

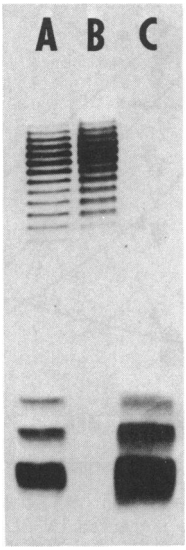

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel of (A) unseparated, (B) long-chain, and (C) short-chain LPS.

TABLE 1. Elemental composition of fractions of magnesium LPS from *E. coli* 0111:B4

Fraction	Ca-P ratio (mol/mol)	Mg-P ratio (mol/mol)	P-LPS ratio (mmol/g)	P-LPS ratio (mol/mol)[a]
Unseparated	0.0	0.52	0.61	6.7
Long chain	0.0	0.88	0.28	5.6
Short chain	0.0	0.71	1.01	5.9

[a] Assumes average molecular weights of 11,000 for the unseparated, 20,000 for the long-chain, and 5,800 for the short-chain fraction as determined from the known structures of the core lipid A (28) and the O-antigen (8) and the average number of O-repeats in each sample (27; Fig. 1).

levels of associated Mg^{2+} (0.11 μmol/mg). Thus, the excess of Mg^{2+} in the long-chain fraction may have resulted from metal ion association with the polysaccharide chains. Small variations in the Mg^{2+} content of LPS fractions between preparations did not significantly alter the ESR probing results.

The motion of the ESR spin probe 5DS in the LPS lipid regions appeared to differ only slightly when comparing the three magnesium LPS fractions at temperatures between 0 and 50°C (Fig. 2). The slightly lower degree of order detected in the short-chain and unseparated samples may reflect the fact that the probe intercalated deeper into the lipid region in these samples. The polarity of the spin probe environment above 25°C indicated that the probe sensed an increasing hydrophobicity in the samples in the order long-chain < short-chain < unseparated LPS (Fig. 3). These differences in polarity among the samples may have resulted either from differences in the depth to which the probe partitioned into the lipid or from differences in hydration of the lipid region, either of which would suggest differences in LPS packing between the fractions.

Measurement of polarity with a pure phospholipid indicated that the gel-to-liquid crystalline phase transition was marked by a large, rapid increase in hydrophobicity as the doxyl group of 5DS partitioned into the bilayer. 1-Palmitoyl-2-oleoylphosphatidylethanolamine had a gel-to-liquid crystalline transition at approximately 23°C, and 5DS partitioned from a highly hydrated environment below 23°C ($A_o > 17$) to a hydrophobic environment above 23°C ($A_o = 14.8$ [Fig. 3]). The change in polarity measured by 5DS in the LPS samples, however, decreased relatively slowly with increasing temperature. This gradual partitioning of 5DS into LPS suggests a broad phase transition from approximately 25°C to greater than 50°C. Such a broad transition is also indicated by the increase in slope of the temperature-dependent change in order parameter at 25°C with no corresponding decrease in slope between 25 and 50°C (Fig. 2b).

The mobility of the spin probe CAT_{12} was greater in the long-chain fraction than in the short-chain fraction, indicating that the head-group region (the phosphorylated diglucosamine of lipid A and the 2-keto-3-deoxyoctulosonic acids) of the short-chain sample was more rigid or more tightly packed (Fig. 4). The mobility of CAT_{12} in the magnesium salt of all samples was lower than in their respective sodium salts, although the same differences in mobility between fractions were observed. This suggests that the head-group packing of the long-chain fraction is inherently more open than that of the short-chain fraction, regardless of the LPS salt form. For both salt forms, mobility in the head group of unseparated LPS was intermediate between the two fractions below 25°C (in the gel phase) and less than either above 25°C (within the gel-liquid crystalline phase transition).

