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Physical Properties of Defined Lipopolysaccharide Salts†

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ABSTRACT: The electron spin resonance probes 5-doxylstearate and 4-(dodecyldimethylammonio)-1-oxy-2,2,6,6-tetramethylpiperidine bromide were used to characterize the fluidity of the acyl chain and head-group regions, respectively, of defined salts of lipopolysaccharide (LPS) from *Escherichia coli* K12. The removal of the weakly bound divalent cations from native LPS by electrodialysis and their replacement by sodium had little effect on the midpoint of the lipid-phase transition or on head-group mobility. In contrast, lipopolysaccharide acyl chain mobility increased following electrodialysis. The replacement of most of the remaining cations with sodium resulted in a further dramatic increase in mobility in both the polar and nonpolar regions of lipopolysaccharide. Head-group mobility of the sodium salt of LPS was shown to be reduced with the addition of divalent cations. Furthermore, evidence is presented which suggests that low magnesium concentrations may induce phase separations in the sodium salt. The magnesium salt of lipopolysaccharide closely resembled the native form in both head-group and acyl chain mobility although the cation charge to phosphorus ratio in the magnesium salt was greater than that detected in the native isolate. Analyses of other lipopolysaccharide salts support our hypothesis that many of the observed differences in the physical and pathological properties of lipopolysaccharide salts may simply be explained by the degree of charge neutralization.

The physical state of lipopolysaccharide (LPS)[1] has been reported to have a profound impact on its endotoxicity. A strong correlation seems to exist between the particle size of enteric LPS aggregates and their ability to inactivate serum complement (Galanos, 1975). LPS salts which have low particle size (e.g., triethylamine) do not interact with the complement system, whereas high particle size salts (e.g., sodium) are potent inactivators of complement. This pattern may not be common to all LPS. For example, the native and sodium salts of *Chromatia vinosum* LPS are equally effective in interacting with complement (Hurlbert & Hurlbert, 1977).

Another indication of the importance of the physical state of LPS on endotoxicity is the effect that mild alkaline treatment has on the ability of LPS to act as a mitogen (Goodman & Sultzer, 1977). Although high pH has been reported to cleave esterified fatty acyl chains from LPS, mild treatment simply lowers particle size without apparent chemical denaturation. In this case, alkaline-treated LPS of low particle size had enhanced B cell mitogenicity.

Attempts to characterize LPS aggregates by sedimentation coefficients or particle sizes can be complicated by gross changes in the morphology of the LPS aggregate. Furthermore, correlations between LPS aggregate size or shape and endotoxicity do not give attention to subtle differences in the intermolecular interactions between LPS within these aggregates.

We have attempted to characterize the differences between various LPS salts in their head-group and acyl chain mobilities. This was done in the hope that such differences may point toward a better understanding of the molecular interaction of cations with this complex anionic lipid and its involvement in bacterial pathology.

Materials and Methods

Cell growth and LPS isolation and electron spin resonance (ESR) probing were carried out as described earlier (Janoff et al., 1979; Coughlin et al., 1981, 1983). Defined LPS salts were prepared as described in the preceding paper (Coughlin et al., 1983). The temperature dependence of ESR spectral parameters was analyzed as described by Brunder et al. (1981). All temperature-dependent ESR parameters were reversible in the temperature range indicated. We observed that LPS solutions which had been frozen and thawed did not resuspend well. In addition, some samples showed age-dependent alterations in their physical properties. Consequently, all ESR experiments were performed on fresh or lyophilized and resuspended LPS samples stored in solution under nitrogen at 4 °C.

LPS samples were prepared for electron microscopic examination by negatively staining with 1% (w/v) sodium phosphotungstate, pH 7.0. The samples were visualized by using a Philips 300 transmission electron microscope operating at 80 kV.

Results

ESR Probing of D21-Defined LPS Salts. The proposed structure of the LPS from the two *Escherichia coli* strains used in this study is given in Figure 1 of the preceding paper (Couglin et al., 1983). Defined salts of strain D21 LPS were

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[1] Abbreviations: LPS, lipopolysaccharide(s); CAT_{12}, 4-(dodecyldimethylammonio)-1-oxy-2,2,6,6-tetramethylpiperidine bromide; 5DS, 5-doxylstearate; nLPS, native LPS; edLPS, electrodialyzed LPS; Tris, tris(hydroxymethyl)aminomethane.

