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A pH titration study on the ionic bridging within lipopolysaccharide aggregates

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The packing of lipopolysaccharide aggregates from rough strains of *Escherichia coli* was examined at different pH values. Lipopolysaccharide head-group motion, measured with an electron spin resonance probe, was found to be dependent on pH, and indicated the existence of multiple ionizable groups. Lipopolysaccharide from a rough (Ra) and a heptose-less (Re) mutant were more rigid at pH 5 than at pH 10.5. In addition, head-group mobility of the magnesium salt of Ra lipopolysaccharide was substantially less than that of the sodium salt at pH 7.0, whereas at high pH (pH 12) the two salts were equally fluid. Changes in head-group packing were also reflected in pH-dependent changes in the phase transition measured with differential scanning calorimetry. The enthalpy of the transition, ΔH_t, for the sodium salt of Re lipopolysaccharide was greatest at pH 7.5 and approached zero in both the acidic and the basic pH ranges. We propose that fixed charges in the core and lipid A regions significantly influence lipopolysaccharide head-group motion and the lipopolysaccharide aggregation state. Furthermore, ionic bridging among phosphate groups dramatically rigidifies head group interactions in the neutral to acidic pH ranges

Introduction

The lipopolysaccharide (LPS) of Gram-negative bacteria is the major lipid in the outer monolayer of the outer membrane, and its interactions within this membrane help to form a permeability barrier against bile salts and hydrophobic antibiotics [1] During infection, bacterial membranes tend to bleb into the surrounding fluid The lipopolysaccharide in these outer membrame blebs and in membrane fragments released during cell lysis constitutes the endotoxin that produces disseminated intravascular coagulation and induces shock as well as other pathogenic effects in septic patients [2] Since lipopolysaccharide toxicity is dependent on its salt form [3], alterations in the physical form of LPS may be critical in modulating its biological and toxic activities

Ionizable groups within lipopolysaccharide such as amino and acidic sugars, and phosphate, and ethanolamine moieties [4] contribute to produce a three-dimensional zone of high charge density on the outer surface of the bacterium The net nega-

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Abbreviations CAT_{12}, 4-(dodecyl dimethylammonium)-1-oxyl-2 2,6,6-tetramethylpiperidine bromide, ESR, electron spin resonance, NaEDTA, sodium (ethylenediamine)tetraacetate, DSC differential scanning calorimetry, KDO, 3-deoxy-D-*manno*-octulosonic acid

tive charge gives LPS a high capacity for binding metal ions [5,6], these cations stabilize the membrane by decreasing the electrostatic head group repulsion in LPS When depleted of divalent cations, the outer membrane releases up to half of the associated LPS and becomes permeable to antibiotics and lytic enzymes such as lysozyme [7] In addition, electron micrographs of purified LPS have revealed a large diversity of aggregate shapes depending on the type of LPS, temperature, and ions present [8,9]

We have examined the lipopolysaccharide aggregation state as a function of pH in an effort to determine the influence of ionizable groups on LPS aggregate structure We propose that hydrogen bonding and cation bridging between ionic groups are responsible for stabilizing the lamellar structure of LPS in the Gram-negative outer membrane At pH extremes isolated LPS may lose its lamellar structure and form nonbilayer structures

Materials and Methods

Lipopolysaccharide preparation

Escherichia coli strains D21 (a K12 rough strain) and D21f2 (an Re LPS-producing strain) [10] were grown at 37°C in nutrient broth (1% tryptone, 0 2% yeast extract, 0 4% NaCl) Lipopolysaccharide was isolated using either hot aqueous phenol (D21) or phenol/chloroform/petroleum ether (D21f2) as previously described [6] The sodium salt of LPS (NaLPS) was obtained by dialyzing electrodialyzed LPS against three to five changes of 10 mM NaEDTA, pH 7 0, at 4°C, followed by extensive dialysis against double-distilled water Samples used for differential scanning calorimetry (DSC) were passed through a Chelex-100 column preequilibrated at pH 8 2 The magnesium salt of LPS (MgLPS) was prepared by dialyzing the NaLPS against 10 mM $MgCl_2$, followed by extensive dialysis against double-distilled water Elemental analysis of the salts was carried out as described elsewhere [6] Levels of LPS were quantitated using the thiobarbituric acid analysis for KDO [11]

