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Rapid isolation method for lipopolysaccharide and lipid A from Gram-negative bacteria

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A fast, convenient extraction method for lipopolysaccharide (LPS), using a commercial RNA isolating reagent, allows the isolation of LPS or lipid A from low milligram (dry weight) quantities of bacterial cells. The method avoids the use of specialized equipment and has been used for processing relatively large numbers of samples. The major components of the commercial RNA isolating reagent, Tri-Reagent, are phenol and guanidinium thiocyanate in aqueous solution. The bacterial cell membranes are disrupted with guanidinium thiocyanate, which eliminates the need for mechanical cell disruption (*e.g.* French press) or heating. LPS and its degradation products, with particular attention paid to its bioactive lipid A portion, were measured and compared with those from the most common conventional extraction method, hot phenol–water. Negative ion quadrupole ion trap and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, fatty acid composition analysis by capillary gas chromatography, total and free phosphate by UV spectrophotometry and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed that LPS and lipid A isolated using the Tri-Reagent approach were cleaner and suffered less degradation through loss of phosphate and (or) fatty acyl side chains from lipid A. The Tri-Reagent extraction method generated low free phosphate contamination, 11% of the total phosphate concentration, whereas the hot phenol–water extraction method gave approximately 58% as free, inorganic phosphate. Similar results were observed for the degradation of fatty acyl side chains. The time required by the new method is considerably shorter (two or three days) than that required by conventional hot phenol–water extraction (about two weeks).

Introduction

The external layer of the cell envelope of Gram-negative bacteria, including *Salmonella typhimurium*, *Pseudomonas aeruginosa* (PA) and *Porphyromonas gingivalis* (PG), is a membranous structure that contains lipopolysaccharide (LPS) in addition to phospholipid and protein.[1] LPS is normally described in terms of three covalently linked chemical structures: lipid A, core polysaccharide and O-antigen. The development of techniques for the separation of this outer layer from the underlying cytoplasmic membrane has been important with respect to investigations of LPS structure and function.[2,3] In the field of microbial pathogenesis, much recent attention has been focused on the molecular events that occur when the bacterial cell encounters the mammalian host innate immune system.[4,5] LPS, and most importantly its lipid A functionality, is highly immunogenic and plays an important role in a number of diseases of bacterial origin, including Gram-negative sepsis and periodontal disease.[6]

Chemically, lipid A consists of a diglucosamine sugar backbone substituted with varying numbers of ester- or amide-linked fatty acyl side chains, of varying fatty acid composition (Fig. 1, see later). Phosphate and (or) additional carbohydrate groups are linked to carbons at the 1 and (or) 4′ position of the glucosamine dimer.[1–5] It is now known that the structure of lipid A, the most immunologically important part of LPS, is to some degree dependent on the external environment sensed by the bacterial cell, and that these structural changes may be of relevance to the pathogenesis of a number of diseases.[7,8] We have observed that lipid A structure in PA isolated from the lungs of cystic fibrosis (CF) patients differs markedly depending on how many generations of laboratory propagation have occurred since the bacteria were originally isolated from the lung.[9,10] These structural changes correlate with functional changes as measured by laboratory assays which measure the ability of LPS and lipid A to stimulate an inflammatory response from cultured mammalian cells.[7–9] The clinical role of LPS or lipid A induced inflammatory processes in the lungs of CF patients, suffering from PA related airway disease, is not clear at present, but is a question of active research interest. Thus, our laboratory maintains a long-term interest in developing scaled-down analytical procedures for LPS and lipid A that will ultimately allow the characterization of these molecules from bacteria isolated directly from human patients, without passage on growth medium in the laboratory. Broadly speaking, this analytical research effort has taken three different, but interdependent, directions. First, it includes the optimization of our detection method of choice, negative ion mass spectrometry.[11,12] Secondly, it includes the development of a capillary HPLC separation for complex lipid A biological extracts that is compatible with mass spectrometry.[13,14] Thirdly, it involves the development of a scaled-down extraction and cleanup procedure that is suitable for relatively small numbers of bacterial cells, corresponding to whole cell dry weights in the range of 100 μg to 10 mg. This paper describes our efforts to date in the area of isolation and cleanup procedures. The well-established hot phenol–water extraction method,[15–17] perhaps the most common LPS isolation method, has major limitations in our hands when attempting to manipulate such small quantities of cells. It also has a reputation for preferentially isolating smooth-type LPS, *i.e.* LPS which contains O-antigen side chains, as opposed to rough-type, that lack this functionality. The method of Darveau and Hancock[18] is less widely known, but has proven to be equally effective for extracting both rough and smooth LPS, while also minimizing degradation problems associated with hot phenol–water. Their approach is based on the mechanical disruption of the intact cells and sodium dodecyl sulfate (SDS) solubilization, followed by magnesium precipita-