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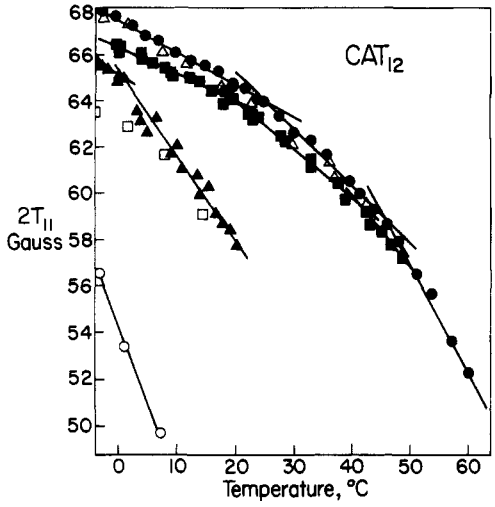

FIGURE 1: Temperature dependence of the hyperfine splitting parameter, $2T_{\parallel}$, of CAT_{12} bound to defined salts of LPS from *E. coli* strain D21. The LPS salts included native LPS (●), electrodialyzed LPS neutralized with NaOH (■), NaLPS (▲), Tris-LPS (□), MgLPS (△), and TbLPS (○). All LPS salts were resuspended in double-distilled water at an approximate concentration of 0.5 mM and mixed with approximately 10 μM CAT_{12} (final concentration).

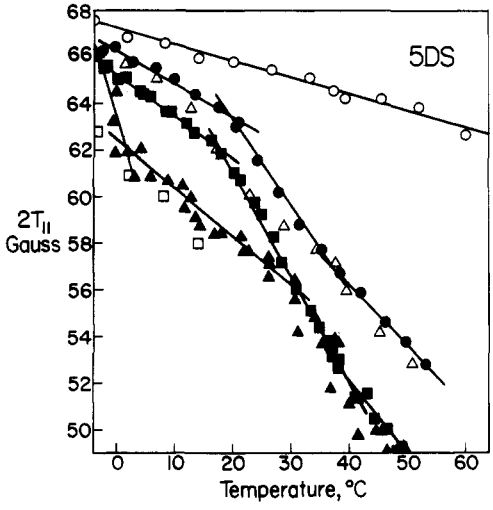

FIGURE 2: Temperature dependence of the hyperfine splitting parameter, $2T_{\parallel}$, of 5DS bound to defined salts of LPS from *E. coli* strain D21. The LPS salts studied were native LPS (●) electrodialyzed LPS neutralized with NaOH (■), NaLPS (▲), Tris-LPS (□), MgLPS (△), and TbLPS (○). All LPS salts were suspended in double-distilled water at an approximate concentration of 0.5 mM and mixed with approximately 5 μM 5DS (final concentration).

probed with either 4-(dodecyldimethylammonio)-1-oxy-2,2,6,6-tetramethylpiperidine bromide (CAT_{12}) or 5-doxylstearate (5DS) to measure head-group and acyl chain mobility, respectively. Spectral parameters were measured as a function of temperature. We found that native LPS (nLPS) was only slightly more restricted in head-group mobility than electrodialyzed LPS (edLPS) neutralized to pH 7 with NaOH (Figure 1). Even this small difference diminished with increasing temperature. In contrast, acyl chain mobility of these two samples probed with 5DS was significantly different as determined when either $2T_{\parallel}$ (Figure 2) or order parameter S (Figure 3) was plotted as a function of temperature. Both probes indicated that a broad temperature-dependent transition occurs in native and electrodialyzed LPS with T_m at 29 ± 9 and 24 ± 5 °C, respectively.

The magnesium salt (MgLPS) closely resembled nLPS particularly with respect to acyl chain order (Figure 3).

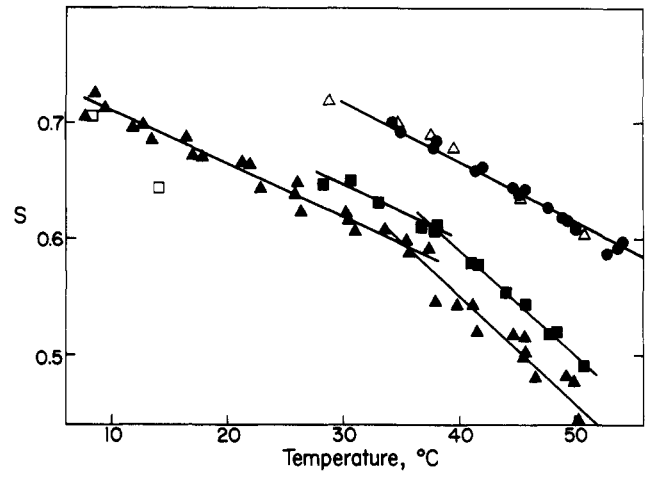

FIGURE 3: Temperature dependence of the 5DS order parameter in native (●), electrodialyzed (■), sodium (▲), magnesium (△), and Tris (□) LPS. The LPS to 5DS molar ratio was 50.