Electron micrographs of the long-chain LPS fraction

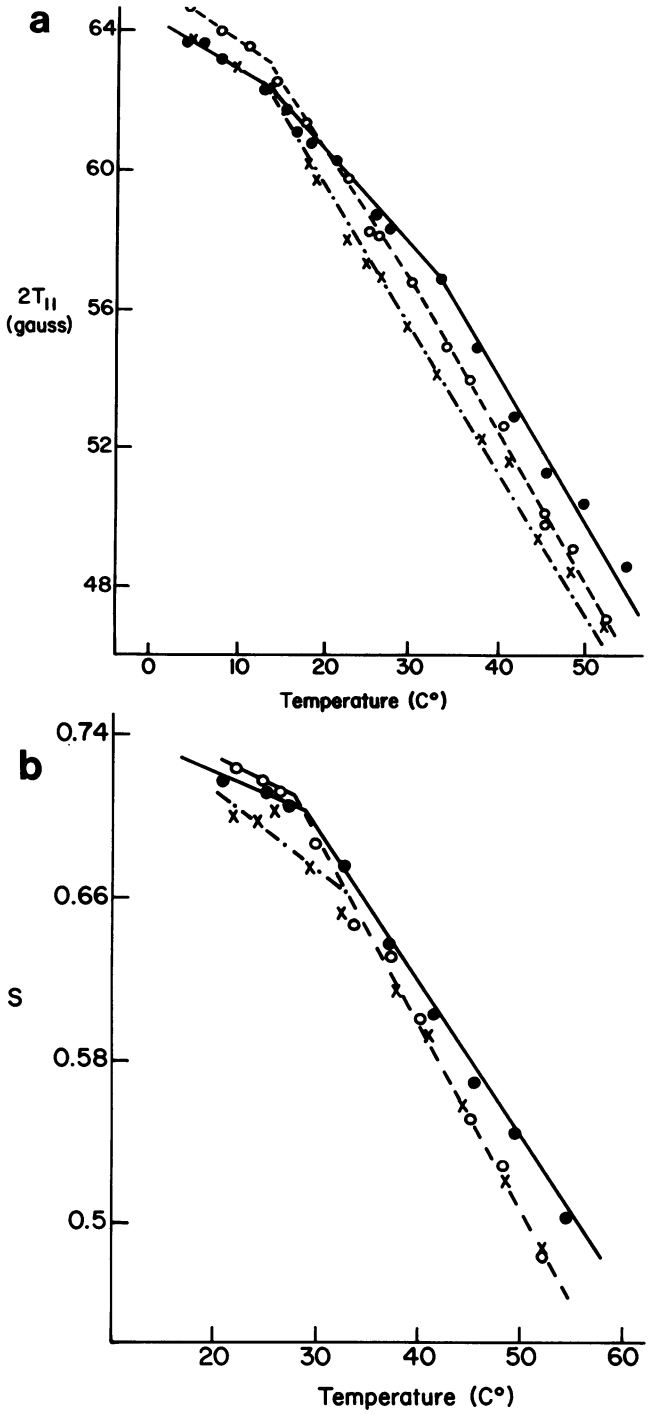

FIG. 2. Mobility of the lipid probe in unseparated (○), long-chain (●), and short-chain (X) LPS. Mobility was measured by the parameters $2T_{\parallel}$ (a) and S (b) as a function of temperature.

stained in buffer at 37°C revealed structures that appeared to be tubular micelles (Fig. 5A), whereas micrographs of the short-chain fraction revealed lamellalike structures (Fig. 5B and C). The tubular micelles had diameters of approximately 4 to 5 nm for the unstained lipid region, which is consistent with other studies (10, 18). These tubular structures were separated from each other by approximately 15 nm, suggesting that each micelle had an average O-antigen length of approximately 7.5 nm. The lamellar sheets formed by the short-chain LPS fraction were often separated by <3 nm, consistent with the presence of a much shorter O-antigen.

The results described above suggest that there are differences in LPS aggregate structure depending on O-antigen length and heterogeneity of length. To examine whether O-antigen length modulated polycation binding to LPS, magnesium LPS samples containing CAT_{12} were titrated with the cyclic peptide antibiotic polymyxin B, the aminoglycoside antibiotic gentamicin, and the polyamine spermine (Fig. 6). Of these polycations, polymyxin B displaced the greatest amount of probe from all samples as indicated by the large value of the partitioning parameter Ψ_i. Less probe was displaced from the long-chain and unseparated LPS fractions by polymyxin B than from the short-chain fraction, suggesting that the presence of the long O-antigen may decrease polymyxin B binding to the LPS core region. Gentamicin and spermine each displaced similar amounts of probe from all samples. There was somewhat less probe displaced by spermine from the long-chain fraction than from the short-chain fraction (Fig. 6) and no difference in probe displacement by gentamicin between the fractions (data not shown). Addition of cations up to a concentration of approximately 0.3 cation per LPS (mol/mol) induced a slight rigidification or closer packing of the LPS head groups as indicated by CAT_{12} mobility (Fig. 7). Further addition of the antibiotics increasingly rigidified all three fractions, whereas the polyamine fluidized the samples.