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tion in cold ethanol, and involves a heating step (85 °C, pH 9.5) under alkaline conditions that is necessary for complete removal of SDS-resistant proteins. The Darveau and Hancock method also requires an ultracentrifuge. Although we prefer to avoid the use of mechanical cell disruption, ultracentrifugation and the application of heat, we use the cold magnesium ethanol step in our own work, as described in the Experimental section, when we are interested in examining whole LPS rather than lipid A only. We have developed a microscale isolation procedure for LPS that is broadly applicable among different Gram-negative bacteria and appropriate for the small numbers of cells found in clinical samples, *e.g.* sputum from CF patients. Our target sample size in such cases to date has been roughly 10^9 to 10^{11} bacterial cells, although we hope that in the future we can go lower. The method has been used to process up to 24 samples per batch, and is amenable to high throughput screening for changes in LPS or lipid A structure. The basis of the method is the use of a commercial extraction reagent. The active components contained in this product consist of guanidinium thiocyanate and phenol in a monophase aqueous solution.

Experimental

Bacterial strains and reagents

Bacterial strains grown in-house at the University of Washington were *Salmonella typhimurium* (strain CS341, from the laboratory of S. I. Miller), *Pseudomonas aeruginosa* (wild-type PAO1, from the laboratory of Steven Lory) and *Porphyromonas gingivalis* (strain 33277, from the laboratory of R. J. Lamont). Purified LPS from *S. typhimurium* (lot #96H4021) was purchased from Sigma (St. Louis, MO, USA). Lipid A from *S. typhimurium* Re-mutant was purchased from RIBI ImmunoChem Research, Inc. (Hamilton, MT, USA). Tri-Reagent was purchased from Molecular Research Center, Inc. (Cincinnati, OH, USA). Electrophoresis and staining reagents (electrophoresis grade) were obtained from Bio-Rad (Hercules, CA, USA). Solvents were of HPLC analytical grade and solutions were prepared with organics free, microbiologically sterile, 18 MΩ water (Nanopure UV, Barnstead, Thermolyne Corporation, Dubuque, IA, USA). Methanol and chloroform were used without further purification (Burdick & Jackson, Muskegon, MI, USA). Magnesium chloride was ACS reagent grade (Sigma, St. Louis, MO, USA). 5-Chloro-2-mercapto-benzothiazole (CMBT) (Aldrich, Milwaukee, WI, USA) was recrystallized in absolute ethanol and kept at −20 °C. Monobasic potassium phosphate was labeled as enzyme assay grade (FisherBiotech, Fair Lawn, NJ, USA) and used as received. HCl, ACS reagent grade (J. T. Baker, Phillipsburg, NJ, USA), was used as received.