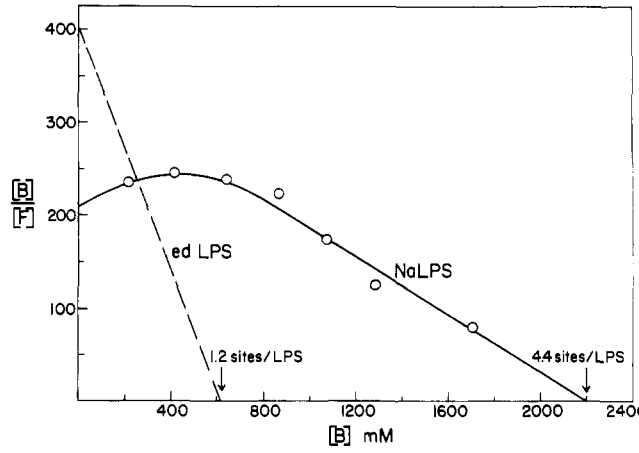

FIGURE 4: Scatchard analysis of CAT_{12} binding to electrodialyzed LPS (dashed line) and NaLPS (○) from *E. coli* K12 grown at 37 °C. CAT_{12} partitioning was measured at 37 °C on samples with an LPS concentration of 500 μM as a function of probe concentration.

Head-group mobility appeared to be intermediate between native and electrodialyzed LPS (Figure 2).

The hydrophobic acyl chain region of NaLPS probed with 5DS was shown to be more fluid than that of edLPS up to 30 °C (Figure 2). Above 30 °C, the differences in acyl chain mobility between NaLPS and edLPS was slight. In contrast, head-group mobility detected with CAT_{12} in NaLPS was dramatically greater than that of edLPS over the entire temperature range examined. Again, a broad acyl chain transition was observed with a T_m at 15 ± 12 °C. Tris-LPS appeared to be similar to NaLPS in its physical properties (Figures 2 and 3).

The terbium salt of LPS from strain D21 represented something of an anomally. The acyl chain mobility of this salt was more severely restricted than that of nLPS. The spectra at all temperatures examined were like that of a rigid glass (Schreier et al., 1978). In contrast, the head-group mobility of TbLPS proved to be the most fluid of the LPS salts. Even at −5 °C, CAT_{12} reported an unusually fluid environment.

Scatchard Analysis of CAT_{12} Binding to NaLPS. The above results suggested that the interaction of CAT_{12} with NaLPS may be complex. Thus, we examined the affinity of CAT_{12} for NaLPS by Scatchard analysis. Figure 4 indicates that CAT_{12} has 4.4 binding sites on NaLPS isolates from strain D21. In addition, some of these sites show strong positive

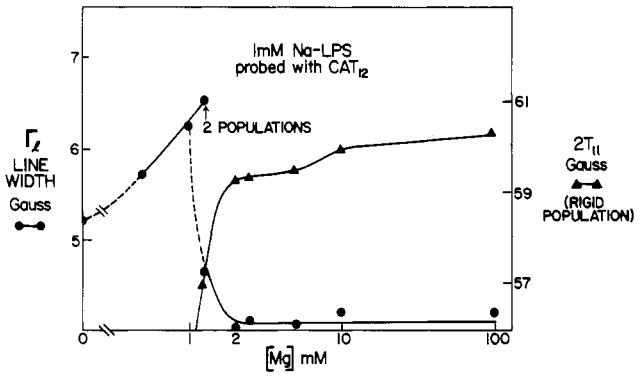

FIGURE 5: Titration of NaLPS from *E. coli* strain D21 with $MgCl_2$. A 1 mM solution of NaLPS suspended in double-distilled water and mixed with approximately 20 μM CAT_{12} (final concentration) was tritrated with increasing amounts of $MgCl_2$. The line width of the low-field peak indicates increasing heterogeneity of the probe environment as $MgCl_2$ is added.

cooperativity (Cantor & Schimmel, 1980). This is in contrast to our previous results with edLPS neutralized with NaOH which indicated that CAT_{12} bound to a single site in a noncooperative fashion. Consequently, the spin probes were added to LPS samples at very low levels. The CAT_{12} to LPS weight ratio used throughout was 0.074. This is equivalent to 1 mol of probe per 50 mol of LPS or 300 mol of LPS fatty acyl chains. At this level, there was no evidence that the probe perturbed its local environment.

Magnesium Titration of NaLPS from Strain D21. The sodium salt of LPS from strain D21 probed with CAT_{12} at 37 °C was mixed with increasing concentrations of magnesium chloride (Figure 5). The low-field line width (Γ_l) was used as an indicator of the formation of multiple spin probe domains. At 37 °C, Γ_l of the probed sodium salt is already rather high, presumably due to lifetime broadening (Mason et al., 1977). As the magnesium concentration approached that of the NaLPS, Γ_l increased dramatically. The observed broadening could be explained by either of the two following mechanisms: (1) as the level of Mg increased, CAT_{12} was forced into increasingly smaller "pure" NaLPS domains resulting in spin–spin exchange broadening, or (2) CAT_{12} partitioned randomly between both pure NaLPS domains and domains bound with Mg. Figure 5 provides evidence which strongly supports the latter interpretation. At least two distinct low-field peaks could be resolved when the concentration of Mg equalled that of NaLPS. As the Mg to NaLPS molar ratio approached 2, the line width of the rigid population reached a value of about 4 G and did not change even at very high Mg concentrations. In the rigid spin probe population, the hyperfine splitting parameter ($2T_{\parallel}$) could be measured. CAT_{12} mobility was greatly reduced in this population and was nearly equal to that of CAT_{12} in the defined salt of MgLPS (vide infra).