DISCUSSION

Variations in head-group motion and lipid polarity between short- and long-chain LPS fractions and unseparated LPS from a smooth strain of *E. coli* suggest differences in

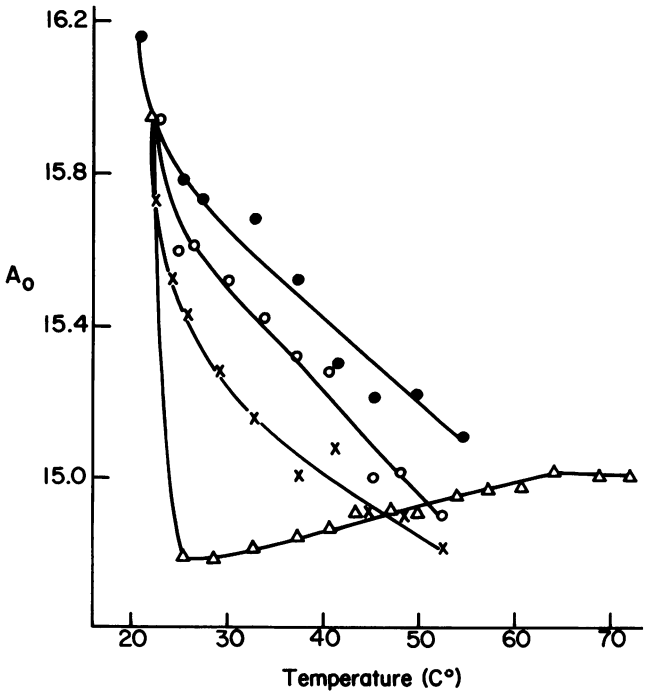

FIG. 3. Polarity or hydration (A_o) of the LPS lipid region as a function of temperature measured by 5DS added to unseparated LPS (X), the long-chain fraction (●), and the short-chain fraction (○) of LPS, and to pure 1-palmitoyl-2-oleoylphosphatidylethanolamine (△).

LPS aggregate packing dependent on O-antigen length. The fluidity of LPS aggregates is known to be dependent on ionic composition and pH (4; Coughlin, et al., in press); thus, we examined uniform LPS salts in buffered solutions. Magnesium LPS was studied because of its similarity in fluidity and ion content to isolated native LPS (4) and because the outer membrane is naturally high in Mg^{2+} content (5). We found no significant differences in the number of phosphates per LPS among the fractions; thus, it appears that the core lipid A region has the same degree of phosphorylation regardless of O-antigen length. The slightly higher amount of phosphate detected in the unseparated sample may be from contamination by nucleic acids which were removed from the separated fractions during gel filtration. Differences observed in the physical properties of these samples, then, are assumed