Electron spin resonance probing

The head-group mobility of LPS, suspended at a concentration of 10 mg/ml, was monitored with the ESR probe CAT_{12} The sample pH was adjusted either by suspending the sample in 50 mM of potassium citrate (pH 5 5–6 5), potassium phosphate (pH 6 5–8 5 and pH 11–12 5) or potassium borate (pH 8 5–10 5) buffers, or by the addition of HCl or NaOH to unbuffered solutions Head-group mobility was determined by measuring the hyperfine splitting parameter, $2T_{max}$ The head-group motions detected in samples in different buffers at the same pH values were identical regardless of the buffers used, indicating that the buffer did not differentially alter LPS structure Heterogeneity of the bound probe's environment was calculated by measuring the half-width of the low-field peak at half-height (Δ_1) All ESR experiments were carried out at 37°C on a Varian ESR Spectrometer model E-112

Differential scanning calorimetry

Differential scanning calorimetry (DSC) of LPS was performed on a Perkin-Elmer DSC-2 5–10 mg of LPS in 0 07 ml of double-distilled water were placed in a DSC pan and sealed An equal volume of double-distilled water was placed in the reference pan Temperature scans were made from −5°C to 75°C with heating rates of 1 25 Cdeg/min All calorimetry data were obtained from samples during the heating phase and represent the average of at least three scans In all cases the scans were completely reversible Molar enthalpies were obtained from the molar concentration of LPS as determined by assaying for KDO [11] in the sample pan following calorimetry

^{31}P-NMR analyses

^{31}P-NMR spectra were obtained with a Varian XL-200 spectrophotometer operated at 81 MHz Samples were prepared by dissolving LPS in 2% deoxycholate, 5 mM EDTA, pH 8 0 Measurements were made at 45°C and are the averages of (2–4) 10^5 transients

Light scattering

The intensity of 90° scattered light was measured by simultaneously scanning the incident and scattered light from 230 nm to 500 nm using LPS samples suspended at different pH values as described for the ESR experiments

Results

The content of phosphate and pyrophosphate groups bound to LPS from *E coli* strains D21 (Ra strain) and D21f2 (Re strain) was determined by applying ^{31}P-NMR spectroscopy The types of phosphate groups were identified by their chemical shifts as phosphomonoester (0 5 to 4 5 ppm), pyrophosphomonoester (-6 0 to -4 5 ppm and -11 5 to -10 ppm), pyrophosphodiester (-10 0 to -9 0 ppm, Fig 1) [12,13] The spectrum of LPS from strain D21 is similar to previously published spectra of LPS from other *E coli* K12 strains [8] and is consistent with the presence of approximately 7 5 phosphates per 3 KDO units as measured by elemental and sugar analysis [6] Integration of this spectrum shows that 40% of the signal can be attributed to monophosphate, 44% to pyrophosphomonoester, and 16% to pyrophosphodiester (Fig 1A) There was no indication of monophosphodiester substitution The pyrophosphodiester is presumably substituted with an ethanolamine group

The ^{31}P-NMR spectrum of LPS from strain D21f2 (Fig 1B) was similar to that of another heptose-less *E coli* mutant [13] and revealed that of the 2 4 phosphates per 2 KDO residues present [6] 73% were monophosphate and 27% pyrophosphomonoester Thus, in contrast with the D21 strain, there was no evidence of ethanolamine substitution on D21f2 LPS, since phosphodiester linkages were not detected

The ESR spectra of CAT_{12} in LPS aggregates from the Ra strain (D21) showed large differences in mobility depending on the LPS salt form and pH (Fig 2) To examine the influence of pH on the motion within LPS aggregates, sodium salts of LPS from both the D21 and D21f2 strains were adjusted to various pH values with buffers or with the direct addition of NaOH or HCl and then probed with CAT_{12}

CAT_{12} mobility in LPS from strain D21f2 increased as the pH was raised from 5 0 to 10 5, indicating that head-group mobility was dependent on pH Addition of sodium hydroxide to NaLPS, initially at approx pH 7, indicated a single titratable group at approx 2–4 OH^-/LPS (Fig 3A) In addition, head-group mobility of this NaLPS in buffered solutions measured by the hyperfine splitting parameter ($2T_{max}$) of CAT_{12} resolved two titratable groups in the basic range with pK_a values of approx 8 5 and 10 (Fig 3B) A third ionization was detected in the acidic range between pH 5 0 and 7 5 Between pH 7 5 and 8 5, where there was no significant change in head-group mobility, there was an increase in heterogeneity of the probe signal as measured by the width of the low-field peak, indicating multiple

