PHD THESIS

NOVEL CAPILLARY AND MICROCHIP ELECTROPHORETIC METHODS ALONG WITH MASS SPECTROMETRIC STRATEGIES FOR MONITORING BACTERIAL ENDOTOXINS

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1. Introduction

The bacterial species (almost all) can be differentiated into two groups, called Gram-positive and Gram-negative based on their cell-wall structure (Figure 1). Endotoxins are important virulence factors synthesized by Gram-negative bacteria only. These microorganisms are naturally found in our normal intestinal flora, as well as in the environment, including the human pathogens such as *Escherichia coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Haemophilus*, *etc*. Endotoxins gained their name from the Greek word *endo* meaning '*inner*', as they represent the integral constituents of the Gram-negative bacterial outer membrane.

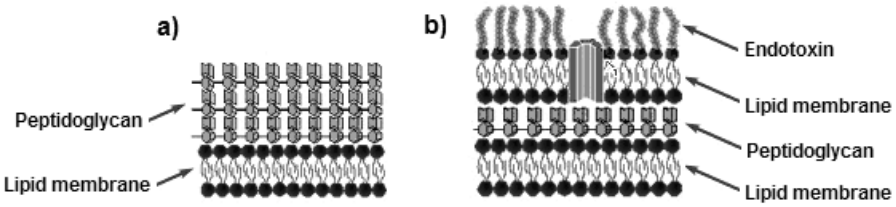


Figure 1. Schematic of a) Gram-positive and b) Gram-negative cell-wall arrangement.

1.1. Chemical construction of endotoxins

Chemically, endotoxins are a mixture of lipopolysaccharide (LPS) molecules. The basic structure of *smooth* (*S*-type) intact endotoxins consists of a hydrophobic lipid part termed "Lipid A", covalently attached to a hydrophilic polysaccharide part composed of a "core" region and a long "O-polysaccharide" chain (Figure 2). The Lipid A part is integrated into the cell-wall and the polysaccharide part extends outward from the bacterial cell surface. In *rough* mutant strains the O-specific chain is missing and such endotoxins are termed R-form LPS or lipooligosaccharides (LOS). All regions of LPS display heterogeneity (intrastrain differences). Thus, *S*-type endotoxins normally consist of populations of molecular components with different length in the O-polysaccharide chain, including components without the O-chain, and with incomplete core oligosaccharide. The structure of LPSs varies widely between bacterial strains (interstrain differences). For that reason, endotoxins can be used for serological classification of bacteria. LPS preparations can be obtained by simple procedures, like extraction, washing and purification. The molecular mass of endotoxin monomers are in the range of 2-20 kDa. The amphipathic LPS molecules form aggregates in aqueous solutions with micellar weight up to 1 million Da. These aggregates can be broken down by different surfactants or proteins.

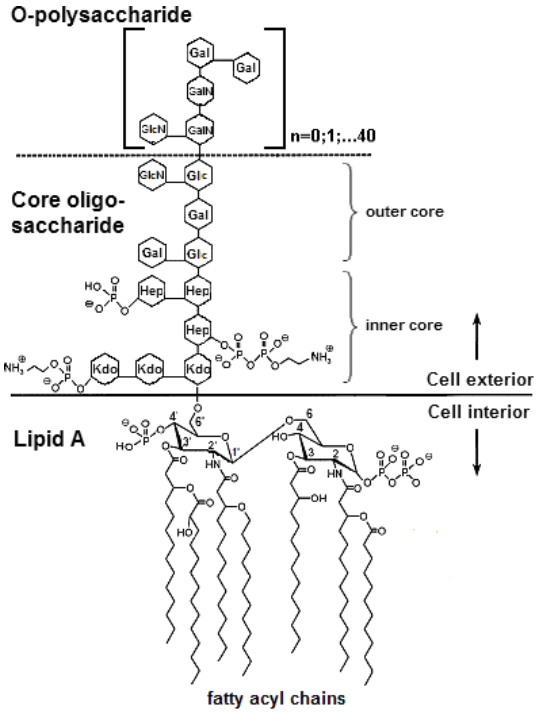


Figure 2. General structure of the intact LPS molecule. Gal: galactose, Glc: glucose, Hep: heptose, GalN: N-acetyl-D-galactosamine, GlcN: N-acetyl-D-glucosamine, Kdo: 2-keto-3-deoxy-octonic acid. The fatty acyl chains are generally saturated and their chain lengths vary between 12 and 18 carbon atoms. Anionic functions are contributed by phosphate and Kdo and (sometimes) hexuronic acid residues. Mild acid hydrolysis preferentially splits Kdo linkages in the LPS molecule, thus, it is usually used to separate Lipid A from the carbohydrate moiety.

1.2. Biological definition of endotoxins

When bacteria grow or lyse, endotoxins are set free into their surroundings. The O-side chains are primary targets of the immune receptors on macrophages and the epithelial cells in humans. The presence of higher than 1 EU (endotoxin unit = 0.1 ng endotoxin)/kg of body weight in the bloodstream is highly toxic, called endotoxemia. The toxicity is related to the Lipid A moiety of LPS. The resulting pathophysiological reactions are high fever, hypotension, intravascular coagulation, endotoxin shock and even death by lung or kidney failure, as regularly observed during Gram-negative bacterial sepsis. In addition, endotoxins are very heat stable molecules (the standard laboratory autoclaving procedures cannot break down these substances) therefore, it is obligatory to screen endotoxin levels in parenteral solutions, *e.g.*, infusion fluids. Besides the harmful effects, the immunostimulatory activity of endotoxins of low concentration in normal conditions may be beneficial for the human health.