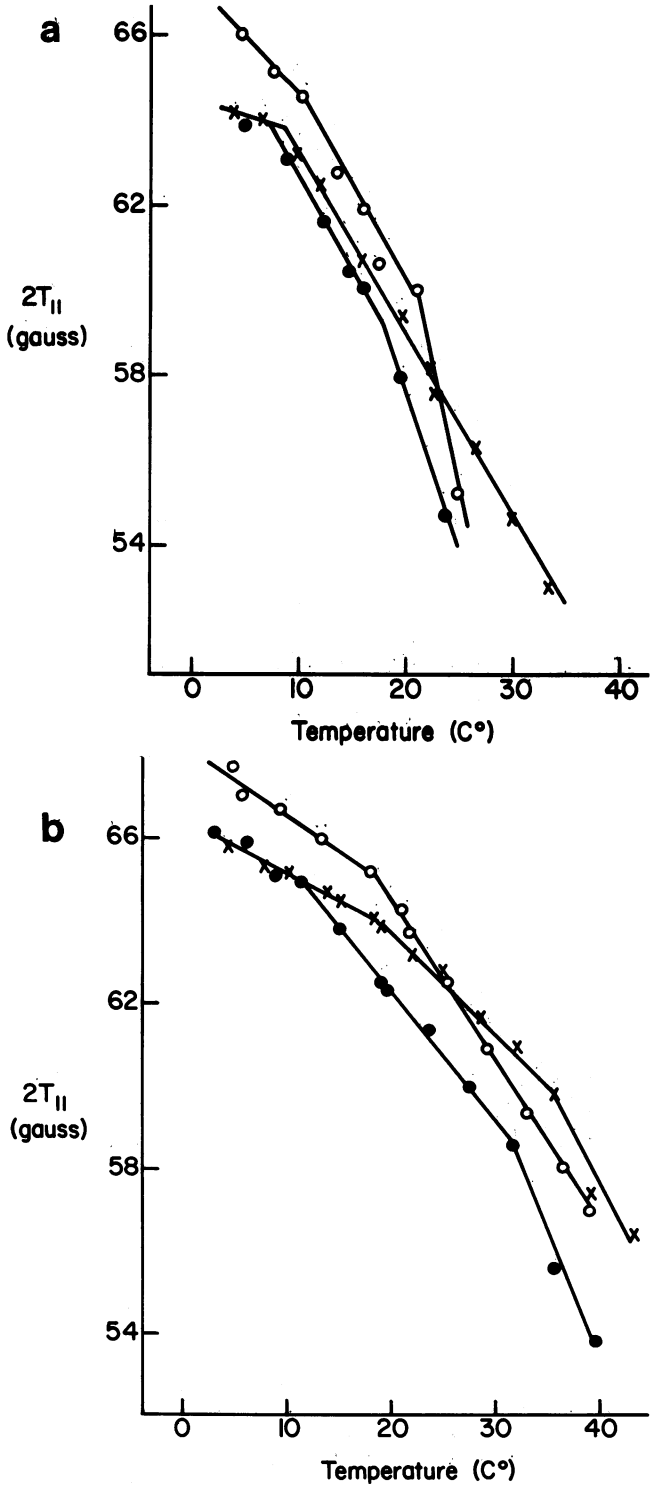

FIG. 4. Mobility of the head-group probe CAT_{12} in unseparated (X), long-chain (●), and short-chain (○) LPS. Probe motion was measured by using (a) sodium LPS and (b) magnesium LPS as a function of temperature.