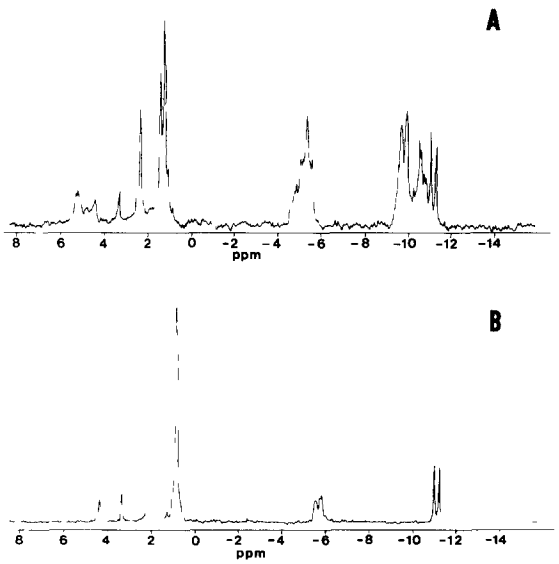

Fig 1 ^{31}P-NMR spectra of the NaLPS from (A) strain D21, and (B) strain D21f2

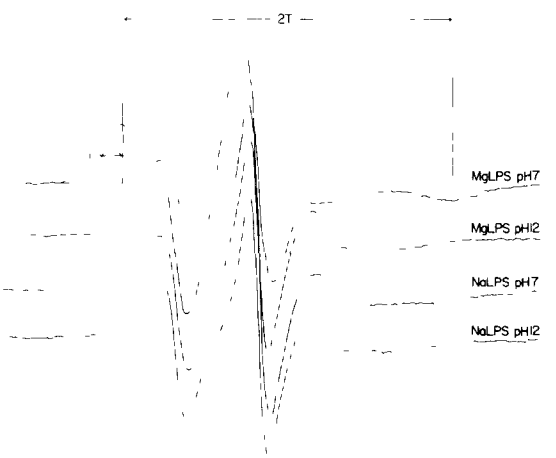

Fig 2 ESR spectra of CAT_{12} in LPS from *E coli* strain D21 at 37°C

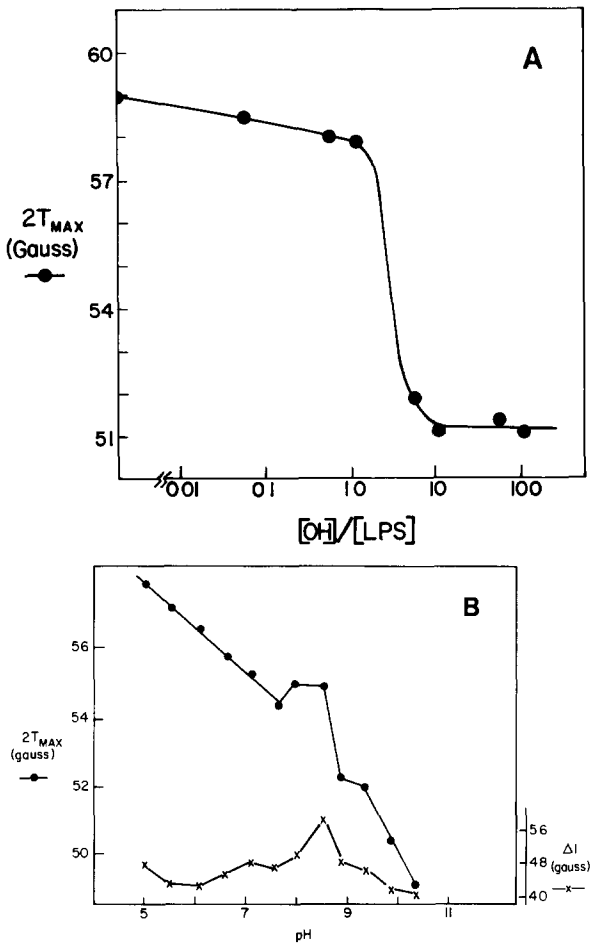

Fig 3 The pH dependence of the hyperfine splitting parameter ($2T_{max}$) and half-width at half-height of the low-field peak (Δ_1) of CAT_{12} bound to NaLPS from strain D21f2 (A) LPS was suspended in distilled water and aliquots of NaOH were added (B) LPS was suspended in buffer adjusted to the appropriate pH

populations of probe residing in slightly different environments

The magnesium salt of LPS from the Ra strain (D21) underwent large changes in head-group motion with changes in pH At pH 7, MgLPS was much more rigid than NaLPS, whereas at pH 12 the two salts were nearly identical (Fig 2, Table I) The effect of high pH on MgLPS fluidity was completely reversed by neutralization of the sample with HCl This indicated that the increase in head-group mobility at pH 12 was not the result of alkaline hydrolysis during the brief time of this experiment The slightly increased head-group mobility of MgLPS after neutralization, compared to MgLPS with no additives, could be accounted for by the presence of added NaCl (Table I)