The observed decrease in Γ_l of the rigid population accompanied by an increase in $2T_{\parallel}$ is taken as evidence that the head-group mobility of NaLPS decreased upon addition of Mg. These changes cannot be explained by altered polarity of the spin probe environment since Γ_l has been shown to be insensitive to such changes (Mason & Freed, 1974).

Electron Micrographs of D21 LPS. The structure of various LPS salts was characterized in the electron microscope with sodium phosphotungstate, pH 7.0. Native isolates of LPS from strain D21 appeared in the electron microscope as a regular array of pits within a single-layered structure (Figure 6A). Upon electrodialysis, the array appeared swollen, and irregular tubes or ribbons connected to the pitted arrays were visualized (Figure 6B). Upon formation of the sodium salt, long tubular structures approximately 9 nm in diameter were seen. These tubes or ribbons were frequently swollen to twice this width (Figure 6C). There were also a number of irregularly shaped sheets or vesicles present. The Tris salt had a nearly identical structure (Figure 6D).

The addition of 1 mol of calcium per mol of NaLPS resulted in the complete conversion of the tubes to bilayer sheets (Figure 6E). Tubes could be seen at several places on most of these bilayer structures, and these tubes appeared to loop back onto the sheets. The diameter of these tubes was equal to the minimum tube diameter seen in NaLPS before the addition of calcium. Although most complexes were approximately 60 nm in diameter, several very large aggregates were also present.

Magnesium was less effective than calcium in inducing bilayer structure when added to NaLPS. In fact, 4 mol of magnesium per mol of NaLPS was required to completely convert tubes into bilayers (Figure 6F). Again, in virtually every case, there were one or more looped tubes present on each aggregate structure. In contrast to the round shape of calcium-induced structure, the magnesium-induced complexes appeared irregular.

Both terbium chloride (data not shown) and ferric chloride (Figure 6G) produce similar results when added to NaLPS. When either was added at molar ratio of 1, the tubular structure of NaLPS appeared to stabilize. Furthermore, there appeared to be fewer regions of swelling within tubes of either the terbium- or the iron-treated NaLPS. The minimum diameter remained 9 nm.

ESR Probing of a Heptoseless Mutant of Strain D21. The sodium salt of LPS from strain D21f2, a deep rough mutant of strain D21, was probed with CAT_{12} and 5DS. Figure 7 indicates that the heptoseless mutant produced LPS which in the sodium salt form was substantially more restricted in head-group mobility than the head group of NaLPS from the parent strain. Above 45 °C, however, head-group mobility of NaLPS from strain D21f2 increased dramatically. In contrast, 5DS probing showed the acyl chain mobility and order to be virtually identical in the sodium salts of D21 and D21f2 (Figures 8 and 9). Furthermore, both sodium salts showed an abrupt change in the temperature dependence of the order parameter around 35 °C (Figure 9).

Discussion

In the preceding paper (Coughlin et al., 1983), we reported that *E. coli* LPS extracted and purified according to standard protocol remains heavily contaminated with a variety of cations. Using sodium (ethylenedinitrilo)tetraacetate, we were able to obtain a sodium salt of LPS which was relatively free of these contaminants. NaLPS was then used to obtain various chemically defined salts of LPS. We report here that these specific salts are very distinct in their physical properties. These differences are interesting in that they suggest independent behavior of the polar and nonpolar regions of LPS.

When acidic edLPS was neutralized with different bases, the various salts were greatly altered in their ability to inactivate serum complement (Galanos & Lüderitz, 1976). A strong correlation exists between endotoxin activity and the apparent aggregate weight of these salts. One important difference between our defined LPS salts and those described by Galanos & Lüderitz (1976) is that our preparations were converted to a relatively pure sodium salt with low levels of contaminating ions. Thus, our edLPS neutralized with NaOH may be analogous to the sodium salt of LPS described by Galanos & Lüderitz (1975).

