

A Versatile High-Recovery Method For Removing Detergents From Low-Concentration Protein or Peptide Samples For Mass Spectrometry Sample Preparation And Analysis

Babu Antharavally, Ryan Bomgarden, John Rogers
Thermo Fisher Scientific, Rockford, IL



Overview

Purpose: To efficiently remove detergents from low-concentration protein or peptide samples with high protein or peptide recovery for downstream applications.

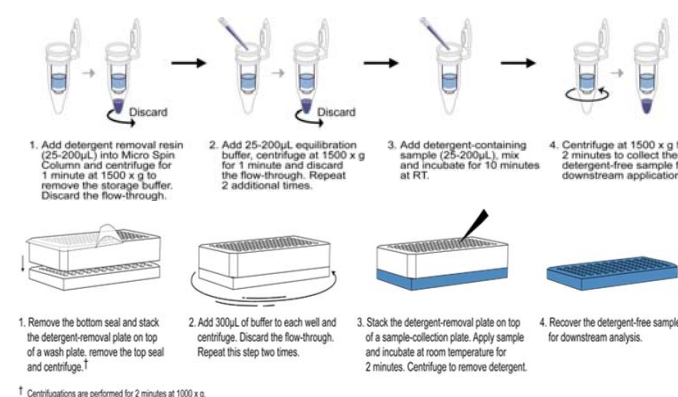
Methods: Colorimetric assays, A275 nm absorption and mass spectrometry (MS) analysis were used to measure the efficiency of detergent removal. Micro bicinchoninic acid (BCA) protein assays and sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) analysis were used to determine the protein recovery.

Results: The method successfully removed >95% of nonionic, ionic, and zwitterionic detergents from 0.5%-1% solutions with high recovery of proteins or peptides. Tandem mass spectrometric analysis of 2.5–10 µg of bovine serum albumin (BSA) enzymatic digests at 25–100 µg/mL, prepared in the presence of detergents and processed to remove detergent, revealed sequence coverage and Mascot™ scores as good as or better than control BSA samples processed without detergent. The method significantly removes detergents from very low concentration protein or peptide samples and eliminates the interference by detergents with the peptide mass spectral profile. The method can also be used in 96-well plate format to remove detergents with >95% efficiency at concentrations of 0.125%–1% with high protein/peptide recovery. The advantage of this method is the speed (less than 15 minutes), efficient detergent removal and high recovery of proteins and peptides.

Introduction

Mass spectrometry plays a central role in high-throughput proteomic analysis and provides a greater understanding of cellular regulation and global protein expression levels [1]. However, sample complexity, interfering substances, and the large dynamic range of protein and peptide concentrations are major hurdles in MS-based proteomic analysis. Sample preparation upstream of MS analysis is pivotal to success, and protein isolation methods commonly utilize reagents that are not compatible with mass spectrometry [2]. As a result, it is often necessary to modify procedures to avoid the use of interfering compounds. This is particularly true for protein isolation methods that utilize detergents. Detergents are commonly used in protein-chemistry protocols and may be necessary for protein extraction, solubilization, and denaturation [3]. To enable downstream analysis by mass spectrometry, it is critical to remove unbound detergents from protein and peptide samples while maintaining good protein or peptide recovery, especially for low abundant proteins. Methods such as precipitation, solid phase extraction, desalting, or dialysis can be time consuming, inconsistent, and result in poor sample recovery. As an alternative to redeveloping these types of sample isolation procedures, we developed a detergent removal resin optimized for samples with very high detergent loads and with protein or peptide concentrations >100 µg/mL [4]. An alternative resin and protocol was later developed for samples with lower concentration ranges – Thermo Scientific HiPPR (High Protein and Peptide Recovery) Detergent Removal Resin (Figure 1). The HiPPR™ Detergent Removal Resin is fast and effective at removing >95% of up to 1% detergents from protein or peptide samples with very low protein or peptide concentrations (1–100 µg/mL) with excellent sample recovery in less than 15 minutes. The spin column format is simple and efficient. Pre-filled 96-well plates enable fast and efficient high-throughput detergent removal of 192 samples in a single run.

