MALDI Mass Spectrometry Analysis of High Molecular Weight Proteins from Whole Bacterial Cells: Pretreatment of Samples with Surfactants

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The use of surfactants as additives in conjunction with on-probe whole cell bacterial protein analysis employing MALDI-TOF-MS is described. Nonionic and zwitterionic surfactants were used to enhance the detection of high molecular weight proteins. Three nonionic, *N*-octyl-*B*-D-glactopyranoside, *N*-decyl-*B*-D-maltopyranoside, and *N*-dodecyl-*B*-D-maltoside, and two zwitterionic surfactants, *N*,*N*-dimethyldodecylamine-*N*-oxide and zwittergent 3-12 were evaluated with five different MALDI matrix systems. New peaks in the mass range of 2 to 80 kDa were produced with all of the various combinations of matrix and surfactant from both whole cell gram-positive and gram-negative bacteria. Ferulic acid used in conjunction with a 1.0 mM solution of *N*-octyl-*B*-D-glactopyranoside produced the highest quality spectra with high signal to noise ratios and peaks up to 140 kDa. (J Am Soc Mass Spectrom 2005, 16, 1422–1426)

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been shown to be a powerful tool for the detection of bacterial proteins [1–3]. Sample preparation for the bacterial proteins for the MALDI-TOF-MS analysis has ranged from separation using HPLC and two-dimensional polyacrylamide gel electrophoresis (2D PAGE) [4, 5] to direct analysis of whole bacterial cells without preseparation [6, 7]. Holland et al. first reported on the successful analysis of five individual microorganisms by simply mixing the bacteria with α-cyano-4-hydroxycinnamic acid matrix on the MALDI probe followed by MALDI-TOF-MS analysis [6]. Using this approach, bacterial protein profiling was simple and rapid (time <5 min). A majority of the early research published on whole cell bacterial MALDI analysis described protein profiles up to 30 kDa [2, 3]. The signals in this range were attributed to cellular proteins or cell wall associated proteins [8, 9].

Numerous studies were reported to expand the upper mass range for bacterial proteins obtained from whole cell MALDI-TOF-MS analysis [7, 9]. The influence of matrix, solvent, and spotting techniques on the mass range and the quality of the MALDI spectra were reported.

The addition of detergents has a positive effect on sample preparation of bacterial lysates for 2D PAGE. Detergents such as sodium dodecylsulfate (SDS), Triton X-100, and octylglactopyranoside, as well as inorganic and organic buffers, have been postulated to play a major role in stabilizing protein solutions and minimizing adsorption losses [10]. However, for MALDI-TOF-MS analysis of proteins, the presence of ionic surfactants can be problematic and cause signal suppression and peak broadening [10, 11]. Therefore, many recommend that detergents be removed before MS analysis [12–14].

Nilsson reported on the use of nonionic surfactant with different matrices for the analysis of proteins contained in the bacterial lysates and extracts using MALDI-TOF-MS [11]. *Helicibacter pylori*, a gastrointestinal pathogenic bacterium, was investigated using MALDI-TOF-MS in conjunction with *N*-octyl-*B*-D-glactopyranoside surfactant [11]. Sinapinic acid and ferulic acid matrices produced bacterial lysate spectra with peaks up to 27 kDa without using surfactants in the sample preparation. When a 20-mM *N*-octyl-β-D-glactopyranoside surfactant was used with ferulic acid matrix, peaks up to 40 kDa were observed. Strain specific biomarkers were observed in the mass range of 20 to 40 kDa from the lysates and extracts from six different bacterial strains.

Here we describe the use of three nonionic surfactants, *N*-octyl-*B*-D-glactopyranoside (OGS), *N*-decyl-*B*-D-maltopyranoside (DMS), and *N*-dodecyl-*B*-D-maltoside (DdMS), and two zwitterionic surfactants, *N*,*N*-dimethyldodecylamine-*N*-oxide (LDMS) and zwittergent 3-12 (ZG) with five different matrices to determine an optimal combination to produce spectra containing protein

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peaks above 30 kDa with high signal-to-noise ratios. The dependence of the surfactant concentrations on obtaining optimum MALDI-TOF-MS spectra from whole cell bacterial proteins will also be discussed.