1.3. Methods used in endotoxin monitoring

The recognition of the very significant disease-causing potential of these interesting microbial constituents provided the basis for studies directed at the isolation, purification, and detailed chemical characterization of the active constituent(s). The existing methods for the detection and analysis of endotoxins can be divided into two groups, to detect bioactivity of endotoxins, or to provide structural information about the molecules. The so called “endotoxin assays” are generally carried out by diverse *in vitro* and *in vivo* biochemical tests, *e.g.*, the USP Rabbit test and the LAL (*Limulus* Amebocyte Lysate) test.

The structural analysis of endotoxins was problematic for a long time, since the amphipathic nature and the strong tendency of LPSs to aggregate in solutions hindered the investigation. Generally, LPS substructures (for example, core sugar and Lipid A anchor) obtained by mild and strong acidic hydrolysis are analyzed by gas-chromatography, nuclear magnetic resonance (NMR) and soft ionization mass spectrometry (MS), which usually complement each other in resolving the entire molecule. However, a disadvantage of the chemical degradation of LPS is the high risk of loosing labile, but biologically important functional groups.

SDS-polyacrylamide gel-electrophoresis method with silver staining is used for revealing the heterogeneity of intact LPS molecules. The characteristic 'ladder' pattern (with the 'ladder steps' representing the number of O-chain repeating units) provides the basis for differentiation of *smooth* and *rough* type endotoxins. However, the silver stain detection has limited resolution since the visualization often results in doublets or diffuse/broad staining rather than distinct LPS bands.

The fast, sensitive and quantitative characterization of endotoxins using instruments with standard optical detectors is challenging due to the lack of chromophores in LPS structures. Capillary electrophoresis (CE) and microchip electrophoresis, both employing conventional UV/VIS or fluorescence detectors, are high-resolution separation methods widely used for the analysis of biological molecules like drugs, peptides, nucleotides, saccharides, viruses, cells. The question arose, whether these techniques can be applied also for the analysis of endotoxins. To our knowledge, only a few attempts by CE, while no detection methods based on microchip electrophoresis were used to study endotoxins earlier.

Another essential problem faced in LPS research is the high heterogeneity of the endotoxin molecules. The direct identification of structural microheterogeneities of intact LPSs is a particular challenge. This thesis focuses on the molecular characterization of enterobacterial LPS molecules by CE and microchip electrophoresis, along with mass spectrometric strategies – with delimitations to MALDI-TOF MS – in order to provide fast and precise structural elucidations of the LPS molecular components from certain bacteria.

2. Aims

The overall aim of this study was to develop new and fast screening methods by the use of standard optical detectors for the separation and analysis of bacterial endotoxins (lipopoly- or lipooligosaccharides, LPSs or LOSs, respectively) in the field of immunochemistry or clinical vaccinology, for example antigen structure analysis. Furthermore, to know the exact molecular masses and chemical structures of certain intact LPSs and that of the separated LPS components. For this, our ideas and challenges were:

1. To visualize endotoxins by their protein complexes in capillary electrophoresis in order to overcome the solubility and detection problems of the amphiphilic and UV-inactive LPSs or LOSs, since it is known, that proteins not only disaggregate but also form UV-.detectable complexes with endotoxins.

2. To develop a qualitative and quantitative microchip electrophoretic method with high sensitivity and speed for detection of pure endotoxins based on the facts, both, that endotoxin aggregates are dispersed by SDS in aqueous solutions and that SDS interacts with fluorescent dyes. In addition, to investigate the capability of such microchip electrophoresis systems to replace the time-consuming and laborious SDS-PAGE with silver staining routinely used for the detection of endotoxins.

3. To use the new microchip technique in the characterization of endotoxins prepared directly from whole-cell lysates (without further purification of the LPS samples), *i.e.*, to develop a fast-way method to detect *S* and *R* type endotoxins. To study the correlation of the electrophoretic profiles with those of purified LPSs.

4. To determine the molecular masses of endotoxins from certain species of the *Enterobacteriaceae*, and to clarify their intrinsic heterogeneity by use of mass-spectrometry.

3. Materials and Methods

3.1. Bacterial strains and endotoxins

Bacterial strains of smooth *Escherichia coli* NCB O21, O55, O83, O111, O112, 102, ATCC 25922, *Proteus morganii* O34, *Salmonella urbana* O30, *Salmonella adelaide* O35, *Salmonella minnesota* wildtype, *Shigella sonnei* phase I, *Shigella dysenteriae* 2, *Yersinia enterocolitica* O9 and of rough *Escherichia coli* NCB D31, *Salmonella minnesota* R595, *Shigella sonnei* phase II (4303), 41, 562H and 4350 were obtained from the collection of the Institute of Medical Microbiology and Immunology, University of Pécs, Hungary.

The bacteria were grown at 37°C in a fermentor and collected by centrifugation. LPS was extracted from smooth bacteria by the hot phenol/water method according to *Westphal et al.* (1952), and from rough strains by the phenol-chloroform petrol ether method of *Galanos et al.* (1969) and lyophilized. The LPS solutions were prepared by dispersing 1 mg of the lyophilized LPSs.

In order to obtain the Lipid A, LPSs were hydrolyzed with 0.1 M sodium acetate buffer (pH 4.4) at 100°C for 2 h, lyophilized, extracted with in HCl/ethanol and ultracentrifuged. The sediment (containing Lipid A) was washed with water and lyophilized.

Partially purified endotoxins samples were prepared from whole-cell lysates. Briefly, bacterial strains were cultivated in 1 ml medium (bouillon), heated at 100°C, lysed with lysozyme and "LPS lysing buffer" and subjected to proteolytic digestion (proteinase K enzyme were added). The protein-free LPS samples were directly analysed (without further purification).