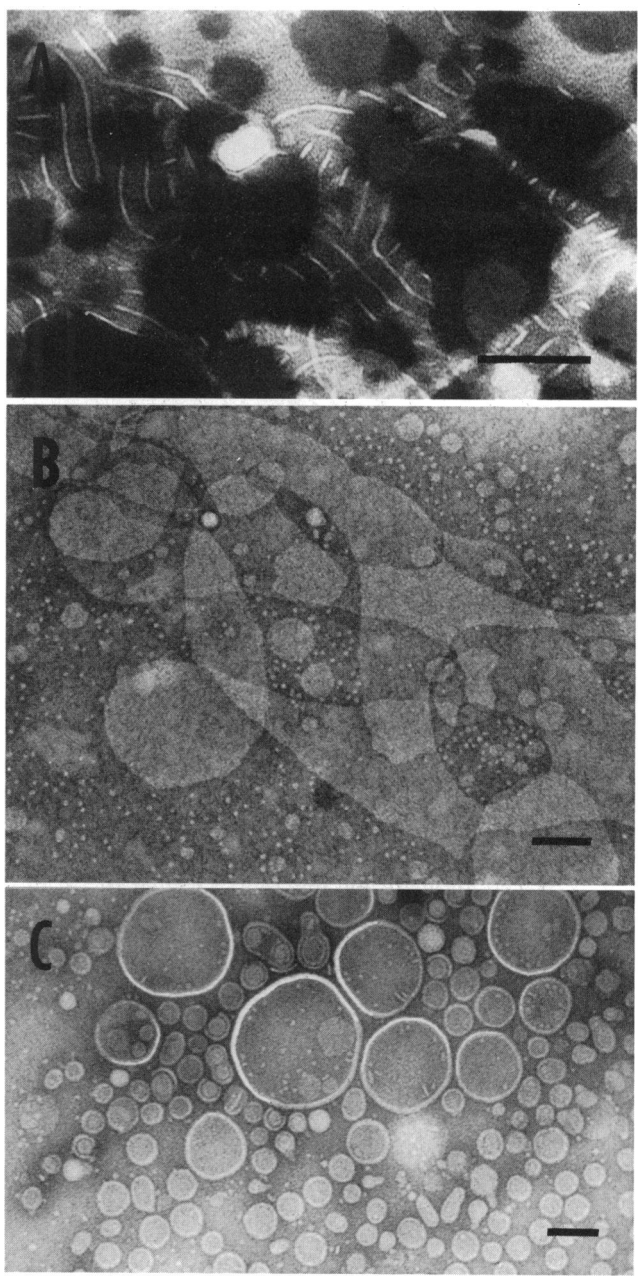

FIG. 5. Electron micrographs of (A) the long-chain LPS fraction and (B and C) the short-chain LPS fraction. Samples were stained with ammonium molybdate (A and B) or phosphotungstate (C). Bars, 100 nm. Dark areas in A are staining artifacts.

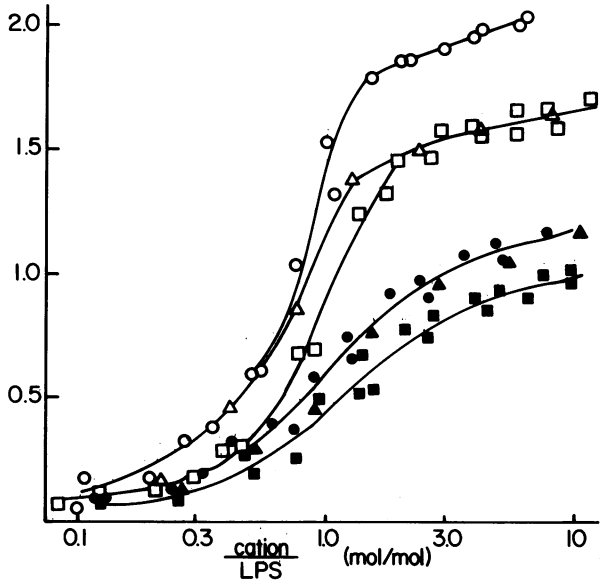

FIG. 6. Partitioning of CAT_{12} in unseparated (△, ▲), long-chain (□, ■), and short-chain (○, ●) LPS as a function of the added cations polymyxin B (open symbols) and spermine (closed symbols). Molar concentrations of LPS assume average molecular weights of 11,000 for the unseparated, 20,000 for the long-chain, and 5,800 for the short-chain fraction.

to result from differences in the length of the attached O-antigen.

As visualized in the electron microscope, the aggregate structure of the two fractions and the distance between aggregates were dramatically different. The closest spacing between particles may indicate the approximate dimensions of the O-antigen chain. The separation between micelles of long-chain LPS suggested that the O-antigen extends out 7.5 nm from the lipid region, whereas the polysaccharide length of short-chain LPS appeared to be <1.5 nm. While this long-O-antigen length is much shorter than the possible 30 nm to which a polysaccharide of 54 residues may extend, it is twice the length measured by some other investigators (10, 18). Perhaps the difference in measured lengths reflects differences in the conformation of long O-antigen in unseparated LPS and purified long-O-antigen LPS.

According to results from ESR studies, the head-group probe had greater motion in the long-chain LPS fraction than in the short-chain fraction, whereas the lipid probe reflected similar or lower mobility in the long-chain sample. In addition, the lipid region of the long-chain fraction detected by the probe was more hydrated. These observations suggest a greater surface curvature in long-chain LPS aggregates in which the head groups are spaced further apart compared with the short-chain fraction, while the lipid region is packed more tightly in the long-chain fraction, preventing the probe from intercalating as deeply. Other studies have suggested that LPS can assume both lamellar and hexagonal packing arrangements, depending on the effective head-group size (32; Coughlin et al., in press). The presence of long O-antigen attached to all of the molecules in the long-chain fraction may increase the overall head-group size and hence increase the tendency for the formation of hexagonal I or tubular micelle structures. This is supported by visualization of aggregates resembling tubular micelles in electron micrographs of the long-chain fraction compared with lamellar sheets seen in the short-chain LPS fraction.

