PosterREPRINT

Waters

A NOVEL METHOD FOR DESALTING AND PURIFICATION OF PEPTIDE SAMPLES PRIOR TO NANOSPRAY-MS ANALYSIS

Santiago Vazquez, Jeff. W. Finch, John. C. Gebler and Steven. A. Cohen
Waters Corporation, Milford, MA, USA

Presented at ASMS, Montréal, Canada • June 8-12, 2003

Overview

- The use of a hydrophobic surface for the isolation of peptides from solutions prior to nanospray-MS analysis was investigated.
- The peptide isolation method is simple, robust and can be performed in a manual or automated fashion.
- The surface was effective in the isolation of peptide solutions containing 1M TRIS, 0.1% SDS and 2M urea.
- Protein sequence coverage from a trypsin BSA digest (667 fmol) following treatment with the hydrophobic surface was 55% using static nanospray-MS
- The surface was also effective in the isolation of peptides generated from in-gel digests from gels stained with either Silver, Coomassie or Sypro Ruby™ stains.

Introduction

Nanospray-MS is routinely used in identification of biologically significant proteins from proteolytic digests. Sample preparation is a critical step in these analyses since high concentrations of buffers, salts, detergents and other contaminants can suppress ionization of peptides and proteins, and may cause the nanospray emitter to plug.

Current technologies employ pipette tips or micro columns packed with reversed-phase material for the purpose of sample purification and concentration prior to nano-ESI-MS analysis. The use of these techniques, however, is not without problems, for example; ionic detergents such as SDS can bind and concentrate with analyte peptides during the purification procedure resulting in loss of sensitivity. Poor sample recovery and column robustness are also potential disadvantages.

In this study we present results demonstrating a novel sample preparation method for static nanospray-MS. This approach utilizes a hydrophobic surface designed to allow both the pre-concentration of peptides and removal of contaminants. The surface also facilitates manipulation of low sample volumes without significant sample loss and the process is readily automated.

Methods

Samples: A bovine serum albumin (BSA) digest standard was utilized for testing peptide purification protocols.

1D and 2D-Gels: A mixture of BSA, enolase, lysozyme and myoglobin (2 pmol of each protein) was separated using linear 15% SDS-PAGE gels. Gels were stained with either Coomassie, Silver or Sypro Ruby protein stains. Yeast cytosolic proteins (3.24 mg) were separated on linear 3-10 pI,15% 2D-gels and stained with Coomassie. Protein bands and spots were excised from the gels and digested with a Waters® MassPREP™ robotic workstation using the standard in-gel digestion protocol.

Peptide Purification and On-Plate digest methods: The peptide purification and on-plate digest methods used are outlined in Figure 1. The steps shown in Figure 1 were also incorporated into a MassPREP Station program for automation.

Sample Purification Surface: A prototype Sample Purification Surface (SPS) manufactured from polypropylene is shown in Figure 2.

Mass Spectrometry: Nanospray-MS was acquired on both a Waters Micromass® Q-Tof II™ and a Q-Tof micro™. DDA MS/MS experimental conditions used were those recommended for peptide LC/MS/MS.

Results

Initial evaluation of the SPS was undertaken by examining the protein sequence coverage of a tryptic digest of BSA following treatment using the peptide purification method shown in Figure 3. The direct nanospray-MS of the same BSA digest showed 60% protein sequence coverage. Following treatment with the SPS the protein sequence coverage was found to be 55%.

The ability of the prototype SPS to purify peptides from solutions containing contaminants was also investigated. The contaminants tested included 2M Urea, 0.1% SDS and 1M TRIS. SPS treatment of these solutions resulted in effective isolation of peptides on the plate surface and removal of contaminating peaks from the mass spectra as shown in Figure 4.

Protein stains commonly used for detection of proteins in gels can cause ion suppression if not removed from the digest mixture prior to MS analysis. The effectiveness of the SPS in the analysis of in-gel digested protein from 1D-gels initially stained with either Coomassie, Sypro Ruby or silver stain showed that SPS treatment was able to effectively isolate peptides from these different gels (Figure 5). SPS treatment also enhanced the ability to detect multiply-charged peptide precursor ions during automated DDA MS/MS experiments. As a result, high quality MS/MS spectra resulted in the ability to identify the proteins from 2D-gel spots with greater confidence as shown in Figure 6.