TABLE I

HYPERFINE SPLITTING OF CAT_{12} IN *E COLI* D21 LIPOPOLYSACCHARIDE AT 37°C

Preparation	Half-width (G) [a]
NaLPS pH 7	18 9
NaLPS, pH 12	18 6
MgLPS pH 7	25 1
MgLPS, pH 12	19 0
MgLPS pH 7 → pH 12 → pH 7	24 7
MgLPS pH 7 +58 mM NaCl	24 8

[a] Low-field peak to mid-field splitting

The effects of pH on the phase-transition temperature and enthalpy of LPS from strain D21f2 were also characterized Scanning calorimetry

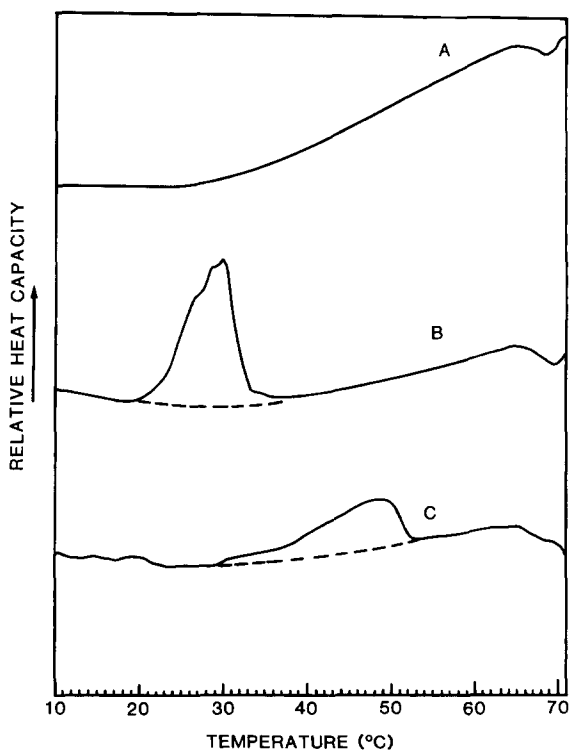

Fig 4 DSC endotherms of NaLPS from strain D21f2 (A) upon the addition of 3 25 mol NaOH per mol LPS (B) without the addition of acid or base and (C) upon the addition of 3 57 mol of HCl per mol LPS The LPS samples were suspended in double-distilled water prior to any additions

showed that the peak in the heating isotherm reached a maximum near neutral pH and decreased upon the addition of either NaOH or HCl to unbuffered samples (Fig 4) The enthalpy, ΔH_t, of the phase transition for NaLPS of strain D21f2 was greatest at approx pH 7 5 and decreased to zero at high pH and almost to zero at low pH (Fig 5) The enthalpy of the LPS phase transition is dependent on the number of acyl-chain methylene units that interact cooperatively in the gel-to-liquid-crystalline melt *E coli* LPS has six or seven acyl chains per molecule compared to two on phospholipids, a comparison of the ΔH_t for D21f2 LPS at pH 7 (8 45 kcal/mol LPS) with that for dimyristoylphosphatidylcholine and dimyristoylphosphatidylethanolamine (5 4 and 5 8 kcal/mol phospholipid, respectively) [14] indicates a lower degree of cooperative association for LPS on a per acyl chain basis The phase-transition midpoint of D21f2 NaLPS, measured by DSC heating isotherms, decreased with added base and increased with added acid (Fig 6) The temperature of the phase-transition midpoint at pH 5 1 was about