Experimental Procedures

Chemicals

N-Decyl-*B*-D-maltopyranoside (DMS) and zwittergent 3-12 (ZG), which is *N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate, were obtained from Calbiochem (La Jolla, CA). *N*-dodecyl-*B*-D-maltoside (DdMS), *N*,*N*-dimethyldodecylamine-*N*-oxide (LDMS), and N-octyl-*B*-D-glactopyranoside (OGS) were from Sigma-Aldrich (St. Louis, MO). Fisher Scientific (Fair Lawn, NJ) HPLC grade acetonitrile solvent was used in the sample preparation. Five MALDI matrices including α-cyano-4-hydroxycinnamic acid, sinapinic acid, 2,5-dihydroxybenzoic acid, dithranol, and ferulic acid were from Sigma-Aldrich. Formic acid (88%) and trifluoroacetic acid (TFA) were from Fisher Scientific and from Sigma-Aldrich, respectively.

Bacteria Growth

Several bacteria were purchased from the American Type Culture Collection (ATCC), including *Escherichia coli* (ATCC 11,775), *Staphylococcus aereus* (ATCC 12,600), *Bacillus circulans* (ATCC 61), *Bacillus cereus* (ATCC 14,579), *Bacillus licheniformis* (ATCC 14,580), *Bacillus megaterium* (ATCC 14,581), *Staphylococcus epidermidis* (ATCC 12,228), and *Pseudomonas aeruginosa* (ATCC 10,145). *Bacillus subtilis*, *Salmonella* spp., *Enterobacter aerogonas*, *Proteus mirabilis*, *Pseudomonas strutzi*, and *Staphylococcus pyogenes* were used from stock cultures maintained in-house.

Lyophilized cells of each bacteria isolate (stored at −20 °C) were added to the growing nutrient broth suspension, 30 g/L (Difco, Detroit, MI), and after 24 h, this suspension was used to inoculate culture plates containing solid nutrient agar (Difco, Detroit, MI). Bacteria were cultured and incubated at 37 °C.

Sample Preparation

Throughout this study, bacteria samples were removed directly from agar plates with a tungsten wire loop and placed onto the MALDI plate. After the transfer step, 1 μL of the surfactant aqueous solution was added and mixed thoroughly with the bacteria spots followed by evaporation at room-temperature. The surfactant concentration used for most of the experiments was 1.0 mM with the exception of *N*-dodecyl-β-D-maltoside which was used at a concentration of 0.17 mM. For the concentration studies, the surfactants were prepared at concentrations from 0.1 to 20 mM. Solutions of α-cyano-4-hydroxycinnamic acid, sinapinic acid, 2,5-dihydroxybenzoic acid, dithranol were prepared by dissolving 10 mg of the matrix into a 30:70 solution of water and acetonitrile containing 0.1% TFA. Ferulic acid (12.5 mg/1.0 mL) matrix was dissolved in a solution of 17% formic acid, 33% acetonitrile, 50% water. In all cases, a 1.0 μL aliquot of MALDI matrix solution was used to overlay the surfactant-bacterial sample spot and the resulting droplet was then left to crystallize by air-drying. The last step was repeated twice. The entire process of sample preparation, including transferring the bacteria from the agar plate to the MALDI probe, treating the sample spots with surfactant, and applying the matrix solution, was accomplished in 5 min or less. Samples were prepared in a Biosafety Level II hood with careful attention paid to using proper sterilization techniques.

