Low Molecular Weight Proteome Analysis: SDS Gels Vs Reversed Phase

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Objectives

- To reduce complexity by removing high molecular weight and hydrophobic proteins.
- To develop LC workflow to identify low molecular weight proteins.
- To concentrate proteins derived from mass spectrometry incompatible buffer (EDS).
- To identify low abundant proteins by using 10 to 100 fold higher sample concentration compared to in-gel digestion.

Introduction

One challenge facing protein identification in proteomics studies is the huge dynamic range difference in protein expression level. This renders the detection of peptides generated from less abundant and low molecular weight proteins in a complex sample such as a cell lysate. We have tested two strategies to mitigate this problem: SDS gel electrophoresis and reversed phase protein separation. In pilot studies we determined that the elution of intact proteins from SDS gels, proteins could be concentrated from dilute SDS solutions using ion exchange. Reversed phase chromatography can also be used as an alternative separation strategy instead of SCX, and it could also be used to further separate proteins based on their hydrophobicity. In this poster, we compare these techniques using E. coli lysate as a model system. Intact protein molecules either by peptide mass fingerprinting (PMF) or by MALDI-MS-MS following microbore peptide separation. It was found that these techniques are advantageous in studying low molecular weight proteins because large, abundant proteins are efficiently removed.

Methods

(1) Whole Gel Elution of E. coli Lysates: a) Phast study to remove SDS from a standard protein: 1 microgram of BSA was diluted in 2.5 µl SDS gel running buffer, 1.5 ml of 10 mM Tris at pH 3 was added to reduce the ionic strength and shift down the pH. The sample was loaded through an ion exchange column (POROS® HSP) and washed with 3 ml of 10 mM Tris buffer at pH 3. The protein was eluted with 0.5 µl 1 M Tris buffer at pH 6.5. The sample was digested by adding porcine trypsin (2 µg) and incubated at 37°C overnight. Reversed phase HPLC (RPR) beads were used to clean up the sample before checking the results by MALDI TOF mass spectrometry (Voyager DE™ STR). b) Lysates of E. coli (200 µg) were separated on an SDS gel and material was eluted from the gel into 14 chambers using a whole gel elution apparatus (BioRad, Mini Whole Gel Eluter). About 5% of each fraction was run on a second SDS gel, which confirmed that a substantial amount of protein was present in each fraction that was distinguishable by molecular weight. The remaining material was purified by ion exchange as above. The eluted proteins were then digested by tryptic, desalted and submitted to PMF for protein analysis.

(2) Fractionation of E. coli lysates by Reversed Phase HPLC: Lysates of E. coli (160 µg) were injected onto a reversed phase C8 (150x1 mm) column. A two hour gradient was run from 5% ACN / 0.5% formic acid to 70% ACN / 0.5% formic acid, and was then maintained at 80% ACN / 0.5% formic acid for 10 minutes. Fractions were collected every 2 ½ minutes to a microtiter plate, a total of 50 fractions. The samples were dried by speed-vac and digested by trypsin. Each fraction was first analyzed by PMF with a MALDI TOF reflector mass spectrometer (Voyager DE™ STR). One fraction (Fraction 40) was then further separated by a 75 µm microbore HPLC column, and 94 fractions were spotted directly onto a 2x2 MALDI plate using a ProBolt™ (LC Packings), and 755 spectra (17000 to 30000 laser shots per spectrum) were collected (6 spectra from each spot) using the 4700 Proteomics Analyzer.

Results

(1) SDS Gel: a) To test out the methodology for removing SDS and concentrating the sample, 1 microgram of BSA was diluted starting from 0.5 µl SDS gel running buffer, and purified using ion exchange chromatography. The results (not shown) indicate that a diluted protein solution (28 µM) in the presence of SDS can be effectively concentrated for identification by mass spectrometry by this means. b) When the BioRad whole gel elution apparatus is used, the sample originates across the whole width of the SDS gel, thus the amount of protein is 10 to 100 fold greater than is traditionally isolated from a small slice of SDS gel or a spot from a 2-D gel. Excellent PMF spectra were obtained from 11 of the 14 fractions, resulting in the tentative identification of approximately 100 proteins, including many of the same proteins as we have previously identified by PMF from SDS gel slices or from spots from 2D gels. Figure 1 shows a MALDI spectrum from one gel elution fraction. Because only a small amount of the peptide digest was analyzed, presumably many more proteins could be identified upon further peptide separation using the techniques described below.

(2) Reversed Phase: a) PMF analysis: up to 10 proteins per sample are tentatively identified by searching against database using ChemApplex. The results indicate that very good protein separation is achieved, with minimum protein overlapping among adjacent fractions. Figure 2 shows the MALDI spectrum derived from fraction 29, 30, and 31. PMF analysis using ChemApplex software identified 10 primarily LMW proteins (Table 1) for fraction 30; b) Following separation of peptides by microbore HPLC and MALDI-MS-MS analysis, 241 of the 755 spectra identified 10 primarily LMW proteins. The results indicate that by further separating the sample and employing tandem mass analysis, many more weak peaks that will be missed by PMF can be confidently identified and assigned to additional proteins than the abundant ribosomal proteins.

Acknowledgement:

We wish to thank Steve Hattan for helping in the experiments.

Table 1. PMF database searching results for LC fraction 30.

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Table 2. Partial list of 67 proteins identified from LC fraction 30 by MS/MS. #: number of individual spectra, ParM: Parent mass; of number of masses submitted to Mascot; PSM: number of theoretical ion fragments matched; q/fm: number of actual fragment ion masses matched to: #/q: mass to Mascot score; y: number of y ion; b: number of b ion; pmw: protein molecular weight.

Conclusions

Low molecular weight proteins can be efficiently identified from complex protein mixtures following elution from SDS gels, and following reversed phase chromatography. Prior to this study, we had expected the SDS gel procedure to be far superior to reversed phase chromatography for the removal of high molecular weight proteins. However, our results indicate that even without any ultra-filtration or initial sizing column, many low molecular weight proteins are efficiently recovered from reversed phase columns. The MS-MS analysis that was performed could in principle identify a peptide from any E. coli protein. Nonetheless, only 1 peptide was matched to EF-tu, the most abundant protein in E. coli, compared to 9 and 11 distinct peptides from the two most abundant proteins in fraction 30. Because reversed phase chromatography is so easily scalable, it should be considered the method of choice when analyzing smaller proteins.

Reference: