protein discovery

PPS SILENT® SURFACTANT
PROTEIN PREP REAGENTS

APPLICATION NOTE

Improving Protein Digestion for Shotgun Proteomics

INTRODUCTION

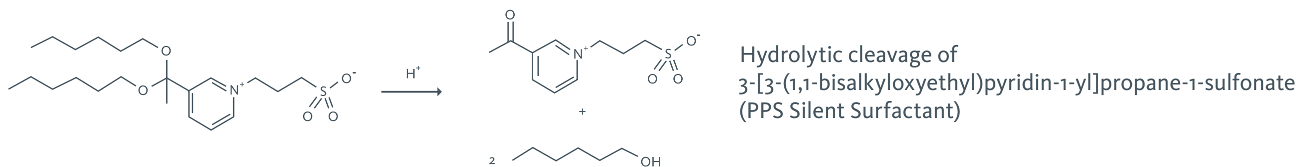
Shotgun proteomics has recently emerged as an alternative to gel-based methods for whole-proteome differential expression analysis. A key experimental objective in shotgun proteomics is to identify as many individual proteins as possible in each sample. Increasing the number of identified proteins enables analysis of a higher percentage of the expressed proteome and increases the odds of finding meaningful proteome differences, regulated proteins, and affected pathways.

BACKGROUND

In shotgun proteomics, the number of unique proteins identified is limited by the quality and completeness of the proteolytic digestion preceding LC-MS/MS analysis. Aaron Klammer and Michael MacCoss of the University of Washington's Department of Genome Sciences recently published the findings of a study designed to evaluate and compare the effects of different digestion schemes on the number of proteins that could be identified from a complex mixture of bacterial proteins.[1] The researchers found that the total number of proteins identified was improved by shorter digestion times and by the use of the reagent RapiGest™SF, which is represented by its supplier as having the ability to increase peptide recovery and coverage and decrease proteolytic digestion time.

OBJECTIVE

Since the publication of Klammer and MacCoss' findings, Protein Discovery Inc. has released PPS Silent® Surfactant (3-[3-(1,1-bisalkyloxyethyl)pyridin-1-yl] propane-1-sulfonate), a reagent for cell membrane disruption and protein solubilization. Continuing their investigation into the development of improved shotgun proteomics methods, the MacCoss lab tested PPS Silent Surfactant to see if digestion conditions could be further optimized.



Hydrolytic cleavage of 3-[3-(1,1-bisalkyloxyethyl)pyridin-1-yl]propane-1-sulfonate (PPS Silent Surfactant)

PPS Silent Surfactant was designed to improve protein solubility without reducing mass spectrometry analyte detection sensitivity caused by conventional detergents.[2] The PPS molecule cleaves at low pH into soluble hydrolysis products with no residual detergent properties.

METHOD

The MacCoss lab evaluated PPS by comparing its performance to RapiGest. Protocols used to compare the effects of the two surfactants are detailed in the table below. The number of identified proteins was determined by nanoLC-MS/MS[1] using a LTQ ion-trap mass spectrometer.

Protocol for Protein Digestion of Complicated Mixtures

SAMPLE PREPARATION WITH PPS

Materials

- PPS Silent Surfactant
(Protein Discovery, Inc., 10 mg vial, 21010)
- 50mM Ammonium Bicarbonate buffer
- 500mM DTT
- 500mM IAA (Iodoacetamide – light sensitive)
- 500mM HCl
- 250 ng/µl Trypsin, modified, sequencing grade (Promega) in 0.01% acetic acid

Procedure

1. Make 0.2% PPS diluted in 50 mM Ammonium Bicarbonate pH 7.8 (1 mg surfactant per 500 µl 50 mM Ammonium Bicarbonate pH 7.8).
2. Using low adhesion microcentrifuge tubes, add 100 µl 0.2% PPS per 100 µl protein mixture (1:1) [final concentration of PPS should be 0.1 % (w/v)]. If protein is in pellet form add 25-50 µl of 0.1% PPS. Vortex the sample. A higher concentration of PPS can be used for particularly difficult to solubilize samples.
3. Add DTT to a final concentration of 5mM.
4. Incubate sample at 50°C for 30 minutes.
5. Cool the sample to room temperature.
6. Add IAA to a final concentration of 15mM.
7. Place sample in dark at room temperature for 30 minutes.
8. Then add trypsin for a final concentration of 1:50 enzyme:protein. If total amount of protein is very low just add 1-2 µg of trypsin.
9. Incubate 4h with shaking at 37°C.
10. Prior to mass spectrometry run, add HCl to a final concentration of 200mM.
11. Incubate at 37°C for 45 minutes.
12. Spin sample at 14K, 4°C for 10 minutes.
13. Analyze supernatant by nanoLC-MS/MS