LPS isolation from whole bacterial cells

Tri-Reagent method. Lyophilized bacterial cells (1–10 mg) were suspended in 200 μl of Tri-Reagent. The cell suspension was then incubated at room temperature for about 10–15 min for complete cell homogenization. After incubation, 20 μl of chloroform per mg of cells was added to create a phase separation. The mixture was then vigorously vortexed and incubated at room temperature for an additional 10 min. The resulting mixture was centrifuged at 12 000*g* for 10 min to separate the aqueous and organic phases. The aqueous phase was transferred into a new 1.5 ml centrifuge tube. Distilled water (100 μl) was added to the organic phase. The mixture was vortexed, incubated at room temperature for 10 min, and centrifuged at 12 000*g* for 10 min. The upper aqueous phases from both steps were combined. Two additional water extraction steps were repeated to ensure complete removal of LPS from the organic phase. The combined aqueous phase was dried using a speed vac (Jouan, Winchester, VA, USA, Model RC10.22).

In cases where it was desirable to examine whole LPS rather than lipid A, we used the cold ethanol magnesium precipitation procedure developed by Darveau and Hancock.[18] Crude Tri-Reagent extracted LPS was dissolved in 500 μl of 0.375 M magnesium chloride in 95% ethanol, stored at −20 °C, followed by centrifugation at 12 000*g* for 15 min. The pellet was suspended in 200 μl of distilled water and lyophilized to give fluffy white solid LPS totaling 15–20% of the starting material dry weight. In the majority of samples, where our interest was primarily in the lipid A group, this step was omitted.

Hot phenol–water LPS isolation. For purposes of comparison with the Tri-Reagent approach, LPS from bacterial cells was also extracted using hot phenol–water.[15–17] In order to help clarify at exactly which stage of the process any degradation might be taking place, the purified LPS standard (Sigma) was also treated with hot phenol-water. The Sigma reference material was itself originally produced using a variant of the hot phenol–water procedure. However, this fact did not prevent it from being a useful standard, because, whatever the origins of the material, it was very sensitive to heat and extremes of pH. This sensitivity to degradation, coupled with its ready availability and modest cost, made it a reasonable choice for many of our experiments, in addition to our own LPS extracts.

Lipid A isolation

Lipid A was isolated from crude LPS by mild acid hydrolysis.[19] LPS was dissolved in 500 μl of 1% SDS in 10 mM sodium acetate (pH adjusted to 4.5 with 4 M HCl) and then placed in an ultrasound bath until the sample was dissolved. It was then heated at 100 °C for 1 h. The mixture was dried by speed vac. SDS was removed by washing the mixture with 100 μl of distilled water and 500 μl of acidified ethanol (prepared by combining 100 μl 4 M HCl with 20 ml 95% ethanol) followed by centrifugation (2000 *g* for 10 min). The sample was then washed again with 500 μl of (non-acidified) 95% ethanol and centrifuged (2000*g* for 10 min). The centrifugation and washing steps were repeated. Finally, the sample was lyophilized to give fluffy white solid lipid A.

Negative ion mass spectrometry

Electrospray ionization (ESI) MS. ESI mass spectrometry was performed with a Finnigan LCQ ion trap (San Jose, CA, USA) modified with a capacitive electrospray device.[12] A syringe pump was used to infuse the lipid A sample. Typical conditions were: infusion rate, 1 μl min^{-1}, spray voltage, 2.4 kV; capillary temperature, 250 °C. The instrument was carefully tuned and optimized with diphosphorylated lipid A (RIBI) for MS^1 data acquisition in negative ion mode prior to the experiments. Much stricter attention to cleanliness with respect to the heated capillary and skimmer is required relative to that required for more routine positive ion experiments, *e.g.* tryptic peptides. Approximately 100 scans (300 to 2000 u) were acquired over a 3 min period and signal averaged post-run. The tuning solution and the lipid A samples were prepared at a concentration of between 2 and 5 pmol μl^{-1} in 1 : 1 $CHCl_3$–CH_3OH.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. Negative ion MALDI-TOF mass spectra were measured on a Bruker Biflex-III reflectron time-of-flight mass spectrometer (Bruker-Franzen, Bremen, Germany) equipped with a SCOUT-384 inlet and gridless delayed