3.2. Proteins and sample preparation

Human cell-free hemoglobin (Hb) was prepared from blood. The Hb solution was diluted 100 times to a final concentration of 1.2 mg/ml (*ca*. 18 μM) with 5 mM Tris-HCl buffer, pH 8.5. Transferrin (Tf) was purchased from Behring Werke (Marburg, Germany).

Mixtures of endotoxins and hemoglobin or transferrin were prepared by adding lyophilized endotoxins to 1 mg/ml final concentration to 1.2 mg/ml Hb solution or to 1 mg/ml Tf solution, respectively. Protein-LPS mixtures were incubated at 37°C to form protein-LPS complexes, and samples were run each hour by electrophoresis.

3.3. Sodium dodecyl sulphate polyacrylamide gel-electrophoresis

The lyophilized LPS sample was dispersed in "LPS sample buffer" (1 mg/ml), sonicated, boiled for 5 min and diluted. Electrophoresis was carried out in a *Laemmli* discontinuous system (4 % stacking gel, 15 % separating gel). A protein calibration kit was run parallel to the samples. Electrophoresis was performed at 40 mA (150V). Silver staining of the gels was performed by the method of Tsai and Frasch (1982).

3.4. Capillary electrophoresis

A BioFocus 3000 Capillary Electrophoresis System was used for capillary electrophoresis (CE) experiments. Fused silica capillaries of 50 μm i.d. were used with effective length of 23.5 cm. The capillaries were coated with non-crosslinked polyacrylamide in order to eliminate electroendosmosis and solute adsorption.

The electrophoresis experiments were conducted in a 5 mM Tris-HCl buffer at pH 8.5. All samples were hydrodynamically injected at 3-4 psi×s (*ca*. 2 nL) at 25°C. The applied voltage was 10 kV. The protein and the protein-containing zones migrated from the cathode (negative pole) towards the anode (positive pole). Separate runs with separate injections were performed for detections at UV (205–340 nm) or at visible (340–800 nm) light. The run times were around 25 min. Peak areas were calculated using the Biofocus Integrator System.

3.5. Microchip electrophoresis

Microchip electrophoretic runs were performed on the commercially available Agilent 2100 Bioanalyzer system equipped with a diode laser for fluorescence detection. The Agilent Protein LabChip kits (Protein 50, 80, or 200) and chips developed for proteins were used in our experiments. To prepare LPS-SDS complex solutions, 1 mg/ml suspension of the lyophilized LPS sample, or LPS sample prepared from whole-cell lysate was mixed with SDS solution of 4 m/v % concentration. Prior to electrophoresis, LPS samples and also the Agilent protein standard mixture were sonicated in water bath, incubated at 100°C, centrifuged and diluted with deionized water.

The chip channels were filled hydrodynamically with running buffer, which contained a fluorescent dye mixed with a polydimethylacrylamide-based linear polymer as a sieving matrix. The samples and the protein standard mixture were loaded on the sample wells and the "ladder" well on the microchip. All samples were electrophoretically injected into the capillaries (*ca*. 40 picoliters) and substances migrated towards the anode (positive pole). The fluorescent dye present in the gel matrix had a strong complexation tendency with SDS. Unbound fluorescent dye present in the buffer filling the capillary channels was diluted at the detection. The LPS–SDS-dye complexes formed in the capillary channel were run using the 2100 Expert software. The run times were *ca*. 50 s for one sample.

For the estimation of molecular masses of peaks appearing in the electropherograms the protein standard mixture was applied of the appropriate LabChip kit. The system software automatically displayed the results of the peak data in the microchip electropherograms as gel-like images.

3.6. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

1 mg of lyophilized LPS or Lipid A was suspended in 1 ml of 0.1 M citric acid solution and sonicated. To reduce the number of salt adducts a rapid desalting step of the analyte was carried out by using Dowex 50WX8-200 (NH_4^+) cation-exchange resin on Parafilm prior to the MALDI crystallization. 1µL from this sample solution was deposited on a stainless steel target and mixed thoroughly with 1µL of saturated 2,5-dihydroxy benzoic acid (DHB) matrix solution (dissolved in 0.1 M citric acid) and analyzed after drying.

MALDI-TOF MS measurements were acquired on an Autoflex II MALDI-TOF/TOF MS instrument. The spectra were recorded in the negative-ion and reflectron or linear mode over the *m/z* 1000–20000 range. Each spectrum was the sum of approximately 1000 laser shots on the same sample spot. The calibration of the instrument was performed externally using protein calibration standards. Data processing was executed with Flex Analysis software packages (version: 2.4.). Evaluation of the endotoxin spectra included the summation of the masses of the sugar, phosphate and fatty acid constituents. The interpretations of the $[M\text{-}H]^-$ quasimolecular ions appearing in the spectra resulted in proposals of LPS structures.

4. Results and discussion

4.1. Development of a capillary electrophoresis method for endotoxin detection

LPSs, having no absorption in the UV region, were detected as complexes with hemoglobin (Hb) or transferrin (Tf), by the UV-absorption of the protein. In each case, the migration of the LPS/protein complex peak was different from the free protein peak. Rough and smooth LPSs extracted from *E. coli*, *S. minnesota* and *S. sonnei* strains provided significantly different electrophoretic profiles when monitoring the migration properties of LPS-protein complexes. Figure 3 shows some of our results using Hb. The detection limit for the rough LPSs was *ca*. 50 µg/ml.

The analysis of LPS/Hb complexes can also be of interest from a medical point of view, as the Hb content in artificial blood preparations are often contaminated with endotoxin.