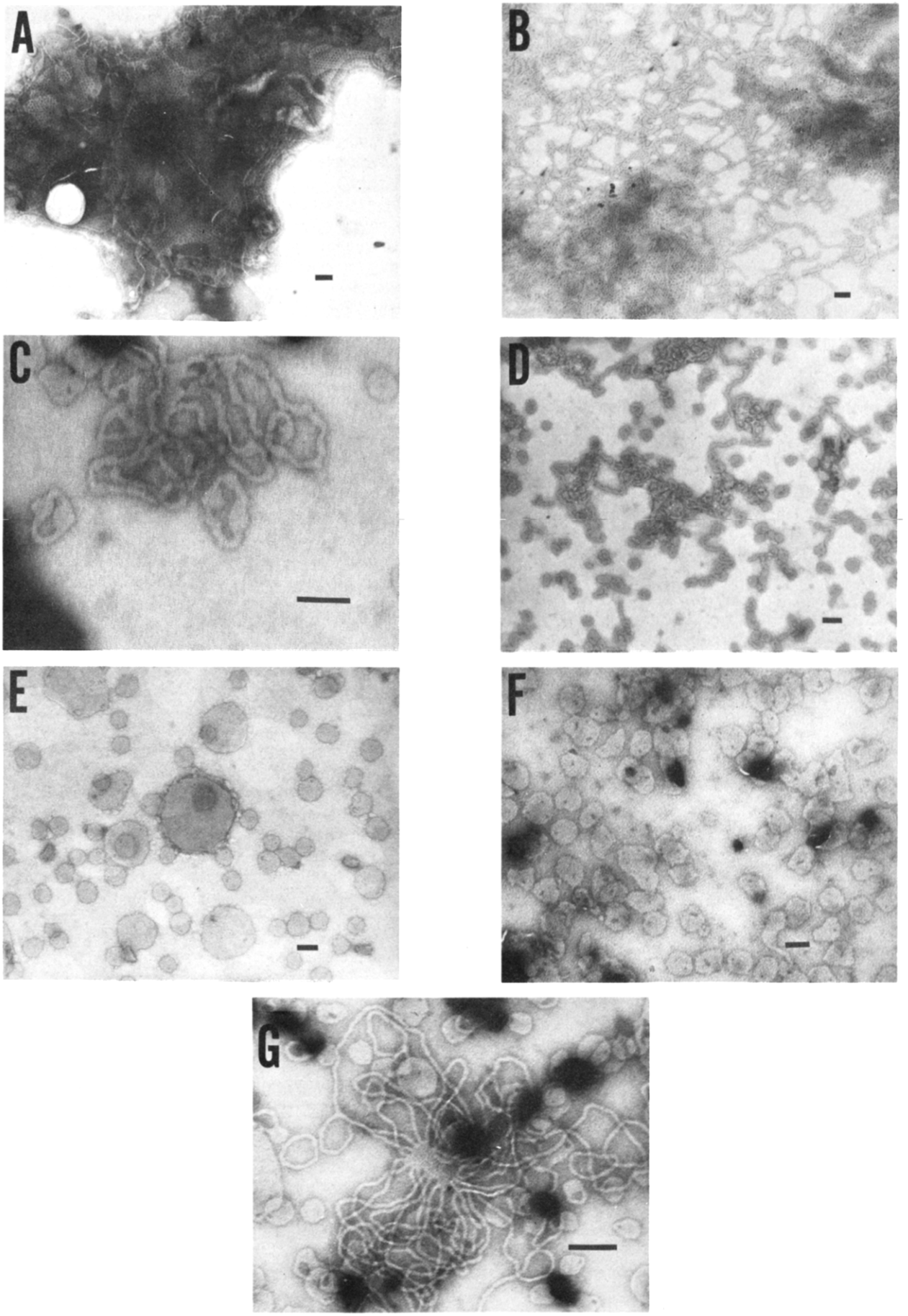

FIGURE 6: Electron micrographs of LPS isolates from *E. coli* strain D21, stained with sodium phosphotungstate, pH 7.0. (A) Native LPS; (B) electrodialyzed LPS neutralized to pH 7.0 with NaOH; (C) NaLPS; (D) Tris-LPS; (E) NaLPS mixed with $CaCl_2$ (1:1 Ca:LPS molar ratio); (F) NaLPS mixed with $MgCl_2$ (4:1 Mg:LPS molar ratio); (G) NaLPS mixed with $FeCl_3$ (1:1 Fe:LPS molar ratio). Each bar represents 100 nm.

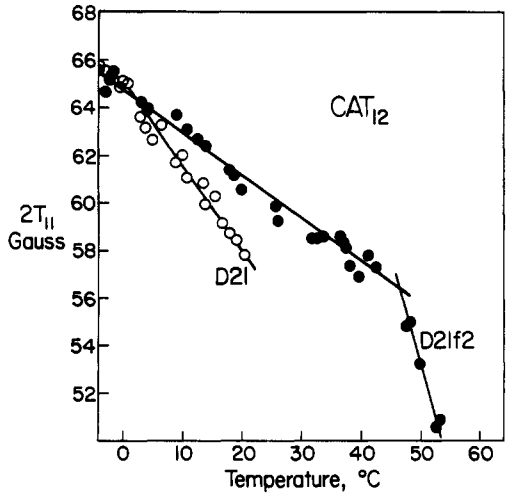

FIGURE 7: Temperature dependence of the hyperfine splitting parameter, $2T_{\parallel}$, of CAT_{12} in the NaLPS salts of D21 (○) and D21f2 (●). The probe to LPS weight ratio was 0.074.