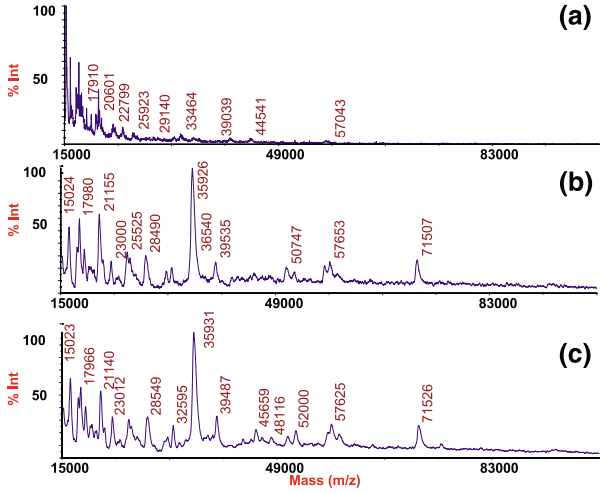

Figure 1. MALDI mass spectra of the whole bacterial cell analysis of *Enterobacter aerogonas*. **(a)** no surfactant, **(b)** 1.0 mM N-decyl-B-D-maltopyranoside, and **(c)** 1.0 mM N, N-dimethyldodecylamine-N-oxide.

MALDI-TOF-MS Analysis

All mass spectra were generated on a Voyager-DE STR+ (PerSpective Biosystems, Inc., Framingham, MA) MALDI-TOF mass spectrometer, operated in positive linear mode. For MALDI-TOF-MS parameters and calibration, see reference [7]. Resolution calculations were made at 50% peak height (FWHM) and signal-to-noise (S/N) was determined as peak intensity to average white noise for all masses across the spectrum. A spectrum for FWHM and S/N calculations was chosen randomly from 10 spectra that had demonstrated statistical reproducibility. Blank controls were analyzed in parallel with the bacterial samples to verify that the protein signals were not originating from either the nutrient agar or unspecified contaminants introduced during sample preparation.

Results and Discussion

Figure 1 shows MALDI mass spectra in the mass range 15 to 100 kDa for pure *E. aerogonas* and *E. aerogonas*

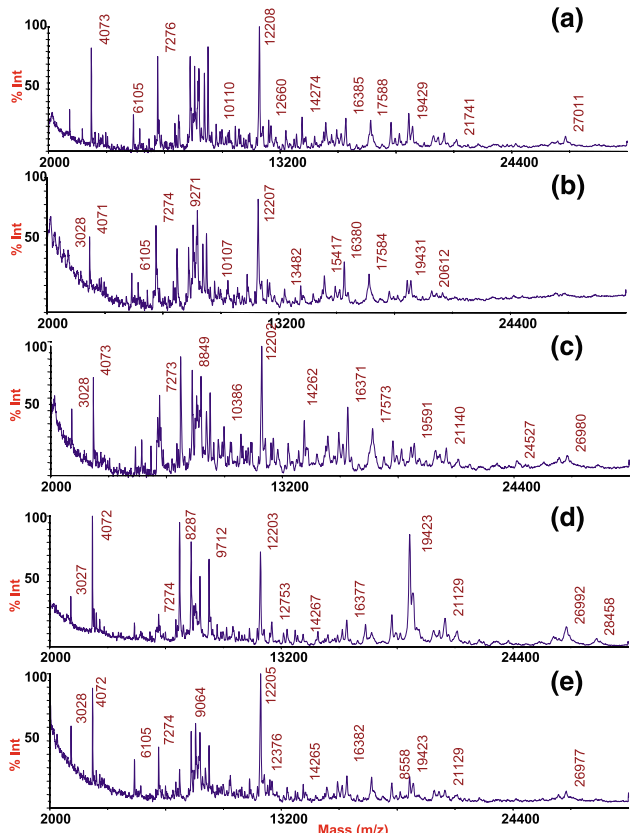

Figure 2. MALDI mass spectra of *Bacillus subtilis* in ferulic acid matrix in the presence of the following surfactants: **(a)** 1.0 mM N-decyl-B-D-maltopyranoside, **(b)** 0.17 mM N-dodecyl-B-D-maltoside, **(c)** 1.0 mM N-octyl-B-D-glactopyranoside, **(d)** 1.0 mM N, N-dimethyldodecylamine-N-oxide, and **(e)** 1.0 mM zwittergent 3-12.