The properties of unseparated LPS appeared to be different than those of either of its component fractions. At temperatures within the phase transition range, above 25°C, the lower head-group mobility and lipid polarity of unseparated LPS, compared with either fraction, suggest that the mixture of short- and long-O-antigen LPS molecules forms a more closely packed lamellar arrangement than is possible in either fraction alone. The lamellar character of unfraction-

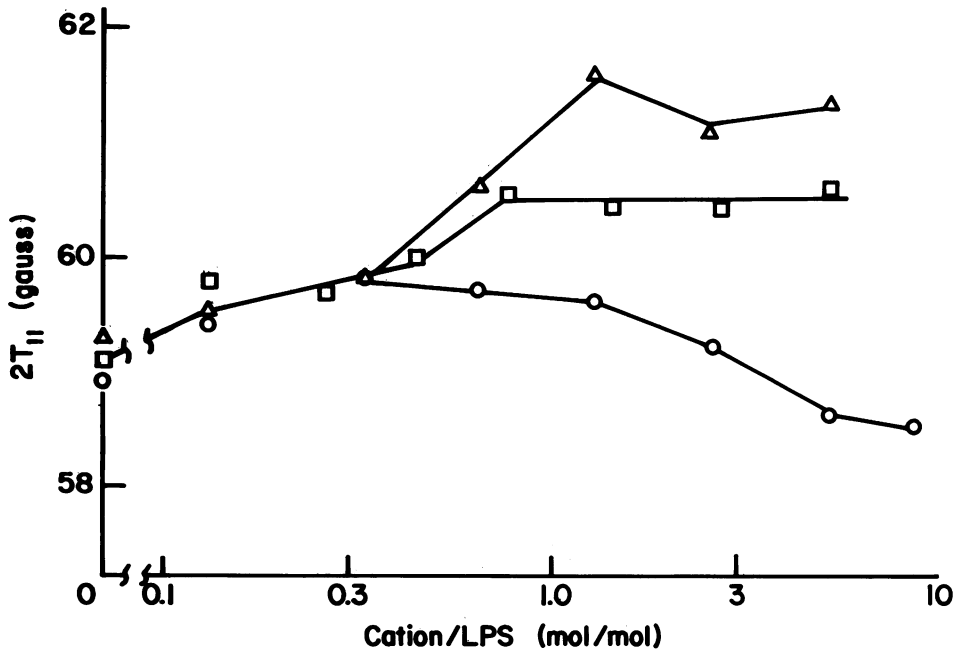

FIG. 7. Mobility of the head-group probe CAT_{12} ($2T_{\parallel}$) bound to short-chain LPS, measured as a function of the added cations polymyxin B (△), spermine (○), and gentamicin (□).

ated LPS from smooth strains has previously been characterized by a variety of physical techniques (10, 18). In the unfractionated samples, short-O-antigen-containing LPS molecules may act as spacers between bulky, long-O-antigen-containing molecules and thus help maintain a lamellar structure. LPS lamellar structures may be stabilized by polysaccharide-polysaccharide interactions between long O-antigens (6). These polysaccharide-polysaccharide interactions may be facilitated by the presence of deoxy and dideoxy sugars in O-antigens (29). The occurence of these interactions is suggested by the fact that LPS dimers, which are stable even during sodium dodecyl sulfate-polyacrylamide gel electrophoresis, tend to be complexes of molecules with approximately the same length O-antigen (27).

The differential cation-induced displacement of CAT_{12} from the three LPS samples indicated that the presence of a long O-antigen can alter cation binding. Cation-induced displacement of CAT_{12} from LPS has been shown to correlate with the ability of cationic antibiotics to increase outer membrane permeability (26). In addition, the level of probe displacement is sensitive to alterations in LPS structure which confer polymyxin resistance (manuscript in preparation). Thus, cation binding as measured by CAT_{12} displacement should be able to detect differences in cation affinity for the different fractions. If the presence of a long O-antigen decreases the affinity of polycations for core lipid A, then a lower level of cation-induced CAT_{12} displacement would be expected for the long-chain fraction. The lower level of CAT_{12} displacement by polymyxin B from the long-chain fraction and unseparated LPS compared with that from the short-chain fraction suggests a decreased affinity of the core lipid A region for this cation, owing to the presence of the longer O-antigen. This decreased affinity may result from a physical block of cation association with the core lipid A region or from a change in aggregate structure. If the long-chain fraction exists in a tubular micelle form, the core-lipid A region would be more exposed than in the unseparated sample. Hence, a decrease in polycation binding by the long-chain fraction owing to the long O-antigen may be negated by the increased surface curvature of aggregates of this long-chain fraction. These variations in cation binding were observed under equilibrium conditions and do not rule out the possibility that, on intact cells, a long O-antigen may also increase cationic antibiotic resistance by decreasing the rate at which antibiotics bind to and cross the outer membrane.