extraction ion source. The ion acceleration voltage was 19 kV and the reflectron voltage was set to 20 kV. For delayed extraction, a 3 kV potential difference between the probe and the extraction lens was applied with a time delay in the range of 200 to 400 ns after each laser pulse. Samples were irradiated at a frequency of 3 Hz by 337 nm photons from a pulsed Laser Science (Cambridge, MA, USA) nitrogen laser. Typically 100 shots were summed into a single mass spectrum. CMBT was used as the matrix.[20] Spectra were calibrated externally using the monoisotopic $[M - H]^-$ ion, *m/z* 1796, of our RIBI lipid A standard and reprocessed by Bruker XMASS 5.0 software running on either a Sun Ultra 5 or Sparc 20 computer.

Carbocyanine colorimetric assay for LPS

The carbocyanine colorimetric assay was performed for LPS for both quantitative and qualitative purposes.[21] For the quantitative estimation of LPS and lipid A, a calibration standard curve (correlation coefficient, 0.99) was generated using a concentration range of 0.1 to 4 μM LPS from Sigma as described below. In the procedure, LPS (0.5 μg) was dissolved in 0.5 ml H_2O in a 1.5 ml microcentrifuge tube. To this solution, 0.3 ml of the dye reagent and 0.2 ml of ascorbic acid were added. The mixture was then incubated at 4 °C in the dark for 10 min. Absorbance readings at 472 nm were made in a 1 cm quartz cell using a Cary 3E UV/VIS spectrophotometer. The procedure was performed in a darkened room. Each measurement was performed in triplicate.

Phosphate determination

Following the method of Ames[22] for the quantitative analysis of free and total phosphate, the phosphate content of LPS isolated using Tri-Reagent was measured and compared with that for LPS isolated using hot phenol–water. A standard curve for external standard calibration (correlation coefficient, 0.99) was generated with 0–100 μM KH_2PO_4 in distilled water. Absorbance (820 nm) readings were made in a 1 cm quartz cell with a Cary 3E UV/VIS spectrophotometer. Each measurement was performed in triplicate.

Polyacrylamide gel electrophoresis (PAGE)

The analysis of bacterial LPS by SDS-PAGE was followed by silver staining, according to the method of Tsai and Frasch.[23]

Fatty acid analysis

Following the manufacturer's instructions (Instant Methanolic HCl, stock no. 18053, Alltech, Deerfield, IL, USA), fatty acyl chains were either hydrolyzed directly from whole LPS or from lipid A, esterified by methanolic HCl, and analyzed by capillary gas chromatography with flame ionization detection as their fatty acid methyl esters.[24] All responses were calculated from peak areas relative to the pentadecanoic acid (Aldrich, Milwaukee, WI, USA) internal standard added prior to the conversion to methyl esters.

Results and discussion

The basis of the method for LPS purification is the use of a commercial RNA isolating reagent, Tri-Reagent, that is normally used for the isolation of total RNA or the simultaneous isolation of RNA, DNA and proteins.[25] Based on our previous experience with LPS isolation by the hot phenol–water method[15–17] and the magnesium precipitation method developed by Darveau and Hancock,[18] we examined the use of Tri-Reagent, in the absence of heating or mechanical disruption of the bacterial cell membranes, as an alternative approach to the isolation of LPS and lipid A from whole cells. In addition, the lengthy LPS isolation procedure using conventional phenol–water extraction can be shortened. The entire procedure can be completed in less than three days. The majority of time is spent waiting for the removal of water during the lyophilization and (or) speed vac steps. With the hot phenol–water method,[15–17], much time is spent with multiple dialysis steps. The LPS isolated using the Tri-Reagent procedure has been observed to be cleaner, containing fewer contaminants and degradation products, than that isolated using the conventional hot phenol–water method. The method of Darveau and Hancock[18] also produces cleaner LPS, but has not been amenable in our hands to high sample throughput or the use of small quantities of cells. Although the work reported here has been with smooth strains, Ernst and Miller[26] have applied our method to rough mutants from various species of *Yersinia*, *Pseudomonas* and *Salmonella* with success, although at present no systematic analytical studies of LPS recovery from rough strains have been performed. Whether or not the Tri-Reagent method discriminates against LPS with either short or missing O-antigen side chains is still an open question.