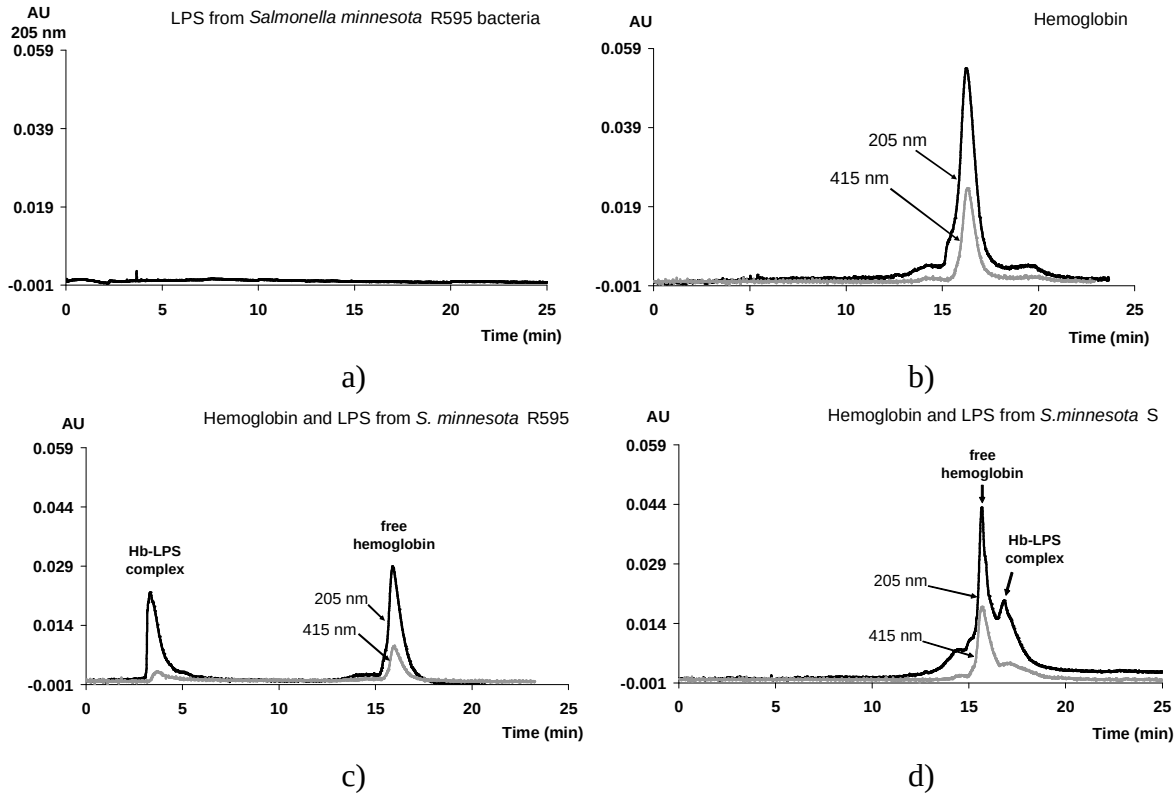


Figure 3. Capillary zone electrophoresis runs of a) LPS alone, b) Hb alone, c) mixture of Hb and an *R* type LPS and d) mixture of Hb and an *S* type LPS. a) LPSs (in general) have no UV-absorption; b) Hb strongly absorbs light at 205 and 415 nm; c) the fast migrating peak corresponds to the *R*-LPS/Hb complex; d) the overlapping peak after the major Hb peak corresponds to the *S*-LPS/Hb complex.

4.2. Development of a microchip electrophoretic method for endotoxin detection

An LPS molecule (similar to proteins) can take part in electrostatic and hydrophobic interactions with surface agents, for instance SDS. We worked out conditions for the application of a microfluidic chip designed for protein assays to the analysis of bacterial lipopolysaccharides.

We found that the LPS aggregates are satisfactorily dispersed in SDS solutions with at least 40-50 mM dodecyl sulphate concentration. LPS-SDS-fluorescent dye complexes were analyzed by a microfluidic separation technique with laser induced fluorescence detection.

The electrophoretic separation of LPS components of the *S* type endotoxins shows a "wave-like" profile with peaks representing the increasing number of O-polysaccharide repeating units. In the profiles of the mutant *R* type LPSs (which synthesize endotoxins without O-side chains and sometimes without portions of the core) only one single or very few peak components are present depending on the length and structure of core parts (Fig. 4). The detection limit of LPSs was *ca*. 6 µg/ml.