FIGURE 1. Protocol summary for Thermo Scientific HiPPR Detergent Removal Spin Columns (upper panel) and 96-well Spin Plates (lower panel)



Methods

Detergent Removal Procedure: Protein (0.375 µg–4 µg in 25 µL–200 µL) + detergent in buffer (0.15M NaCl, 0.05% sodium azide, or 0.2M carbonate-bicarbonate buffer, pH 9.4) was processed with Thermo Scientific Pierce Detergent Removal Resin as shown in the protocol. Residual SDS was measured by colorimetric method using Stains-All dye [5]; Triton® X-100 and NP-40 were measured by absorbance at 275 nm (protein absorbance was subtracted); CHAPS was measured by colorimetric method using concentrated sulfuric acid [6]. The Micro BCA Protein Assay was used for protein determination.

LC-MS/MS Analysis: BSA (25–100 µg/mL) in 50mM ammonium bicarbonate buffer, pH 8.0 was reduced, alkylated and enzymatically digested for 3 hours at 37 °C (enzyme-to-protein ratio, 1:50) in the presence of 0.5% detergents. To prepare the sample containing 0.5% SDS, the detergent was added to the sample following enzymatic digestion. Digested sample (0.1 mL) containing the detergent was processed with 0.1 mL detergent removal resin as shown in the protocol. Control samples (labeled as unprocessed) were not processed with the resin. Samples were loaded (3 pmol) directly onto a C18 capillary column and subjected to LC-MS/MS analysis using a Thermo Scientific LTQ linear ion trap mass spectrometer. No trapping column was used. All data were analyzed using the Mascot search engine (Matrix Science).

Results

Part I: Removal of Detergents from Proteins

BSA sample (25–200 µL) + detergent in 0.15M NaCl, 0.05% sodium azide was mixed with an equal volume of detergent removal resin (2x volume for CHAPS removal) and processed as shown in the protocol. Detergents were effectively removed with high protein recovery (Table 1).

TABLE 1. Detergent removal efficiency and protein recovery

Detergent	Sample Volume (µL)	Protein Quantity (µg)	Detergent Removal (%)	Protein Recovery (%)
SDS (1%)	25	0.375	>99	98
	50	0.75	>99	97
	100	1.5	>99	100
Triton X-100 (1%)	25	0.375	>99	82
	50	0.75	>99	86
	100	1.5	>99	86
NP-40 (0.75%)	25	0.375	95	90
	50	0.75	96	94
	100	1.5	97	91
CHAPS (1%)	25	0.375	95	64
	50	0.75	97	70
	100	1.5	98	78
	100	1.5	98	70
	200	3.0	98	75

A 0.1 mL protein sample (2 µg) + 1% SDS in buffer (0.15M NaCl, 0.05% sodium azide, or 0.2M carbonate bicarbonate buffer, pH 9.4) was mixed with 0.1 mL detergent removal resin and processed as shown in the protocol. Detergent was effectively removed with high protein recovery (Table 2).

TABLE 2. Detergent removal efficiency and protein recovery

Protein	SDS Removal (%)	Protein Recovery (%)
α-Lactalbumin	99	98
Carbonic Anhydrase	99	81
Insulin	99	100

Protein (BSA) samples (15 µg/mL; 25, 50 and 100 µL) in 0.15M NaCl, 0.05% sodium azide, + 1% SDS were mixed with detergent removal resin (25, 50 and 100 µL) and processed as shown in the protocol. Samples (processed and unprocessed) were analyzed by SDS-PAGE and stained with Thermo Scientific GelCode Blue Stain Reagent. As shown in Figure 2, lanes 1–4 were processed samples with sample volumes 25, 50, 100 and 100 µL, respectively. Lanes 5 and 6 were unprocessed samples (1X & 2X). Lane 7 was the molecular weight marker. The data confirms the high recovery of proteins from low concentrated samples (BSA, 15 µg/mL) following the removal of detergent using Pierce Detergent Removal Resin.