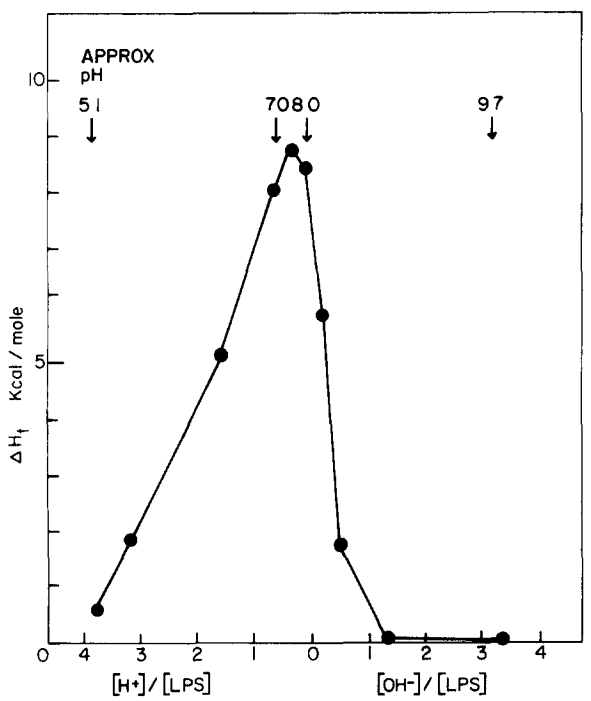

Fig 5 Influence of pH on the enthalpy of transition of NaLPS from strain D21f2

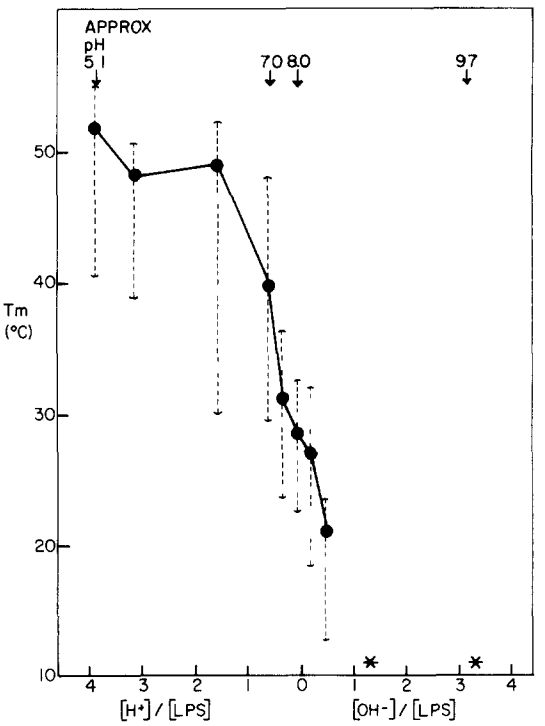

Fig 6 Influence of pH on the transition temperature of NaLPS from strain D21f2 as measured by DSC Data points represent the temperature at the peak in the endotherm curve Brackets indicate the upper and lower limits of the phase transition by extrapolation of the endotherm to the baseline The approximate pH values were determined at room temperature on diluted samples after the DSC scans Asterisks indicate scans which showed no endothermic peak (see Fig 4a)

50°C, while at pH 8 5 it dropped to nearly 20°C and at higher pH values was unresolved (see Fig 4)

Visually, D21f2 NaLPS appeared as a bluish-white, nonviscous solution at neutrality, as a clear, viscous solution at high pH (above pH 8 5), and as a white precipitate at low pH Right-angle scattering of light from 230 to 500 nm by D21f2 NaLPS gave similar results The intensity of light scattering at all wavelengths was more than 4-fold less at pH 10 as compared to that at pH 7 5 In addition, the peak intensity of light scattered by NaLPS at pH 5 and 7 5 was at slightly higher wavelengths than that at pH 10 The decrease in intensity of light scattering of LPS with increasing pH from 7 to 10 was rapid (< 2 min), whereas the change in light scattering upon lowering the pH from 7 to 5 took longer (> 5 min)

Discussion

Our results suggest that the lamellar structure of lipopolysaccharide is stabilized at neutral pH both by ionic interactions within the LPS, and by divalent cation bridges In the basic pH range the loss of hydrogen bonds, increased hydration and increased negative charge of the head group appeared to result in micellar structures, while in the acidic range the diminished charge repulsion and hydration and increase in hydrogen bonds resulted in insoluble complexes