SAMPLE PREPARATION WITH RAPIGEST

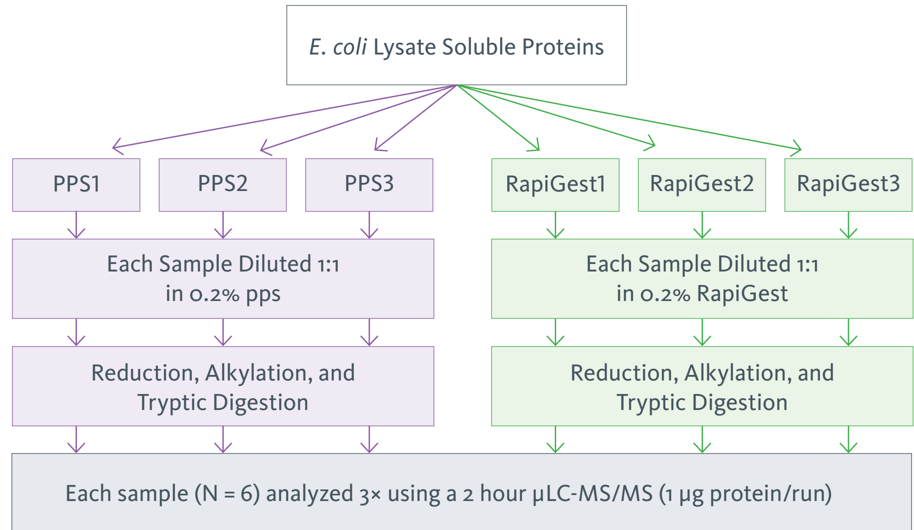
Materials

- RapiGest SF Powder
(Waters Corporation, 5 Pack of 1 mg Vials, 186001860)
- 50mM Ammonium Bicarbonate buffer
- 500mM DTT
- 500mM IAA (Iodoacetamide – light sensitive)
- 500mM HCl
- 250 ng/µl Trypsin, modified, sequencing grade (Promega) in 0.01% acetic acid

Procedure

1. Make 0.2% RapiGest diluted in 50 mM Ammonium Bicarbonate pH 7.8 (1 mg surfactant per 500 µl 50 mM Ammonium Bicarbonate pH 7.8).
2. Using low adhesion microcentrifuge tubes, add 100 µl 0.2% RapiGest per 100 µl protein mixture (1:1) [final concentration of RapiGest should be 0.1 % (w/v)]. If protein is in pellet form add 25-50 µl of 0.1% RapiGest. Vortex the sample.
3. Add DTT to a final concentration of 5mM.
4. Incubate sample at 50°C for 30 minutes.
5. Cool the sample to room temperature.
6. Add IAA to a final concentration of 15mM.
7. Place sample in dark at room temperature for 30 minutes.
8. Then add trypsin for a final concentration of 1:50 enzyme:protein. If total amount of protein is very low just add 1-2 µg of trypsin.
9. Incubate 4h with shaking at 37°C.
10. Prior to mass spectrometry run, add HCl to a final concentration of 250mM.
11. Incubate at 37°C for 45 minutes.
12. Spin sample at 14K, 4°C for 10 minutes.
13. A cloudy pellet may appear. Separate your supernatant from the pellet into a fresh Eppendorf tube.
14. Spin again if needed to make sure you have completely removed the cloudy material.
15. Analyze supernatant by nanoLC-MS/MS.