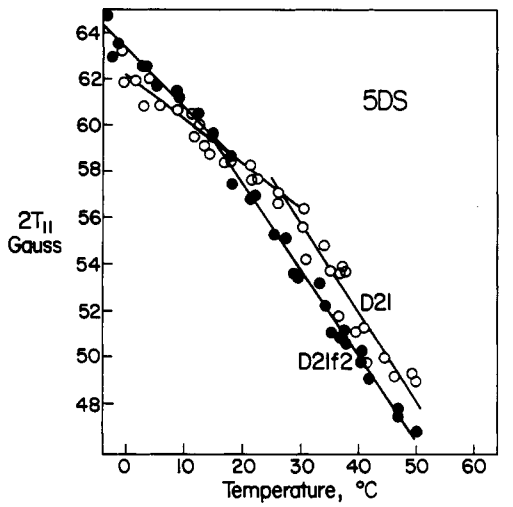

FIGURE 8: Temperature dependence of the hyperfine splitting parameter, $2T_{\parallel}$, of 5DS in the NaLPS salts of D21 (○) and D21f2 (●). The probe to LPS weight ratio was 0.074.

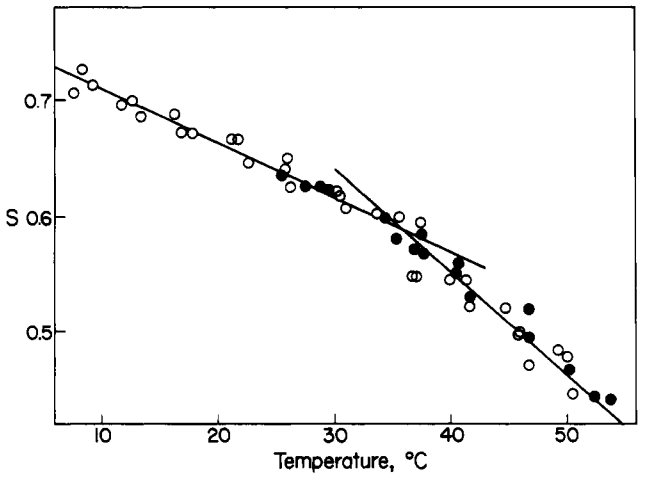

FIGURE 9: Temperature dependence of the 5DS order parameter, S, in the NaLPS salts of D21 (○) and D21f2 (●). The probe to LPS weight ratio was 0.074.

The ESR spin probes CAT_{12} and 5DS were used to monitor changes in head-group and acyl chain mobilities of the LPS salts. Our initial reservations concerning cationic "detergents" as spin probes of a polyanionic lipid system were confirmed. NaLPS has multiple CAT_{12} binding sites, some of which display strong cooperativity. This probe can induce bilayer aggregation from NaLPS tubes at high CAT_{12} to NaLPS molar ratios (data not shown). Nevertheless, CAT_{12} apparently did not alter either the local LPS environment or the morphology of LPS aggregates when applied at or below a probe to LPS weight ratio of 0.074.

The relatively high degree of head-group mobility in the NaLPS compared to edLPS, pH 7, may simply result from the low cationic charge to phosphorus ratio as noted in the preceding paper (Coughlin et al., 1983). Incomplete charge neutralization may also be responsible for the tubelike structures seen in the electron micrographs of NaLPS. The high degree of surface curvature afforded by this conformation would result in less densely packed core sugars. This is consistent with the high degree of head-group mobility observed with CAT_{12}. The head-group structure of the NaLPS from strain D21 may be partially stabilized by proton transfer complexes between phosphate or carboxyl groups and ethanolamines. Proton transfer complexes are now thought to exist at low ionic strength in phospholipids with amine-containing head groups. These noncovalent bonds act as a two-dimensional polymeric network in phosphatidylethanolamine monolayers and are reported to be largely responsible for the observed rigidity of the head group detected with deuterium NMR (Browning, 1981). We suggest that hydrogen bonding between ethanolamine groups and the carboxyl groups of the KDO units or the phosphate groups in NaLPS may contribute to the stability of NaLPS aggregates. The significance of such bonds in the presence of divalent cations is, however, uncertain.

Systematic studies of the association of cations with acidic lipids have only recently been reported (Eisenberg et al., 1979; Lau et al., 1981; Hauser et al., 1976). Mixtures of acidic and neutral phospholipids can form multilamellar tubes which are visible under a light microscope (Lin et al., 1982). Upon exposure to calcium, these tubes can twist back upon themselves into helices. We found that equimolar calcium converted NaLPS tubes into bilayers as did magnesium, albeit at higher magnesium concentration. Calcium has been reported to convert normally bilayer vesicles of sodium phosphatidic acid into the H_{II} phase (Vasilenka et al., 1982; Verkleij et al., 1982). Furthermore, phosphatidic acid can form a calcium ionophore across lipid bilayers by forming an inverted micelle in the presence of calcium (Serhan et al., 1981).