Extraction of LPS from the crude Tri-Reagent isolate

Because our intended purpose for developing the microscale LPS isolation method was to apply it to clinical samples, where LPS yields are usually low, it was important to find optimum extraction conditions for maximizing the yield of cellular LPS from the crude isolate, while minimizing contamination from co-extracted material such as DNA and phospholipid. Following cell homogenization using Tri-Reagent, LPS extractions were carried out at room temperature with pure water, 1 mM EDTA, and pure water at 70 °C, and the yield of LPS under each set of extraction conditions was compared to that observed for hot phenol–water extraction. As summarized in Table 1, the water extraction at room temperature gave roughly a two-fold higher recovery. These results can be rationalized based on the amphipathic nature of LPS and its greater solubility in water relative to the lipid co-extractives that are of greatest concern in our laboratory. Based on the results shown in Table 1,we adopted the water extraction procedure at room temperature for all subsequent work. LPS was quantitatively removed from the homogenized cell suspension after 4 to 5 repetitions of the water extraction step, as determined by UV spectrophotometry. As explained in more detail in the Experimental section, the water extraction can, as an optional step, be followed by magnesium precipitation in cold ethanol[18] to give a cleaner product.

LPS Characterization

According to Tsang *et al.*[27] and others,[28] hot aqueous phenolic solutions degrade the fatty acyl chains of lipid A, which

Table 1 Lipid A recovery as a function of the solution conditions used to extract whole LPS from the crude Tri-Reagent isolate. Determinations were made in triplicate (±*s*) by measuring dry weight, using *S. typhimurium* whole cells as the starting material. The purity of the recovered lipid A was verified by mass spectrometry

Isolation method	Extraction from crude isolate	Cells/mg	Lipid A/mg
Tri-Reagent	Water, 25 °C	10	1.4 ± 0.2
Tri-Reagent	1 mM EDTA, 25 °C	10	0.8 ± 0.05
Tri-Reagent	Water, 70 °C	10	0.4 ± 0.05
Hot phenol–water	Water, 70 °C	10	0.8 ± 0.1

unfortunately is also the part of the LPS molecule of greatest interest in our research, as described in the Introduction. The authors stated that the presence of degradation products is one of the main causes of the heterogeneity characteristic of LPS preparations. This has been our observation as well. However, we believe that there is also a good deal of heterogeneity that is of biological origin. Distinguishing laboratory artifacts from natural heterogeneity remains one of the major analytical challenges when dealing with LPS preparations. The ester and phosphoester bonds of lipid A are easily hydrolyzed, and care must be taken to use as gentle a procedure as possible to hydrolyze the acid labile 3-deoxy-D-manno-octulosonic acid (KDO) group that covalently links lipid A with the carbohydrate portions of LPS. Even with the mild acid hydrolysis procedure,[19] we believe that in certain cases the correct number of monomer units and (or) the identity of functional groups attached to lipid A, as it really exists *in vivo*, are being incorrectly identified due to losses during the isolation procedure. There is clearly much room for improvement methodologically, regardless which isolation approach is currently being used, including ours. With emphasis on identifying losses of acyl chains and phosphate from the lipid A portion, qualitative analysis of LPS was performed using four different analytical methods. Quantitative data for estimating recoveries and degradation were also acquired using three of the methods, the carbocyanine colorimetric assay, fatty acid content (as methyl esters) by gas chromatography with flame ionization detection (GC-FID) and the free and total phosphate determination. We employed negative ion mass spectrometry and SDS-PAGE primarily for qualitative purposes.