Definitive identification of the polymorphic phases that LPS can assume in aqueous solution has not been made Our results support the findings of others which suggest that LPS can assume both lamellar and nonlamellar phases depending on the temperature, pH and salt content Freeze-fracture electron microscopy of *E coli* K12 LPS revealed structures which appeared either as bilayers or tubular micelles depending on the temperature and ions present [8], and X-ray diffraction and film balance measurements of LPS from *Salmonella minnesota* suggested that Re LPS can form both bilayers [15,16] and inverted micelles [15] By measuring the physical properties of LPS aggregates at different pH values it may be possible to identify ionic interactions which play a role in stabilizing LPS aggregates in the intact outer membrane The pK_a values of the phosphate groups on LPS are assumed to be in the ranges 1 0–3 5 and 6 0–9 5 for the first and second ionizations, respectively Soluble phosphates such as phosphoric acid and pyrophosphoric acid have pK_a values of 1–2 and 6–7, while the phosphate group on aggregates of phosphatidic acid has pK_a values of approx 3 5 and 9 0 [17,18] as a result of the high charge density at the micelle surface Thus, the high charge density on the surface of LPS aggregates may also shift phosphate pK_a values up in a similar fashion The LPS from the D21 strain reportedly has approx 7 phosphates, while that from strain D21f2 has 2–3 [6] Thus the charge density on the Ra LPS aggregates is presumably significantly higher than that on the Re isolate *E coli* LPS also contains an acidic sugar, KDO, which should ionize between pH 3 and 5, although the high surface potential on the LPS aggregate could shift the pK_a values up by as much as 3 pH units The LPS of strain D21 is thought to have three KDO residues per molecule [4], while that of D21f2 presumably has only 2 [13]

With these assumptions we propose that the observed increase in head-group mobility of D21f2 LPS between pH 5 0 and 7 5, detected with the ESR probe, is the result of ionization of carboxyl groups on KDO residues Previously we reported that for LPS isolated from D21f2 the ratios of cationic charges per phosphate in electrodialysed LPS (pH 4 0) and NaLPS (pH 7 0) were 1 51 and 1 84, respectively [6] Assuming counter ions bind 1 1 on a charge basis with the negative charges on LPS, we calculate that there are 3 6 net negative charges on D21f2 LPS at pH 4 0, and 4 4 charges on LPS at neutral pH Approx 2 4 of these charges result from the first ionization of phosphate groups (pK_{a1} between pH 1 and 3), leaving one negative charge at pH 4 and two negative charges at pH 7 which may be attributed to ionization of the carboxyls of the two KDO moieties

The observed increase in head group mobility as the pH was increased from 5 0 to 7 0 is thought to result from an increase in charge repulsion of the overall molecule, from an increase in head-group hydration, or from the loss of potential hydrogen bonding as the second carboxyl is ionized The additional increase in head-group motion observed from pH 8 5 to 10 5 is presumed then to result from the second ionization of the phosphate groups The addition of hydroxide ions to LPS at pH 7 resulted in a large mobility increase at 2–4 OH^- per LPS, possibly reflecting a structural alteration resulting from ionization of two phosphate moieties Emmerling et al [19] showed that lipopolysaccharide from *E coli* B/r buffered in the pH range 7–9, and Rosner et al [12] determined, using ^{31}P-NMR, that the pK_{a2} values for phosphomonoesters of LPS from an *E coli* Re mutant ranged from 6 75 to 8 1 The variation in pK_a values reported may be due to salt effects Abramson et al [18] found that the pK_1 of the second ionization of phosphatidic acid shifted from 8 6 to 7 9 in the presence of 100 mM sodium We found that unbuffered ReLPS was much more rigid when compared to LPS suspended in 50 mM buffer

Intermolecular hydrogen bonding has been im-

plicated in affecting both the phase state and overall lipid fluidity in acidic phospholipids [17] Stabilization of phosphatidic acid is greatest when the phosphates are half ionized, when more than half are ionized, charge repulsion pushes the head groups apart, while when less than half are ionized, fewer hydrogen bonds can be formed [17] Thus, hydrogen bonding between head groups of acidic lipids has been shown to rigidify bilayers and increase the gel-to-liquid-crystalline phase transition as compared with neutral phosholipids Furthermore, loss of hydrogen bonding at pH extremes may lead to nonbilayer structures Hydrogen bonds which may form between phosphate moieties in lipopolysaccharide could stabilize the lamellar structure and contribute to the rigidification of the head-group region at near neutral pH In addition, there should be a greater hydration of the LPS head group with increased ionization of the phosphate and carboxyl groups [20,21] This increased hydration may result in increased separation of LPS head groups At low pH values, the decrease in water within the head-group region may facilitate intermolecular hydrogen-bonding, resulting in a closer head group packing Thus the effective size of the LPS head group may be dependent on electrostatic repulsion, hydration, hydrogen-bonding and divalent cation bridging