In the pure sodium salt form, the LPS tube structure is apparently H_I phase whereby the polysaccharide chains extend radially into the aqueous phase (Cullis & DeKruijff, 1979). The minimum diameter of these tubular regions was about 9 nm. The bilayer thickness of LPS reportedly is 10 nm as detected by using X-ray diffraction analysis (Emmerling et al., 1977). The possibility remains that in the presence of divalent cations, the restricted region of the inverted H_{II} phase exists. Evidence supporting the existence of the H_{II} phase is seen in negatively stained samples visualized in the electron microscope in which we have detected pits on the small bilayer sheets (Figure 6E,F). In fact, both nLPS and NaOH-neutralized edLPS consist of bilayer sheets which are regularly and heavily pitted (Figure 6A,B). This sort of structure has also been recognized by van Alphen et al. (1980). In nLPS, the pits are arranged in both random, cubic, and hexagonal arrays. The hexagonal packing of the pits was most commonly observed. The repeat distance for the hexagonal array was 144 Å. Comparison of panels A and B of Figure 6 indicates that the loss of one magnesium through electrodialysis and its replacement with sodium result in the partial degeneration of

these heavily pitted regions into long tubes and ribbons similar to those seen in NaLPS preparations. This morphological change as mentioned earlier is accompanied by a dramatic increase in LPS toxicity (Galanos, 1975). Unfortunately, electron micrographs are often misleading and can lead to erroneous interpretations with regard to molecular organization. These may be particularly true when analyzing what are thought to be lipidic particles and cubic phase lipids (Hui & Boni, 1982).

Surprisingly, the head-group mobilities of nLPS and edLPS were very similar (Figure 1). This similarity detected with CAT_{12} suggested that the removal of one weakly bound divalent cation and its replacement by sodium does not significantly alter the packing of the core sugars. Experiments described in the preceding paper (Coughlin et al., 1983) suggest that the most weakly bound divalent cation in nLPS from strain D21 is magnesium which is presumably located in the KDO core.

CAT_{12} proved to be very sensitive to the binding of cations of NaLPS. The addition of Mg to NaLPS produced a dramatic decrease in the mobility of bound CAT_{12}. This decrease in head-group mobility was not the result of a change in the surface curvature of LPS aggregates. Electron micrographs showed that Mg could not completely induce bilayer structures from NaLPS tubes at molar ratios below 4 (Mg:LPS), and these bilayer aggregates retained regions of tubular LPS which looped back onto the sheets. Furthermore, examination of the low-field line width provided strong evidence that magnesium can promote the formation of separate domains within NaLPS aggregates when the molar ratio of Mg to NaLPS is below 1.

The addition of trivalent ferric and terbium ions seemed to relax the twisted ribbons and tubes of NaLPS into long smooth tubes. This may explain the exceedingly high head-group mobility detected by CAT_1 and the very low acyl chain mobility detected with 5DS in TbLPS. Conversely, the tight packing of the acyl chains of TbLPS may not allow CAT_{12} to completely partition into TbLPS.

Emmerling et al. (1977) reported, using fluorescent probing and X-ray diffraction analysis, that a broad order-disorder transition occurs at 22 ± 5 °C in nLPS from *E. coli* strain B/r. Neither the midpoint nor the width of the observed transition in this strain was altered by electrodialysis. More recently, X-ray diffraction experiments have confirmed that the phase-transition temperature for nLPS from *E. coli* B grown at 37 °C was 25 °C (Nakayama et al., 1980). The broad phase transition we observed in nLPS with both CAT_{12} and 5DS was shifted down 5 °C after electrodialysis, and the temperature over which the transition occurred narrowed substantially. The conversion of the edLPS to the sodium salt resulted in an even further decrease in the transition temperature, consistent with the phase behavior of anionic phospholipids (Verkleij et al., 1974).

Our laboratory has previously shown that outer membranes extracted from *E. coli* grown at different temperatures have altered physical properties (Janoff et al., 1979). Most notably, the beginning and end of the membrane lipid transition seen with 5DS correlated well with the limits of the growth temperature. The acyl chain compositions of both phospholipids (Nakayama et al., 1980) and LPS (Kasai, 1966) have been reported to change in response to growth temperature. We have found no detectable changes in either the acyl chain mobility or the transition temperature of nLPS in response to changes in growth temperature in the range of 12–43 °C (data not shown). The similarity between the midpoints of the phase transitions of nLPS and of outer membranes from *E. coli* grown at 37 °C, therefore, may be coincidental. Emmerling et al. (1977) reached a similar conclusion for the LPS from their *E. coli* strain.