prepared with *N*-decyl-*B*-D-maltopyranoside and *N,N*-dimethyldodecylamine-*N*-oxide using ferulic acid as the matrix. The addition of a surfactant when compared with the bacterial sample without surfactant clearly resulted in the production of new MS peaks in the high mass range above 15 kDa. Differences in S/N values were also observed in the spectra of bacteria-surfactant and the pure bacteria samples. It is speculated that DMS and LDMS surfactants assisted in dissolving certain bacterial proteins [15], such as hydrophobic cell wall proteins, during the on-probe sample pretreatment. If this were true, one would expect a more uniform distribution of the proteins during the formation of the matrix crystals prior the MALDI-TOF-MS analysis, which could result in enhanced ionization and the production of the high molecular weight protein peaks. The presence of the nonionic surfactants did not cause a detrimental effect on the MALDI process. Comparable results were observed with both gram-positive and gram-negative bacteria.

In addition to ferulic acid matrix, four other MALDI matrices (see the Experimental section) were investigated with both the nonionic and the zwitterionic surfactants. As an example, MALDI mass spectra of *B. megaterium* in sinapinic acid without surfactant and with N-decyl-*B*-D-maltopyranoside (nonionic) and zwittergent 3-12 (zwitterionic) showed an improvement in the spectral quality after adding the surfactants; data is not shown. The S/N and resolutions as well as the number of the detectable peaks were significantly enhanced after individually adding the two surfactants (for example, 65 peaks were observed without surfactant, 185 peaks with DMS, and 154 with the ZG surfactant). Similar results were also obtained for all of the other bacteria when they were analyzed with and without surfactant in the other four matrices. After examining the five matrix compounds with the five surfactants, ferulic acid consistently produced the greatest number of peaks, including high mass peaks, with the highest S/N ratio as well as the best shot to shot reproducibility. Therefore, ferulic acid was utilized as the matrix for the remainder of this study.

Figure 2 shows MALDI spectra of *B. subtilis* in the presence of nonionic surfactants (DMS, DdMS, and OGS) and zwitterionic surfactants (LDMS and ZG) in the mass range 2 to 30 kDa. The comparison of the MALDI spectra using the five surfactants showed that the presence of surfactants at the concentration used did not cause detrimental effects to the MALDI spectra and that additional peaks were observed. The nonionic surfactants produced more peaks in the mass range of 2 to 30 kDa than the zwitterionic surfactants. For example, at 10% of the base peak intensity, 138, 137, and 140 mass spectral peaks were produced from the B. subtilis

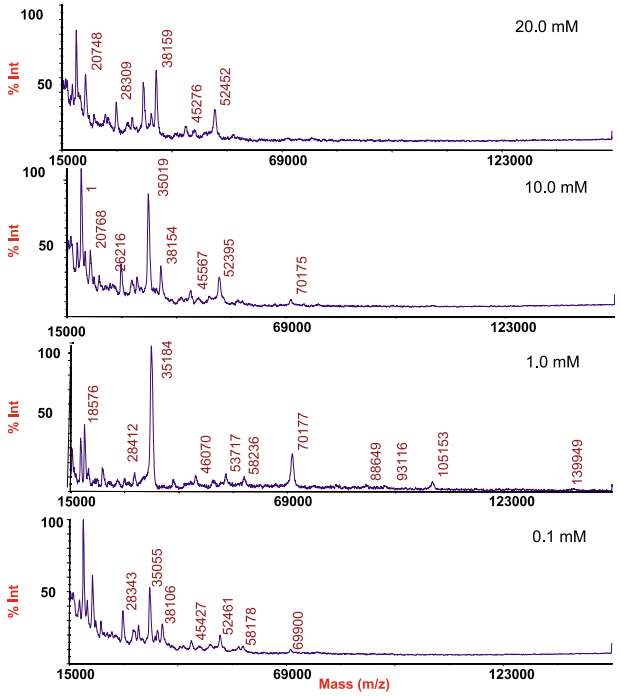

Figure 3. MALDI mass spectra of *E. coli* in ferulic acid treated with different concentrations of N-octyl-B-D-glactopyranoside surfactant.