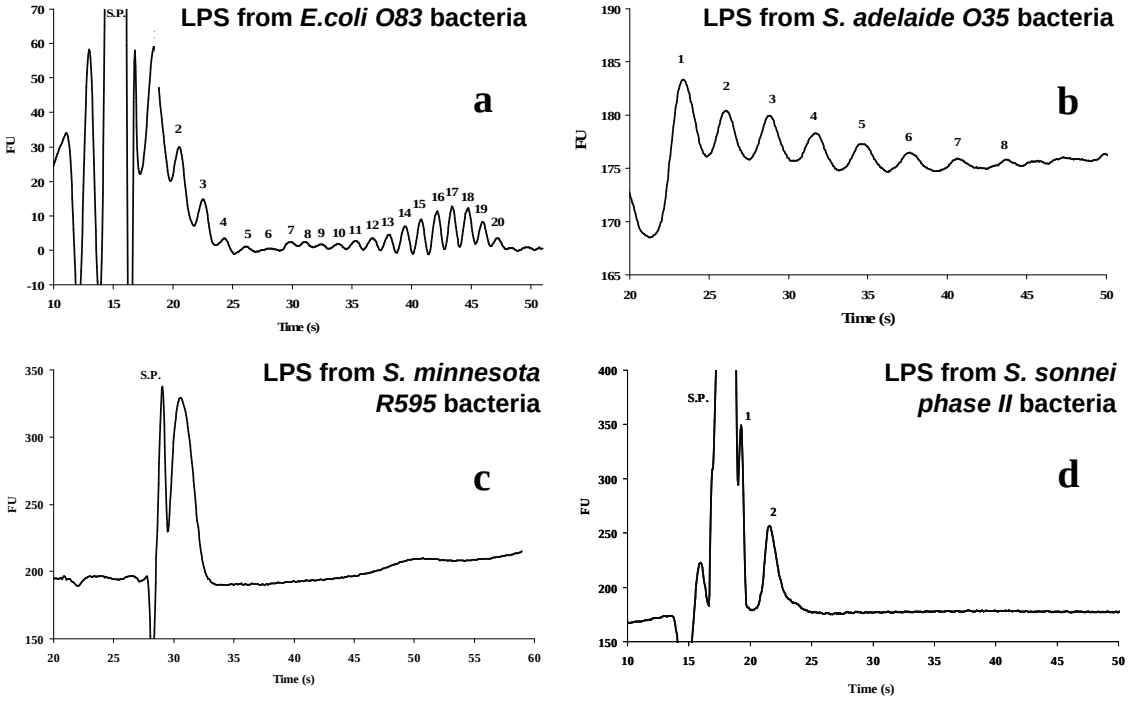


Figure 4. Microchip electropherograms of *S* type LPSs (a-b) and *R* type LPSs (c-d) initially solubilized with SDS-solution. Electrophoresis was carried out in the Agilent Protein 80 LabChip kit. A different distribution in the "wave-like" profiles of LPS microheterogeneity appeared in case of the two *S* type LPSs in a) and b). S.P.= system peak, *i.e.*, free SDS-dye complex.

Electropherograms visualized by the chip software as gel-like images reflected the same size heterogeneity as observed in the conventional SDS-PAGE ladder-patterns visualized by silver staining for the same endotoxin (Fig. 5). The calculated and "estimated molecular masses" for each component of *E. coli O83* LPS are shown in Table 1.

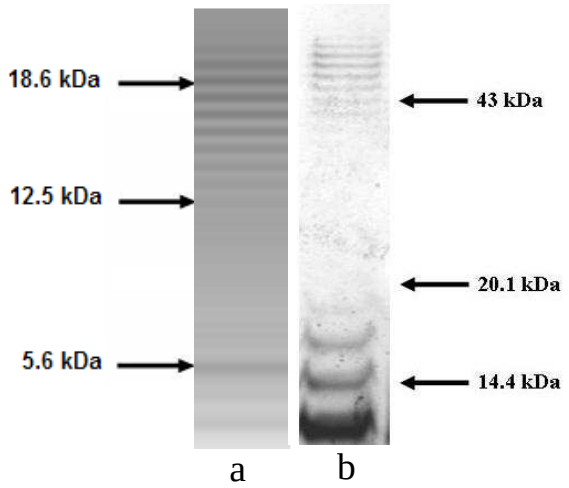


Figure 5. Gel-electrophoretic profiles of *E. coli O83* LPS. a) Gel-like transformation of the microchip electropherogram and b) conventional slab-gel electrophoresis with silver staining. The arrows indicate the positions of protein molecular mass standards run in the respective methods. The two patterns are very similar.

Table 1. Molecular masses respective to the peaks appeared in the electrophoretic pattern of *E. coli O83* LPS.

		SDS-PAGE	SDS-microchip	
Peak number	Theoretical molecular mass (kDa)	Estimated molecular mass (kDa)	Estimated molecular mass (kDa)	Relative peak area
1	3.9		6.1	1000
2	4.7		10.0	243
3	5.6	14.4	14.5	185
4	6.5		17.9	50
5	7.3		22.4	54
6	8.2	20.1	25.9	12
7	9.1		32.1	23
8	9.9		35.7	23
9	10.8		39.5	12
10	11.7		45.0	15
11	12.5		49.0	42
12	13.4		54.5	66
13	14.3		59.1	89
14	15.1	43	64.2	131
15	16.0		71.9	185
16	16.7		79.6	232
17	17.7		87.3	212
18	18.6		95.0	162
19	19.5		100.8	97
20	20.3		107.1	42

The theoretical molecular masses are calculated from the molecular structures of the LPS components, and the estimated molecular mass values are calculated from the positions of the protein MW standards in the respective methods. Yet, for exact molecular mass determination, both methods suffer from the lack of appropriate molecular-mass standards. Thus, it is obvious that the theoretical and estimated molecular mass values seen in Table 1 provide inconsistent values (the net surface charge of the LPS–SDS–dye complexes substantially differ from that of the SDS-denatured proteins, consequently, the migration properties of the two types of complexes are different). However, the quantitative evaluation of the relative amounts of the different LPS components (peaks) by integration of the microchip electropherograms is a leap forward.

When large number of bacterial mutants and their LPS content are to be compared (for instance, in the preparation of vaccines for epidemiological studies) endotoxins are prepared directly from 1 ml bacterial cultures by the relatively short method of *Hitchcock and Brown* (1983), with overall process-time *ca.* 40 h. This results in protein-free LPS without further purification steps (hence it is called partially purified LPS).

Our new microchip electrophoretic method is also applicable for the fast screening of endotoxins prepared directly from whole-cell lysates. The electropherograms showed the characteristic patterns to the respective bacterial origin of eighteen LPSs, thus, assignment of the *S*/*R* chemotypes could be made after blind determination of whole-cell lysate LPS samples. In each case, the classification was proven afterwards with the comparison of the electrophoretic profiles to those of the respective pure endotoxins; they were qualitatively similar to each other (Figure 6).