Carbocyanine colorimetric analysis. The relative concentration of lipid A and LPS was measured by colorimetric assay with carbocyanine dye. Carbocyanine is a dinaphthoylated thiazolium cationic dye known to form complexes with polyanions such as nucleic acids and LPS, depending on the assay conditions.[21] Both whole LPS and lipid A form a complex with the dye under acidic conditions. The ultraviolet absorption maximum of LPS or lipid A shifts from 260 nm to 462 nm under the conditions given.[21] Possible interferences by either proteins or nucleic acids under these assay conditions were investigated with BSA (bovine serum albumin) and a plasmid DNA. These two control materials did not interfere to any extent at 462 nm. Although the absorbance decreases in the dark slowly at a steady rate after mixing, the linearity of absorbance *versus* LPS or lipid A concentration was verified over the range 0.1 to 4 μM, when the experiment was completed within an hour of adding the dye. The response was linear with a correlation coefficient of 0.997.

Analysis of lipid A hydrolyzed from LPS by ion trap ESI mass spectrometry. Fig. 1 shows the negative ion ESI mass spectra obtained from lipid A hydrolyzed from Sigma *S. typhimurium* LPS, after the LPS was treated with the Tri-Reagent method (Fig. 1b) and hot phenol–water (Fig. 1c). For a control, Fig. 1a shows a lipid A spectrum obtained from LPS exposed to mild acid hydrolysis only.[19] Although the expected mass spectral peaks were observed in each case, multiple peaks appeared in the region from *m/z* 1200 to 1700 for the sample exposed to hot phenol–water (see Fig. 1c). The artifact peaks in the mass spectrum and the yellow discoloration that was unique to the sample treated with hot phenol–water suggested that greater sample degradation and (or) contamination was taking place. Mass spectra acquired for less well-characterized isolates from PA and *S. typhimurium* showed similar results, with less degradation and fewer low abundance peaks if either the Tri-Reagent procedure or the Darveau and Hancock[18] method was used.

SDS-PAGE. Analysis by SDS-PAGE followed by silver staining was used to detect and visually characterize LPS. One nanogram of LPS can be detected if the sensitive silver staining method is used to visualize the bands. LPS gives a dark 'staircase' pattern of bands due to the carbohydrate chain length variation of the O-antigen portion.[23] As shown in Fig. 2, LPS usually displays some degree of size heterogeneity. However, LPS exposed to hot phenol–water (Lanes 3 and 5 in Fig. 2) shows some faint bands with dark smearing, especially the cellular extract shown in Lane 5. In contrast, both the LPS standard (Lane 2) and the cellular extract (Lane 4) exposed to Tri-Reagent gave a cleaner pattern. For control purposes, the