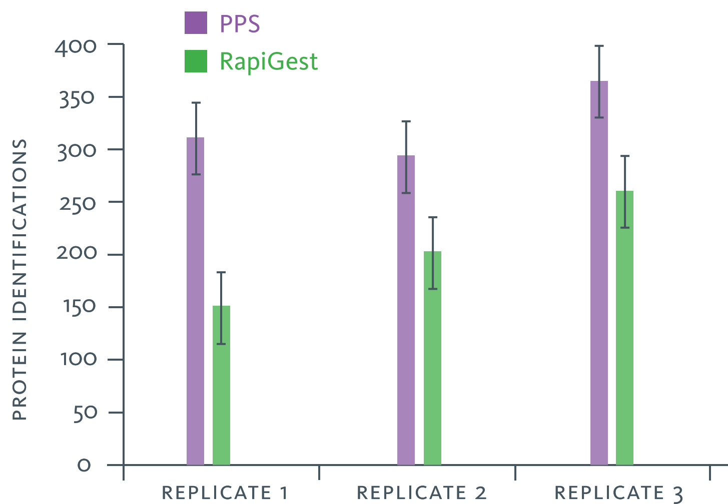
EXPERIMENTAL DESIGN



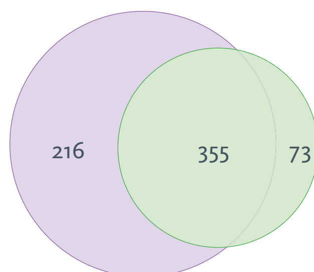
Complex mixtures of proteins derived from *E. coli* were analyzed using PPS Silent Surfactant. Cell extracts were performed using 0.1% detergent in buffer (pH 7.8). Proteins were reduced and alkylated prior to digestion using trypsin. Prior to mass spectrometry run, HCl was added to cleave the detergent. Tryptic peptides were analyzed by nanoLC-MS/MS using a 2 hour gradient on a LTQ ion trap mass spectrometer. The numbers of peptides and proteins identified were compared to evaluate and compare reagent performance. All experiments were repeated in triplicate.

FINDINGS

Different cleavable surfactants yield complementary protein identifications. Proteolytic digestions carried out using RapiGest or PPS Silent Surfactant produced two separate sets of identified proteins characterized by partial overlap. The two digestion conditions resulted in a total of 644 unique protein identifications, an improvement of 50% relative to the 428 proteins identified from the samples prepared using RapiGest alone. Analysis of the samples prepared using PPS Silent Surfactant yielded 571 identified proteins.



Nonredundant protein identifications from three LC-MS/MS runs each of three samples prepared using RapiGest and three LC-MS/MS runs each of three samples prepared using PPS. More proteins are identified from *E. coli* samples prepared using PPS Silent Surfactant. Columns indicate the number of proteins identified in replicate analyses of *E. coli* extract using LC-MS/MS. Error bars indicate the standard deviation of triplicate LC-MS/MS runs of each replicate. Proteins were identified at a <1% false discovery rate.



PPS = 571 Protein IDs
RapiGest = 428 Protein IDS
Total = 644 Protein IDs

Cumulative nonredundant protein identifications from three LC-MS/MS runs each of three samples prepared using RapiGest and three LC-MS/MS runs each of three samples prepared using PPS. Comparative analysis reveals two partially overlapping sets of identified proteins.

CONCLUSION

Researchers in the MacCoss lab identified more individual proteins from a single digest aided by PPS Silent Surfactant than with any other surfactant tested for solubilizing complex protein mixtures prior to in-solution digestion.

Mike MacCoss, PhD
Assistant Professor of Genome Sciences
University of Washington

"PPS outperforms everything else we've used to generate peptides from complex protein mixtures."

ORDERING INFORMATION

PPS Silent Surfactant is available in 1 mg and 10 mg vials. To place an order for PPS Silent Surfactant, please contact Protein Discovery by phone, fax, or e-mail.

Tel: (865) 521-7400
Fax: (865) 521-3548
E-mail: sales@proteindiscovery.com

DESCRIPTION	PART NUMBER
10 mg vial, 1 vial per pack	21010
1 mg vial, 5 vials per pack	21011

Protein Discovery, Inc.
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www.proteindiscovery.com

REFERENCES

1. Klammer A, and MacCoss M. Effects of Modified Digestion Schemes on the Identification of Proteins from Complex Mixtures. *Journal of Proteome Research* 5, 695–700 (2005).

2. Norris J, Porter N, and Caprioli R. Mass Spectrometry of Intracellular and Membrane Proteins Using Cleavable Detergents. *Analytical Chemistry* 75, 6642–6647 (2003).

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