The sensitivity of this method is high, since satisfactory patterns are obtained using 1 ml bacterial cultures, which contain *ca.* 10^8 cells with an LPS content less than 1 nanogram (observe that the actual injection mass of the LPSs is even smaller).

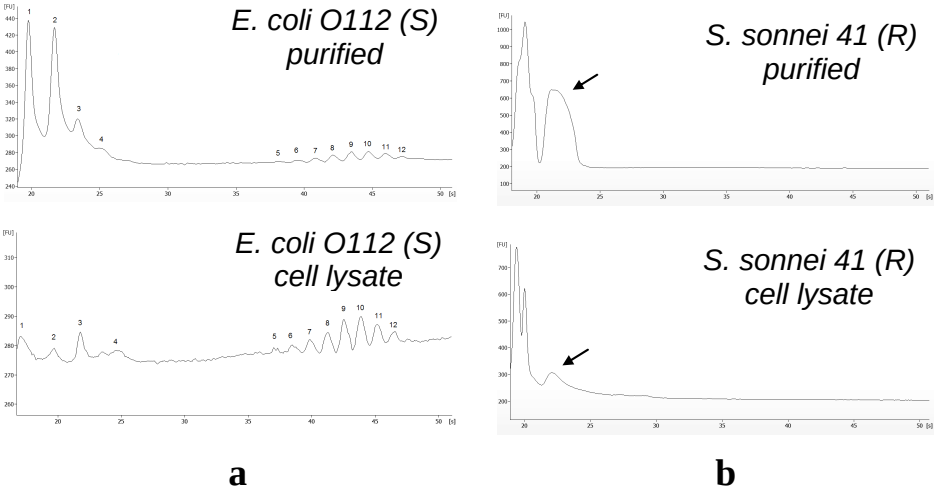


Figure 6. LPSs in cell lysates and purified LPSs can be detected similarly. a) *S* type LPS, b) *R* type LPS.

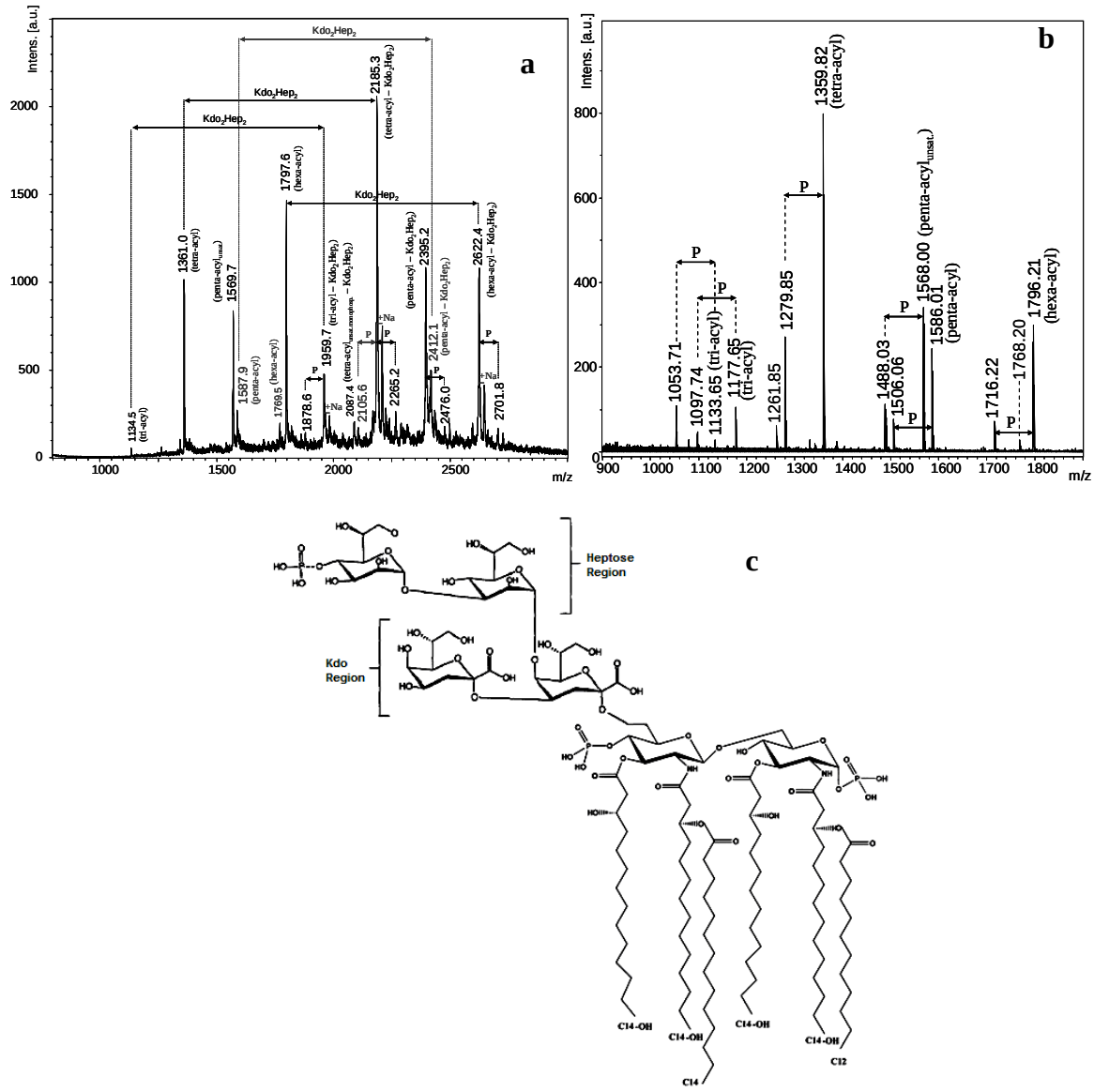
4.3. Endotoxin analysis by MALDI-TOF MS

Studies with endotoxins include another very important and highly challenging area, namely the study of chemical microheterogeneity of the LPS molecules. This is useful for understanding of processes related to pathogenesis of bacterial infection. The detailed structural analysis of the intact native LPS without separation of the different constituents is possible by MS or NMR.