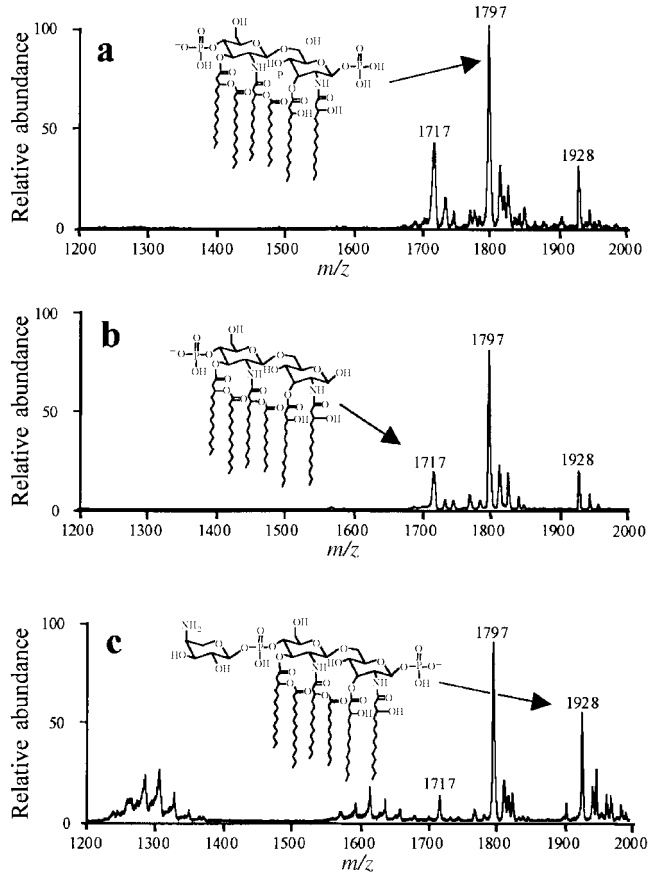

Fig. 1 Negative ion electrospray ion trap mass spectra (MS^1) of lipid A isolated from (a) purified *Salmonella typhimurium* LPS standard, (b) after treatment with Tri-Reagent and (c) after treatment with hot phenol–water.

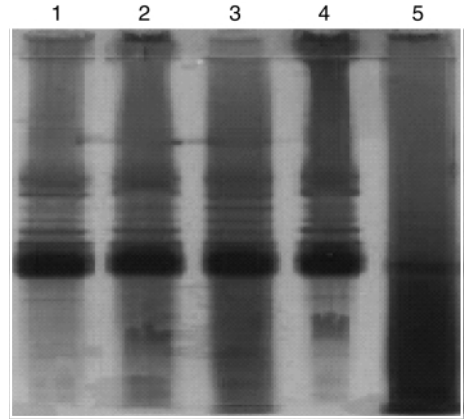

Fig. 2 SDS-PAGE analysis of LPS. Lane 1, purified *S. typhimurium* LPS standard as purchased; Lane 2, the same LPS after exposure to the Tri-Reagent extraction procedure; Lane 3, LPS standard after exposure to the hot phenol–water procedure; Lanes 4 and 5, *S. typhimurium* LPS isolated from whole cells (strain CS341) in-house using the Tri-Reagent and hot phenol–water procedures, respectively.

standard (Lane 1) was run as received from the vendor. These visual observations of the silver stained gels are consistent with the lipid A mass spectrometric data as shown in Fig. 1. The gel data are important in that they suggest that the O-antigen and core polysaccharide groups survive the Tri-Reagent procedure relatively intact.

Free and total phosphate. To examine the degradative removal of phosphate, free and total phosphate were determined quantitatively by the method of Ames[22] after mild acid hydrolysis of *S. typhimurium* LPS. Free phosphate values, as a percentage of total phosphate concentration, from LPS, LPS exposed to Tri-Reagent and LPS exposed to hot phenol–water, were measured to be 2%, 11% and 58%, respectively. These results are consistent with prior observations regarding loss of phosphate as a consequence of exposure to hot phenol–water.[27,28]

Fatty acid analysis. After hydrolysis of fatty acyl chains by methanolic HCl, *S. typhimurium* LPS fatty acids were measured as their methyl esters by capillary GC.[24] The fatty acid profiles for untreated LPS and LPS treated with Tri-Reagent were similar, with a small amount of degradation attributable to the isolation procedure. However, the total fatty acid content of LPS treated with hot phenol–water was only about 30% of that of the LPS control.

Isolation of lipid A from three different cell types

The Tri-Reagent extraction method was applied to the isolation of lipid A from three different bacteria. Fig. 3a is a mass spectrum from *S. typhimurium* (CS341, *pmre*$^-$) acquired with the ion trap. A typical hexa-acylated lipid A signal for the $[M - H]^-$ ion was detected at *m/z* 1797 (average mass) along with other structural variants. This ESI spectrum contains a number of $[M - 2H]^{2-}$ ions in the region below *m/z* 1200, as well as low abundance signals for salt adducts and impurities. Fig. 3b shows the high mass region of an ion trap mass spectrum for lipid A from *Pseudomonas aeruginosa* (wild-type, PAO1) that is dominated by the penta-acylated form with an $[M - H]^-$ ion at *m/z* 1447 (average mass). Fig. 3c is a MALDI-TOF spectrum of lipid A from *Porphyromonas gingivalis*, the dominant structure being a penta-acylated form with an $[M - H]^-$ ion at *m/z* 1691 (monoisotopic). The mass spectrometry data are generally consistent with the literature and with the hypothesis that very little additional sample degradation is introduced by the Tri-Reagent procedure itself, beyond what occurs during the mild acid hydrolysis step in which lipid A is cleaved from LPS.