The analysis of peptides from 2D-gel spots following in-gel digestion is also possible. A representative nanospray-MS, obtained from a 2D-gel of yeast cytsolic proteins, following in-gel digestion and SPS treatment is shown in Figure 7. SPS treatment of the digest mixture allowed the purification of peptides peaks, making DDA MS/MS analysis of the indicated peaks possible. As a result the protein was identified as glyceraldyhyde 3-phosphate dehyrogenase in the database.

In addition to the purification of peptides, the prototype SPS is also able to isolate proteins from solution including those containing contaminants. Following purification of the immobilized protein, in situ enzymatic digestion of this protein can be performed. Figure 8 shows the results of this approach using BSA. Protein isolation, digestion and peptide clean-up steps were performed on one surface. The resulting protein sequence coverage determined by nanospray-MS was 53%, which is similar to a standard BSA following SPS treatment (Fig. 3).

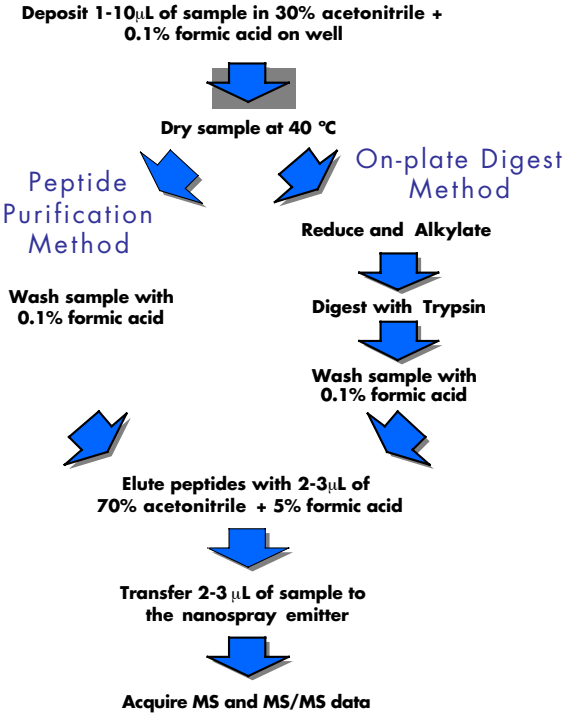

Figure 1: Outline of the SPS protocol for peptide purification and on-plate digestion.

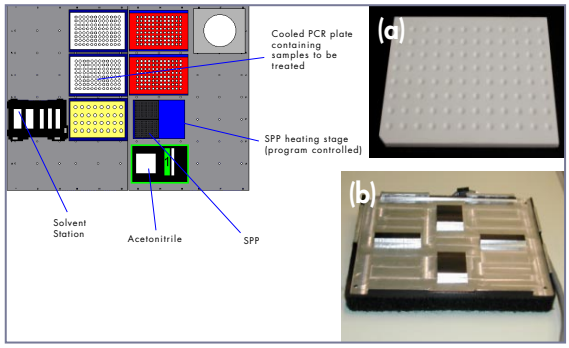

Figure 2: Diagram of the layout of the components for the automated SPS cleanup protocol showing a prototype sample preparation surface (a) and SPS heater (b).

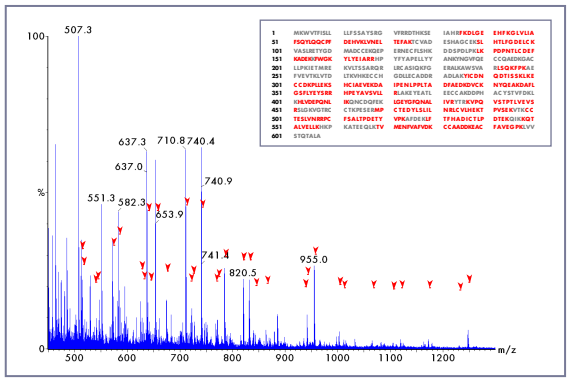

Figure 3: Nanospray-MS of 667 fmole of BSA digest following treatment with the SPS. BSA peaks are denoted with a red arrow and the sequence coverage of BSA is shown inset.