MALDI-TOF mass spectrometry measurements were performed with intact LPSs extracted from the *R* type *S. minnesota R595* strain and the isogenic *S. sonnei 4303, R41, 562H, 4350* mutant strains, as well as the *S* type *E. coli O83* strain. Lipid A parts obtained from the LPSs extracted from *E. coli O83, O112, O157, P. morganii O34, S. urbana O30* and *S. sonnei R41* strains were also analyzed.

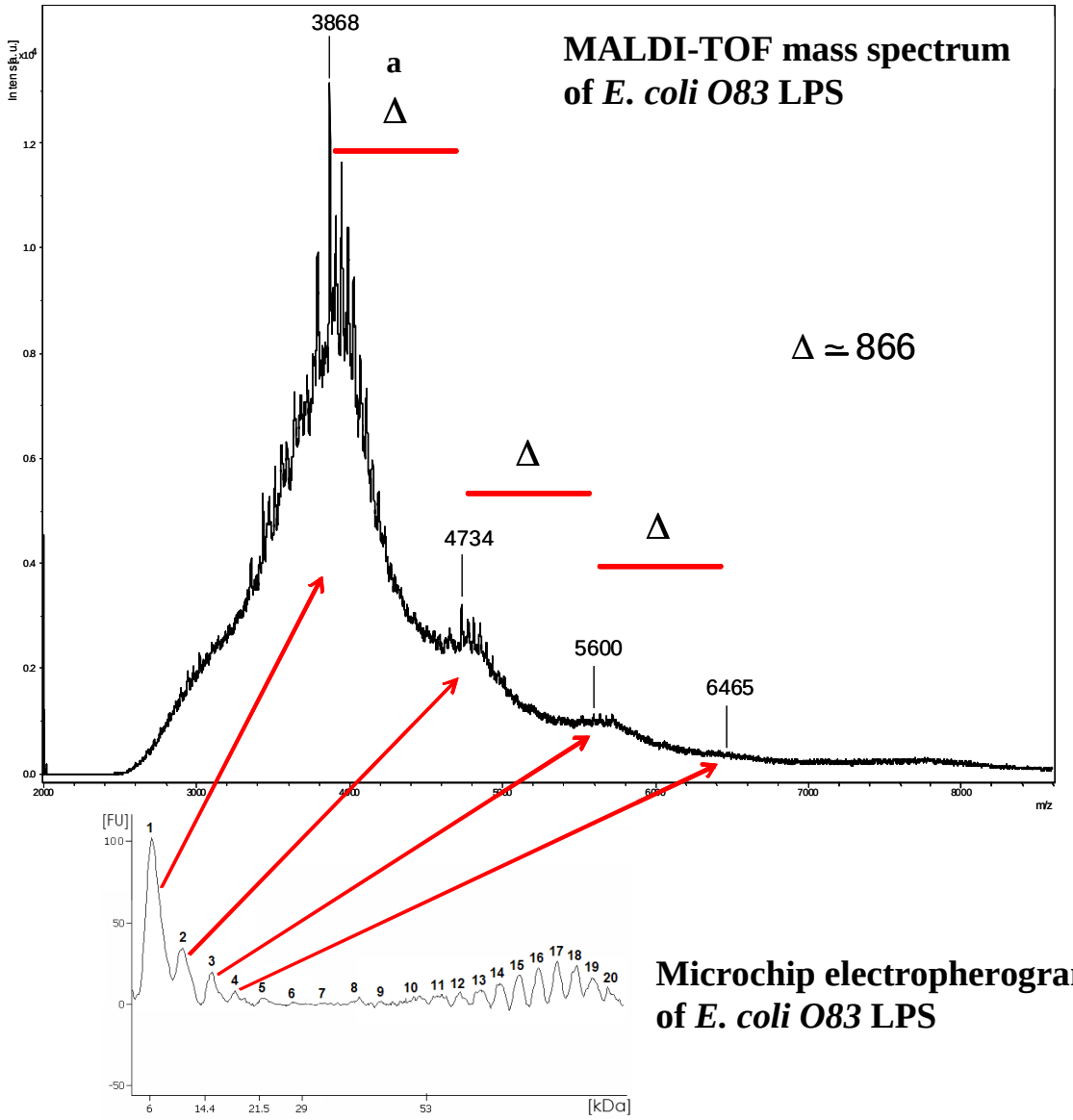
Mass spectra of an *R* type and an *S* type endotoxin are shown in Figures 7 and 8, respectively.

The mass spectra of the intact LPSs exhibit complex pattern of ions indicating the natural heterogeneities of LPS, as well as resulting from fragmentation processes taking place during the desorption and ionization.



<u>Figure 7</u>. Negative-ion MALDI-TOF mass spectra of a) *S. sonnei R41* intact LPS and b) of its Lipid A, and c) is the proposed structure of the *S. sonnei R41* intact LPS according to its mass spectrum.