Within the broader context of progress in mass spectrometry and in separation science, as they relate to these chemically complex polyanions, this isolation method should be a step towards achieving both lower detection limits and a reduction in artifacts due to sample degradation. After two years of experience, we believe it to be a reliable means of extracting small quantities of LPS from bacteria grown in culture media. However, the more complex matrix of CF airway sputum requires an additional stage of cleanup to remove residual co-extractives. Our first results with such samples suggest that matrix interferences and chemical noise are still a problem, when one is trying to push the limits of sensitivity using either negative ion MALDI-TOF or ion trap mass spectrometry. We are presently investigating capillary HPLC and low pressure microcolumn approaches to this problem.

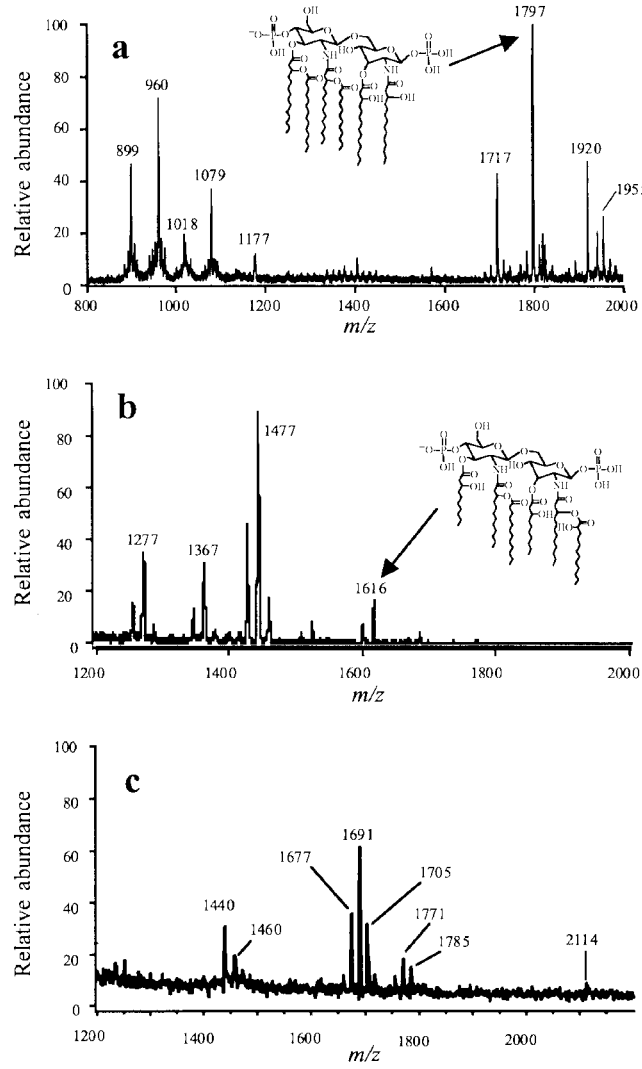

Fig. 3 Negative ion mass spectral analysis of lipid A hydrolyzed from LPS extracted from whole cells using Tri-Reagent. (a) Electrospray ion trap mass spectrum (MS^1) of lipid A from *Salmonella typhimurium*. (b) Electrospray ion trap mass spectrum of lipid A from *Pseudomonas aeruginosa*. (c) MALDI-TOF mass spectrum of lipid A from *Porphyromonas gingivalis*. No structure is given for PG lipid A because these ions remain poorly understood at present.

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