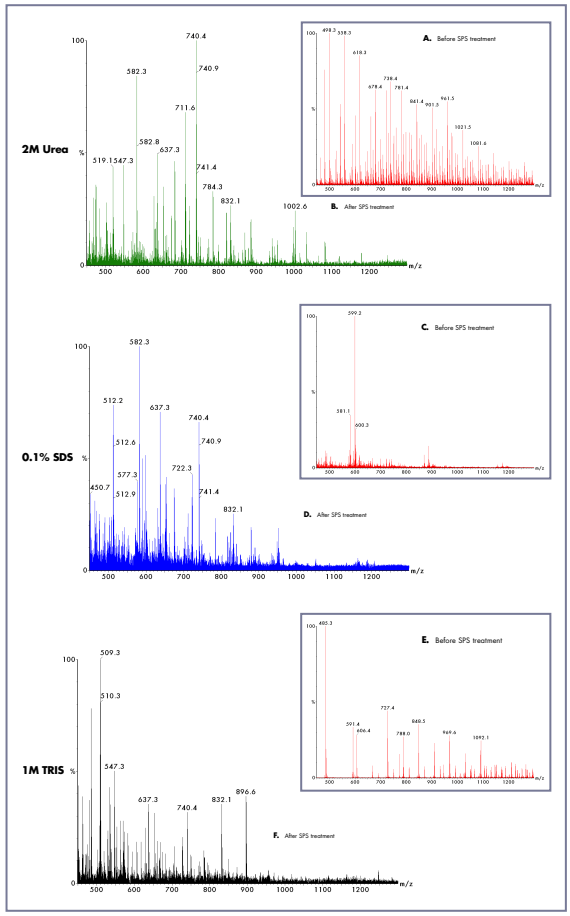

Figure 4: NanoSpray MS of a tryptic digest of BSA with 2M urea, 0.1% SDS or 1M TRIS. A, C, E nanospray-MS prior to SPS treatment; B, D, F nanospray-MS following SPS treatment.

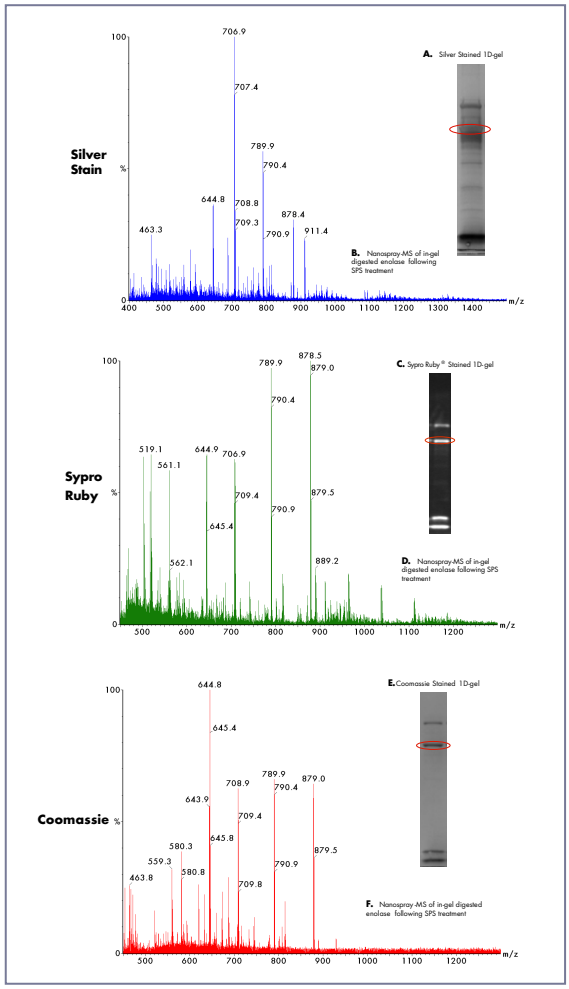

Figure 5: 1D-gels denoting enolase (2 pmol loaded) stained with: A. silver stain; C. Sypro Ruby; and D. Coomassie. The nanospray-MS of the in-gel digested peptides following SPS treatment are shown in B, D & F.