Table 1. Nonionic and zwitterionic surfactants critical micelle concentrations and the optimal concentrations for use with MALDI-TOF-MS

Detergent	CMC[a] (mM)	OSC[b]
nonionic		
N-octyl-*B*-D-glactopyranoside (OGS)	18.5	1.0
N-decyl-*B*-D-maltopyranoside (DMS)	1.8	1.0
N-dodecyl-*B*-D-maltoside (DdMS)	0.17	0.17
zwitterionic		
N, N-dimethyldodecylamine-N-oxide (LDMS)	1.5	1.0
Zwittergent 3-12 (ZG)	2.0	1.0

[a]Critical micelle concentration, CMC, values provided by Anatrace, Inc. and Calbiochem, Inc. and reported in the following references [15]
[b]OSC: Optimal surfactant concentration in final MALDI matrix-bacteria spot (mM).

sample using the surfactants DMS, DdMS, and OGS, respectively, while addition of the zwitterionic surfactants to the bacterial sample produced 90 and 115 peaks for LDMS and ZG, respectively. On the other hand, the use of zwitterionic surfactants showed an improvement of S/N ratios by a factor of 10 for signals in the same mass range. The effect of surfactant concentration on the MALDI-TOF-MS signal quality (resolution, signal to noise ratio) for bacterial lysates and purified bacteria cell proteins has been previously described by Li et al. [13] and by Cadene and Chait [15]. They found that low concentrations of the added surfactants proved to be the most compatible with MALDI-TOF-MS, and enhanced the detection of some of the hydrophobic proteins in the bacterial cell lysate. Therefore, a set of five solutions of each surfactant with concentrations ranging from 0.1 mM to 20 mM was evaluated.

Figure 3 shows MALDI mass spectra of *E. coli* at different concentrations of N-octyl-B-D-glactopyranoside surfactant. The optimum concentration for this surfactant to produce high molecular weight protein peaks with no peak broadening was clearly observed at the 1.0 mM concentration. This concentration of the OGS added to *E. coli* cells produced MALDI spectra containing peaks at 88,649, 105,153, and 139,949 Da. The 1.0 mM concentration of the OGS surfactant is speculated to be optimal because it is below the critical micelle concentration (CMC) for this surfactant [15], and it was low enough to prevent peak suppression and peak broadening. CMC is usually defined as the lowest concentration of a detergent at which molecules aggregate to form micellar structures. Table 1 shows the CMC of the five surfactants and the recommended concentrations established in this study for use with MALDI-TOF-MS. Peaks greater than 30 kDa were also observed when other bacteria (see the Experimental section) were analyzed under the same conditions, i.e., surfactant concentrations were near or below the critical micelle concentration.

Conclusions

The addition of surfactants has resulted in enhanced MALDI spectral quality, additional high mass peaks, and an increased number of peaks for both gram-positive and gram-negative bacteria. The addition of the surfactants during sample preparation is postulated to aid in solubilizing the bacterial proteins that are hydrophobic and/or have high molecular weights. Nonionic and zwitterionic surfactants, at concentrations near or below the critical micelle concentration (CMC), have been found to be compatible with MALDI-TOF-MS of whole cell bacterial analysis using ferulic acid matrix. The sample preparation method which includes ferulic acid matrix dissolved in a solution of 17% formic acid, 33% acetonitrile, 50% water, mixed with 1.0 mM N-octyl-B-D-glactopyranoside produced the best quality spectra with highest S/N ratios and peaks up to 140 kDa.

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