The changes in LPS head-group packing on binding of the different competitive cations appeared to be independent of the length of the O-antigen. As indicated by LPS head-group mobility, polycationic antibiotics rigidified the head group of all LPS fractions, whereas the polyamine spermine either had no effect or slightly fluidized the head groups. Although the presence of the long O-antigen may increase the convex curvature of aggregates owing to its large size, the packing arrangement of the core lipid A region may still be affected by the cations bound.

Our results suggest that purified long-O-antigen-containing LPS may exist mainly as tubular micelles. This may explain why no species of gram-negative bacteria possess LPS which is 100% long O-antigen. In mixtures of short- and long-chain LPS (unseparated LPS), the head-group regions were shown to be more rigid or more closely packed than in either of the purified short- or long-chain fractions. Bacteria may have evolved to synthesize both short- and long-chain LPS because the mixture forms a more stable and impermeable barrier to toxic compounds.

The differences in aggregate structures resulting from differences in O-antigen length may also affect the structure of the intact outer membrane. Microdomains enriched in either LPS size fraction may differ in their tendencies to form blebs and their interactions with membrane proteins. Finally, the differences in the biological activities of LPS from different smooth and rough strains of bacteria may result, in part, from differences in the aggregate structure of the LPS.

LITERATURE CITED

1. **Bader, J., and M. Teuber.** 1973. Action of polymyxin B on bacterial membranes, I. Binding to the O-antigenic lipopolysaccharide of *Salmonella typhimurium*. Z. Naturforsch. Teil. C **28**:422–430.
2. **Coughlin, R. T., C. R. Caldwell, A. Haug, and E. J. McGroarty.** 1981. A cationic electron spin resonance probe used to analyze cation interactions with lipopolysaccharide. Biochem. Biophys. Res. Commun. **100**:1137–1142.
3. **Coughlin, R. T., A. Haug, and E. J. McGroarty.** 1983. Electron spin resonance probing of lipopolysaccharide domains in the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **729**:161–166.
4. **Coughlin, R. T., A. Haug, and E. J. McGroarty.** 1983. Physical properties of defined lipopolysaccharide salts. Biochemistry **22**:2007–2013.
5. **Coughlin, R. T., S. Tonsager, and E. J. McGroarty.** 1983. Quantitation of metal cations bound to membranes and extracted lipopolysaccharide of *Escherichia coli*. Biochemistry **22**:2002–2006.
6. **Dea, I. C. M., A. A. McKinnon, and D. A. Rees.** 1972. Tertiary and quaternary structure in aqueous polysaccharide systems which model cell wall cohesion: reversible changes in conformation and association of agarose, carrageenan and galactomannans. J. Mol. Biol. **68**:153–172.
7. **Dubray, G., and G. Bezard.** 1982. A highly sensitive periodic acid-silver stain for 1,2-diol groups of glycoproteins and polysaccharides in polyacrylamide gels. Anal. Biochem. **119**:325–329.
8. **Edstrom, R. D., and E. C. Heath.** 1967. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. J. Biol. Chem. **242**:4125–4133.
9. **Few, A. V.** 1955. The interaction of polymyxin E with bacterial and other lipids. Biochim. Biophys. Acta **16**:137–145.
10. **Fukushi, K., H. Asano, and J. Sasaki.** 1977. The physical structure of endotoxin extracted from wild-type and R mutants of *Salmonella*. J. Electron Microsc. **26**:95–101.
11. **Galanos, C., and O. Lüderitz.** 1975. Electrodialysis of lipopolysaccharides and their conversion to uniform salt forms. Eur. J. Biochem. **54**:603–610.
12. **Goldman, R. C., and L. Leive.** 1980. Heterogeneity of antigenic-side-chain length in lipopolysaccharide from *Escherichia coli* 0111 and *Salmonella typhimurium* LT2. Eur. J. Biochem. **107**:145–153.
13. **Griffith, O. H., and P. C. Jost.** 1976. Lipid spin labels in biological membranes, p. 454–523. *In* L. J. Berliner (ed.), Spin labeling theory and applications. Academic Press, Inc., New York.
14. **Hancock, R. E. W., V. J. Raffle, and T. I. Nicas.** 1981. Involvement of the outer membrane in gentamicin and streptomycin uptake and killing in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **19**:777–785.
15. **Iida, K., and M. Koike.** 1974. Cell wall alterations of gram-negative bacteria by aminoglycoside antibiotics. Antimicrob. Agents Chemother. **5**:95–97.
16. **Jann, B., K. Reske, and K. Jann.** 1975. Heterogeneity of lipopolysaccharide. Analysis of polysaccharide chain lengths by sodium dodecylsulfate-polyacrylamide gel electrophoresis. Eur. J. Biochem. **60**:239–246.
17. **Kato, N., M. Ohta, N. Kido, I. Ito, S. Naito, and T. Kuno.** 1985.