The carboxyl groups on the KDOs may also be able to participate in hydrogen-bond formation Thus we expect lipopolysaccharide from strain D21f2 to be maximally rigid at approx pH 4 0, at which point one of the two KDO carboxyls is charged and the phosphates are half charged The DSC data confirm the ESR results, in that the gel-to-liquid-crystal phase-transition temperature (T_m) is highest at low pH The change in T_m with pH explains the increased heterogeneity in the ESR spin probe environment observed at pH 8 5, since heterogeneity in spin probe motion appears to be greatest at a phase transition At 37°C, as the pH is increased from 7 to 9, calorimetry results suggest that LPS from strain D21f2 undergoes a phase transition, presumably from gel to liquid-crystalline Isothermal phase transitions have also been demonstrated in the acidic phospholipid, phosphatidylserine, upon the addition of Ca^{2+} [22]

The absence of a DSC-detectable phase transition or enthalpy change in Re LPS at high pH indicates a lack of cooperative association between LPS molecules and may indicate the loss of lamellar structure The large decrease in the amount of light scattered by the sample at high pH is consistent with a dramatic alteration in the aggregate state It has been suggested that phospholipids with small head groups may shift between lamellar and hexagonal phases depending on the charge of the head group and resulting effective head-group size [17,23] We propose that a high pH values lipopolysaccharide from strain D21f2 assumes a hexagonal H_I or tubular micelle structure, this is consistent with our data high pH induced (1) an increase in head group mobility, (2) a loss of endothermic transitions, (3) an increase in solution viscosity, (4) a decrease in intensity of scattered light, and (5) a lowering of the wavelengths of light scattered We suggest that at high pH, charge repulsion between head groups and an increase in head group hydration induces an increase in curvature of the LPS aggregate surface Increased surface charge would also cause a decrease in effective particle size as the LPS aggregates changed from a lamellar to a tubular micelle structure with increasing pH

At low pH there is also a large decrease in enthalpy in D21f2 LPS which may indicate a loss of lamellar structure This may reflect a transition to an inverted micelle or hexagonal H_{II} structure resulting from a decrease in head-group size, concomitant with a decrease in charge, and a maximum in hydrogen bonding Studies with acidic phospholipids have shown that they can undergo an isothermal lamellar-to-hexagonal-H_{II} phase transition with decreasing pH [17,23] That Re LPS can also adopt a hexagonal H_{II} structure at low pH is consistent with the observed precipitation of the sample, its large light scattering, and the decrease in head-group mobility

The lipopolysaccharide from strain D21, when compared to LPS from the D21f2 strain, contains additional sugars, and phosphate, carboxyl and ethanolamine moieties As a result of these additional groups, the spin probe's motion is less restricted in the D21 LPS than in LPS from strain D21f2 Most likely the aggregate packing arrangement is such in the D21 sample that the lipid A portions of the molecules are further separated due to the larger head-group size compared to the

sample from D21f2 The greater spin-probe mobility of LPS from D21 at pH 12 as compared with that at pH 7 is likely the result of the second ionization of approx 5 phosphate groups (4 monophosphates and 1 pyrophosphomonoester) and possibly some carboxyl groups In addition to hydrogen bonding among phosphates and carboxyls, the ethanolamines may be able to form proton-transfer complexes with phosphates [24,25] which may add to the cohesiveness of the aggregates Isoelectric focusing of LPS samples has indicated a p*I* of between 6 1 and 6 6 [26,27] This is unexpected, since the phosphomonoesters on LPS should each carry at least a single negative charge above pH 3 Perhaps these samples of LPS were neutralized with tightly bound cations such as Ca^{2+} or Mg^{2+} so that the complex appeared neutral near pH 6 5

The overall properties of lipopolysaccharide from strain D21 at high pH values were similar to those of LPS from strain D21f2, the samples at basic pH were clear, viscous, and showed high head-group mobility, suggesting that aggregates of Ra LPS may also assume a micellar structure At low pH values, however, D21 LPS exhibited less of a tendency to form a precipitate than did LPS from D21f2 Presumably, the longer polysaccharide chain, as well as the higher charge density on D21 LPS, hinders the formation of inverted micelles