Finally, a comparison of NaLPS of strain D21 and the heptoseless mutant strain D21f2 shows that the two have virtually identical acyl chain mobility. The structure of the lipid A moiety of the two LPS samples has been reported to be the same (Gmeiner & Schlecht, 1980). Consistent with the elemental analysis of these LPS isolates presented in the preceding paper (Coughlin et al., 1983), NaLPS from strain D21f2 was shown to be more restricted in head-group mobility than NaLPS from the parent strain. This decreased head-group mobility is thought to result from a greater charge neutralization by sodium in the core region of the mutant than in the core region of the parent. Furthermore, electron micrographs indicated that NaLPS from strain D21f2 exists as a bilayer aggregate (data not shown).

Acknowledgments

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Registry No. Sodium, 7440-23-5; magnesium, 7439-95-4; calcium, 7440-70-2; terbium, 7440-27-9.

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Effect of Liposomal Size on the Calorimetric Behavior of Mixed-Chain Phosphatidylcholine Bilayer Dispersions†

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ABSTRACT: The effect of liposomal size on the endothermic transition profiles of the saturated mixed-chain phosphatidylcholines 1-stearoyl-2-myristoyl-*sn*-glycero-3-phosphocholine and 1-stearoyl-2-caproyl-*sn*-glycero-3-phosphocholine has been investigated. Liposomal bilayer dispersions of progressively smaller average diameter were prepared by extrusion of coarse multilamellar preparations of these lipids through polycarbonate membrane filters of decreasing pore size from 3- to 0.2-μm diameter. These samples were then investigated by high-sensitivity differential scanning calorimetry and negative-stain electron microscopy. It was found that coarse dispersions of the above lipids are composed of liposomes whose average diameter is considerably smaller than that typically associated with multilamellar liposomes of synthetic phosphatidylcholines. This fact, coupled with an analysis of the size dependence of the transition cooperativity, leads to the conclusion that the small size of the liposomes limits the transition cooperativity in coarse dispersions of these mixed-chain phosphatidylcholines. Thus, the broad and highly asymmetric transition profiles that have been observed in previous studies of these phosphatidylcholines are postulated to arise largely from this size dependence, rather than from characteristic packing properties of the phospholipid acyl chains as has been previously suggested.

Recently, we reported on the calorimetric behavior of a series of saturated mixed-chain phosphatidylcholines whose *sn*-2 acyl chains were varied to be from two carbon atoms to eight carbon atoms shorter than the *sn*-1 acyl chains: C(18):C(16)-PC[1] to C(18):C(10)-PC (Mason et al., 1981a). This study revealed that in multilamellar bilayer dispersions derived from these phosphatidylcholines, the gel ↔ liquid-crystalline phase transition becomes broader, less cooperative, and generally of higher transition enthalpy as the chain length inequivalence is increased.

When high-sensitivity DSC was employed, it could also be seen that the transition profiles of C(18):C(14)-PC and C(18):C(10)-PC were composites of two or more individual transition peaks. A similar observation was made in a high-sensitivity DSC study of C(18):C(14)-PC and C(16):C(14)-PC by Chen & Sturtevant (1981). It has been suggested that these multiple peaks arise from the segregation of the bilayer into regions of interdigitated and random packing of the acyl chains (Chen & Sturtevant, 1981) or from the packing of the acyl chains into more than one interdigitated conformation within the gel state of these phosphatidylcholines (Mason et al., 1981a).

Here, we extend the previous studies by examining the effect of liposomal size on the endothermic transition profiles of C(18):C(14)-PC and C(18):C(10)-PC bilayer dispersions. Liposomal dispersions of progressively smaller average diameter were prepared by extrusion of coarse preparations through polycarbonate membrane filters of decreasing pore size from 3- to 0.2-μm diameter. These samples were then examined by negative-stain electron microscopy and high-sensitivity DSC.

It was found that coarse dispersions of C(18):C(14)-PC and C(18):C(10)-PC form liposomes whose average diameter is considerably smaller than that typically associated with synthetic phosphatidylcholine multilamellar liposomes such as dipalmitoylphosphatidylcholine. This fact, coupled with an examination of the liposomal size dependence of the transition cooperativity based upon the analysis introduced by Marsh et al. (1977), leads to the conclusion that the cooperativity in the thermal transitions of these phosphatidylcholines is largely limited by the size of the liposomes. Thus, the large transition breadth and appearance of multiple peaks that have been

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[1] Abbreviations: C(18):C(18)-PC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; C(18):C(16)-PC, 1-stearoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine; C(18):C(14)-PC, 1-stearoyl-2-myristoyl-*sn*-glycero-3-phosphocholine; C(16):C(14)-PC, 1-palmitoyl-2-myristoyl-*sn*-glycero-3-phosphocholine; C(18):C(10)-PC, 1-stearoyl-2-caproyl-*sn*-glycero-3-phosphocholine; C(16):C(16)-PC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance.

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