Formation of a hexagonal lattice structure by an R-form lipopolysaccharide of *Klebsiella* sp. J. Bacteriol. **162**:1142–1150.

18. **Labischniski, H., G. Barnickel, H. Bradaczek, D. Naumann, E. T. Rietschel, and P. Giesbrecht.** 1985. High state or order of isolated bacterial lipopolysaccharide and its possible contribution to the permeation barrier property of the outer membrane. J. Bacteriol. **162**:9–20.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Lüderitz, O., M. A. Freudenberg, C. Galanos, V. Lehmann, E. T. Rietschel, and D. H. Shaw.** 1982. Lipopolysaccharides of gram-negative bacteria. Curr. Top. Membr. Transp. **17**:79–151.
21. **Mayer, H., and J. Weckesser.** 1984. 'Unusual' lipid A's: structures, taxonomical relevance and potential value for endotoxin research, p. 221–247. *In* R. A. Proctor (ed.), Handbook of endotoxins, vol. 1. Chemistry of endotoxin. Elsevier Biomedical Press, Amsterdam.
22. **Morrison, D. C., and R. J. Ulevitch.** 1978. The effects of bacterial endotoxins on host mediation systems. Am. J. Pathol. **93**:526–617.
23. **Munford, R. S., C. L. Hall, and P. D. Rick.** 1980. Size heterogeneity of *Salmonella typhimurium* lipopolysaccharide in outer membranes and culture supernatant membrane fragments. J. Bacteriol. **144**:630–640.
24. **Nikaido, H.** 1976. Outer membranes of *Salmonella typhimurium*. Transmembrane diffusion of some hydrophobic substances. Biochim. Biophys. Acta **433**:118–132.
25. **Palva, E. T., and P. H. Mäkelä.** 1980. Lipopolysaccharide heterogeneity in *Salmonella typhimurium* analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Eur. J. Biochem. **107**:137–143.
26. **Peterson, A. A., R. E. W. Hancock, and E. J. McGroarty.** 1985. Binding of polycationic antibiotics and polyamines to lipopolysaccharides of *Pseudomonas aeruginosa*. J. Bacteriol. **164**:1256–1261.
27. **Peterson, A. A., and E. J. McGroarty.** 1985. High-molecular-weight components in lipopolysaccharides of *Salmonella typhimurium*, *Salmonella minnesota*, and *Escherichia coli*. J. Bacteriol. **162**:738–745.
28. **Prehm, P., S. Stirm, B. Jann, K. Jann, and H. G. Boman.** 1976. Cell-wall lipopolysaccharides of ampicillin-resistant mutants of *Escherichia coli* K-12. Eur. J. Biochem. **66**:369–377.
29. **Rees, D. A.** 1976. Stereochemistry and binding behavior of carbohydrate chains. MTP (Med. Tech. Publ. Co.) Int. Rev. Sci. Ser. One Physiol. **5**:1–42.
30. **Schindler, P. R. G., and M. Teuber.** 1975. Action of polymyxin B on bacterial membranes: morphological changes in the cytoplasm and in the outer membrane of *Salmonella typhimurium* and *Escherichia coli* B. Antimicrob. Agents Chemother. **8**:95–104.
31. **Vaara, M., and T. Vaara.** 1981. Outer membrane permeability barrier disruption by polymyxin in polymyxin-susceptible and -resistant *Salmonella typhimurium*. Antimicrob. Agents Chemother. **19**:578–583.
32. **Van Alphen, L., A. Verkleij, E. Burnell, and B. Lugtenberg.** 1980. ^{31}P-NMR and freeze-fracture electron microscopy studies on *Escherichia coli*. II. Lipopolysaccharide and lipopolysaccharide-phospholipid complexes. Biochim. Biophys. Acta **597**:502–507.