The head group of NaLPS from strain D21f2 is appreciably more rigid at 37°C than the head group of D21 NaLPS as measured by CAT_{12}, yet the fluidity of the acyl-chain regions of the LPS from the two strains is the same when measured with the ESR probe 5-doxyl stearate [9] Perhaps the head group of D21f2 LPS with its 'open' structure is easier to charge-neutralize by protons and monovalent cations than the head group of D21 LPS, which may be efficiently neutralized only by divalent cations Addition of magnesium ions to D21 LPS significantly decreased head-group motion Previous studies have shown that multivalent salts of LPS were much more rigid than monovalent salts at temperatures from 0°C to 50°C [9] This rigidification may be the result of greater charge-neutralization within the aggregate or divalent bridging between negatively charged groups It has been shown previously by electron microscopy that aggregates of D21 MgLPS at pH 7 appear lamellar whereas NaLPS appears as ribbons (perhaps an intermediate between H_I and lamellar) [9]

While this study is not definitive in assigning polymorphic phases to lipopolysaccharide, it does strongly suggest that it may change between lamellar and nonlamellar phases at different pH values Overall, the aggregate structure of LPS appears to depend on temperature, pH, and the ions present Non-lamellar or hexagonal phases appear to form at pH extremes, indicating that the degree of ionization of the phosphate and carboxyl groups on LPS, and thus the hydration of the LPS, the hydrogen bonding and divalent cation cross-bridging that can form, are critical in stabilizing a lamellar structure

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References

1 Nikaido M (1979) in Bacterial Outer Membranes (Inouye M ed) pp 15–34 Wiley New York
2 Morrison D C and Ulevitch, R J (1978) Am J Pathol 93 526–617
3 Galanos C (1975) Z Immun Forsch Bd 149 S 214–229
4 Luderitz, O , Freudenberg, M , Galanos C , Lehmann V Rietschel E and Shaw D (1982) Curr Top Membranes Transp 17, 79–134
5 Beveridge, T J and Koval, S F (1981) Appl Environ Microbiol 42 325–335
6 Coughlin R T Tonsager, S and McGroarty E J (1983) Biochemistry 22, 2002–2006
7 Leive L (1974) Ann N Y Acad Sci 235 109–129
8 Van Alphen, L , Verkleij A , Burnell, E and Lugtenberg B (1980) Biochim Biophys Acta 597, 502–517
9 Coughlin R T , Haug, A and McGroarty, E J (1983) Biochemistry 22, 2007–2013

10 Boman, H G and Monner, D A (1975) J Bacteriol 121, 455–464
11 Droge, W , Lehmann, V Luderitz, O and Westphal, O (1970) Eur J Biochem 14 175–184
12 Rosner, M Khorana, H and Satterthwait A (1979) J Biol Chem 254 5918–5925
13 Strain, S M , Fesik, S W and Armitage I M (1983) J Biol Chem 258, 13466–13477
14 Mabrey, S and Sturtevant, J M (1978) in Methods in Membrane Biology Vol 9 (Korn, E D ed) pp 237–274 Plenum Press New York
15 Brandenburg K and Seydel, U (1984) Biochim Biophys Acta 775 225–238
16 Labischinski H , Barnickel, G Bradaczek H , Naumann D Rietschel E T and Giesbrecht P (1985) J Bacteriol 162 9–20
17 Eibl, H (1983) in Membrane Fluidity in Biology, Vol 2 (Aloia R C ed) pp 217–236 Academic Press New York
18 Abramson, M Katzman R , Wilson, C and Gregor H (1964) J Biol Chem 239 (12) 4066–4072
19 Emmerling, G , Henning, U and Gulik-Krzywicki T (1977) Eur J Biochem 78 503–509
20 Franks F and Eagland, D (1975) CRC Crit Rev Biochem 3 165–219
21 Hauser H and Phillips M C (1979) in Progress in Surface and Membrane Science, Vol 13 (Cadenhead D A and Danielli, J F eds) pp 297–413 Academic Press New York
22 Portis, A Newton, C Pangborn, W and Papahadjopoulos D (1979) Biochemistry 18, 780–790
23 Cullis, P R , De Kruijff, B Hope M J Verkleij A J Nayar R , Farren, S B , Tilcock C Madden, T D and Bally M B (1983) in Membrane Fluidity in Biology, Vol 1 (Aloia R C ed), pp 39–81, Academic Press New York
24 Browning, J (1980) Biochemistry 20 7144–7151
25 Akutsu H Kyogoku Y Nakahara, H and Fukuda F (1975) Chem Phys Lipids 15 222–242
26 Goldman R C and Leive L (1980) Eur J Biochemistry 107 145–153
27 Goldman R C , White, D Ørskov F , Ørskov I Rick P D Lewis M S Bhattacharjee, A K and Leive, L (1982) J Bacteriol 151 1210–1221