PosterREPRINT

Waters

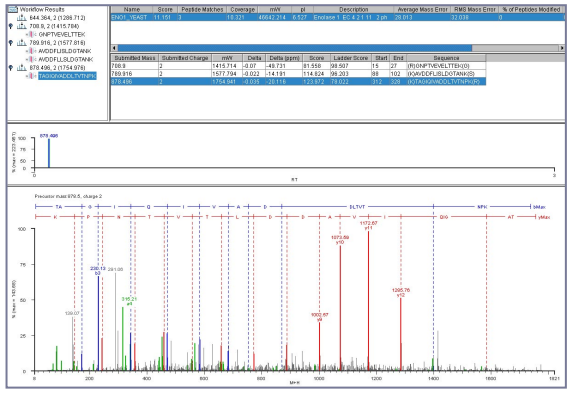

Figure 6: ProteinLynx™ Global SERVER 2.0 search results from the Nanospray DDA analysis of in-gel digested enolase (Coomassie stained) following cleanup with the SPS protocol.

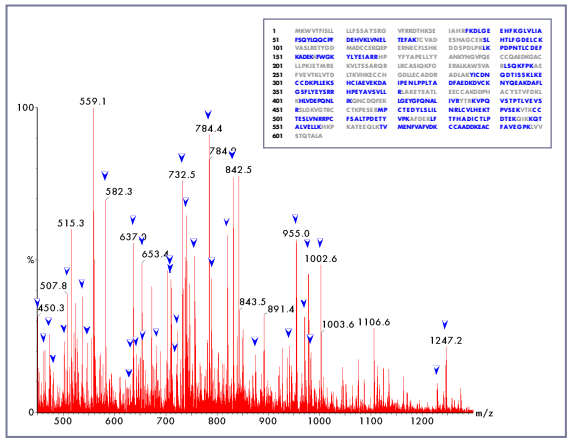

Figure 8: Nanospray of 3 pmol of on-plate digest BSA following extraction of peptides from the SPS. BSA peaks are denoted with a blue arrow and the sequence coverage of BSA is shown inset.

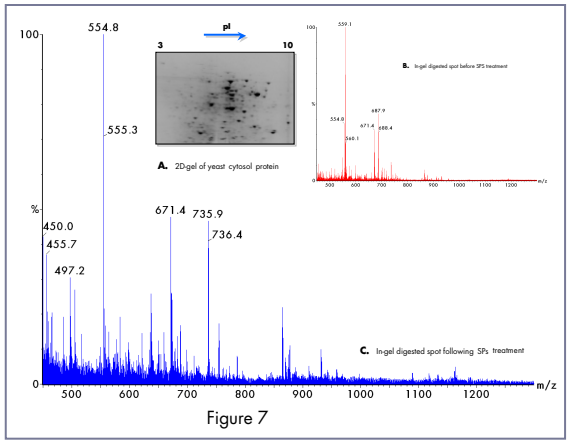

Figure 7

Figure 7: Nanospray-MS analysis of a yeast cytosol 2D-gel spot following in-gel digestion. A. Coomassie stained 2D-gel of yeast cytosol protein. The circled gel spot was chosen for in-gel digestion. B. Nanospray-MS of in-gel digested spot prior to SPS treatment. C. Nanospray-MS of in-gel digested spot following SPS treatment.

Conclusions

- The SPS is a robust and easy-to-use device that is very effective for off-line cleanup and pre-concentration of peptides from samples containing salts, chaotropes and detergents.
- It is effective for the cleanup of peptides from in-gel digests from gels stained with Coomassie, Sypro Ruby or silver stains.
- The SPS is also able to capture proteins from solution, allowing on-surface digestion with good sequence coverage.

WATERS CORPORATION
34 Maple St., Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

Waters
RIGHT ON TIME.

Opt-In! My Profile www.waters.com