The evaluation of the mass spectra with the help of previously described detailed structural interpretations of analogous mass spectral values of Lipid A parts and proposed core structures resulted in compositional analyses and proposals for LPS structures from *S. minnesota R595*, the isogenic rough mutant strains: *S. sonnei phase II (4303)*, *R41* (Fig. 7c), *562H*, *4350*, and *E. coli* O83 bacteria.



<u>Figure 8.</u> Negative-ion MALDI-TOF mass spectrum of LPS from *E. coli* O83 in relation to the microchip pattern of the same LPS. The first peak in the microchip electropherogram corresponds to the Lipid A and core component of the LPS, while the following peaks indicated with arrows correspond to the most ionizable LPS components carrying 0, 1, 2 and 3 O-polysaccharide repeating units. Their mass difference in the mass spectra is equal to the mass difference of the O-side chain repeating unit of known composition ($\Delta m/z$ = 866 Da). From the number of peaks in the microchip electropherogram it is possible to deduce the number of repeating units present in the intact LPS. The highest molecular mass assuming 19 repeating units in *E. coli O83* LPS is 20328 Da.

5. Discussion

Analysis of bacterial lipopolysaccharides is important in both, the identification of bacteria and characterization of mutants. In our studies, new, fast and sensitive electrophoretic methods – both capillary and microchip electrophoresis – were introduced for the detection and quantification of endotoxins extracted from an assortment of bacterial strains of the *Enterobacteriaceae* family. The reveal of intrastrain microheterogeneities of intact LPSs were approached with MALDI-TOF MS measurements.

New results:

1. We conclude that both, capillary electrophoresis (CE) and microchip electrophoresis can now be applied for endotoxin analysis, especially to differentiate the *S* and *R* endotoxins.

2. The application of the relatively fast migrating proteins, hemoglobin (Hb) or transferrin (Tf) in the LPS sample enables analyzes of LPS in aqueous solution (based on the strong interaction between LPS and proteins) by capillary electrophoresis using a simple Tris-buffer and UV/VIS detection.

3. Our newly developed microchip technique made the identification of bacterial chemotypes simpler, faster and more sensitive compared to – and able to replace – the commonly used SDS-PAGE with silver staining. Results are comparable with the SDS-PAGE patterns of endotoxins.

4. Main advantages of this microchip technique are high sensitivity (less than a few ng of LPS can be analyzed), high speed and simplicity (sample preparation for the microchip analysis and analyses of 10 different samples are carried out within 1 h), the requirement of small amount of samples and reagents (picoliters are injected in the chip), specificity (it is suitable for fast fingerprinting of *R*/*S* chemotypes of LPSs allowing the complete and high resolution separation of LPS glycoforms), and quantification (the determination of relative amounts of LPS components is possible.

5. The microchip technique is unique and provides characteristic profiles for the bacterial strains and their genetic variants. Thus, it is suitable for a very fast fingerprinting of LPS chemotypes when whole-cell lysates from large number of bacterial mutants are used.

6. The mass spectrometric measurements along with microchip electrophoretic analysis of the LPS or LOS preparations can be used to deduce the structures and the molecular masses of the "low-molecular-mass LPS molecular components". This can help to study the structure–function relationship of bacterial endotoxins.

Future perspectives

- To clarify the migration properties of endotoxins with small structural differences with CE method.
- Elimination of the presence of the system peak in the microchip electrophoretic profile, since this peak disturbes the quantitative evaluation of the *R* type LPSs.
- To find appropriate LPS molecules (or other standards) with known chemical structure, which might serve as molecular mass standard for estimation of molecular masses the of LPSs in electrophoretic runs.
- Further mass spectrometric studies are required for the structural evaluation of the molecular species with high number of the repeating units.

6. Publications, presentations

Publications related to this thesis

Kilár, A., Kocsis, B., Kustos, I., Kilár, F., Hjertén, S.: CE to monitor endotoxins by protein complexation. *Electrophoresis* 27 (2006) 4188 – 4195. IF: 4.101, citation (independent): 3 (2)

Kilár, A., Farkas, F., Kovács, K., Kocsis, B., Kilár, F.: Novel quantitative electrophoretic analysis of endotoxins on microchips *Electrophoresis* 29 (2008) 1713-1722. IF: 3.509

Kilár, A., Péterfi, Z, Csorba, E., Kilár, F., Kocsis, B.: Capillary electrophoresis chips for screening of endotoxin chemotypes from whole-cell lysates. *Journal of Chromatography A* 1206 (2008) 21-25. IF: 3.756

Bui, A., Szabó, Z., Poór, V., Kovács, K., **Kilár, A.**, Kocsis, B., Kilár, F.: Structural variability of endotoxins from *R* type isogenic mutants of *Shigella sonnei. Journal of Chromatography A* (2009) – *submitted*

Lectures related to this thesis

Kilár, A., Farkas, V., Kocsis, B., Kilár, F.: Development of two electrophoretic methods for the analysis of bacterial lipopolysaccharides
7th International Symposium and Summer School on Bioanalysis, June 10-15, Pécs, Hungary 2007

Kilár, A., Farkas, V., Kilár, F., Kocsis, B.: Endotoxin analysis by electrophoresis and chip technology
15th International Congress of the Hungarian Society for Microbiology, July 18-20, Budapest, Hungary 2007

Kilár, A.: Az endotoxinok biológiai és kémiai különlegességei
XXXIX. Kromatográfiás Továbbképző Tanfolyam, January 28-30, Szeged, Hungary 2008

Kilár, F., Bui, A., **Kilár, A.**, Kocsis, B., Szabó, Z., Farkas, V.: Study of structure-function relationship in endotoxin analysis by microchips and mass spectrometry
22nd International Symposium on Microscale Bioseparations, March 9-13, Berlin, Germany 2008

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