FIGURE 2. SDS-PAGE analysis of samples before and after detergent removal



A 0.1 mL BSA sample (1.5 µg) + detergent in 0.15M NaCl, 0.05% sodium azide was mixed with 0.1 mL HiPPR resin (0.2 mL for CHAPS, octyl glucoside, and octyl thioglucoside) and processed as shown in the protocol. For the 96-well plate, samples (0.1 mL) containing 0.025 mg/mL BSA and detergent were processed through the HiPPR Detergent Removal Spin Plate as described in the protocol (Table 3).

TABLE 3. Maximum removable detergent concentrations

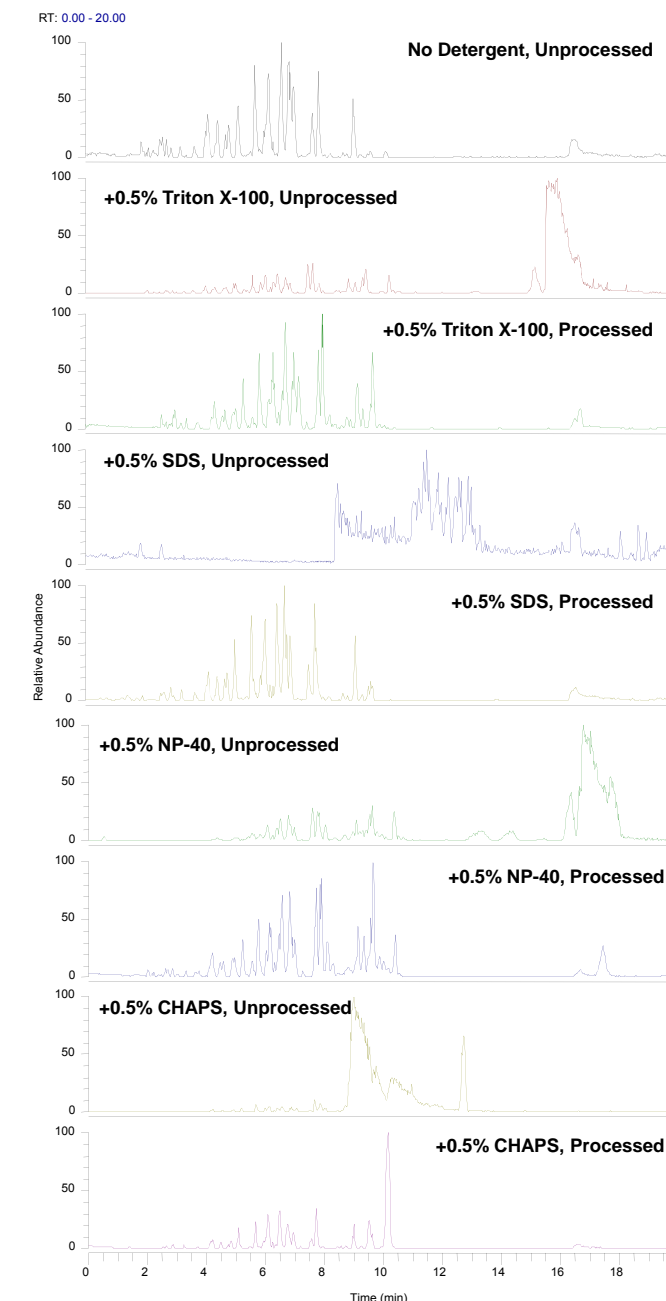
Detergent	Maximum Removable Concentration (%)	
	Spin Column	96-Well Plate
SDS	1	0.4
Sodium Deoxycholate	1	0.5
Triton X-100	1	0.25
Triton X-114	0.5	ND
NP-40	0.75	0.125
CHAPS*	1	1
Octyl Glucoside*	1	1
Octyl Thioglucoside*	1	1

*Maximum processable sample volume is 50 µL. ND: Not determined.

Part II: Removal of Detergents from Peptides

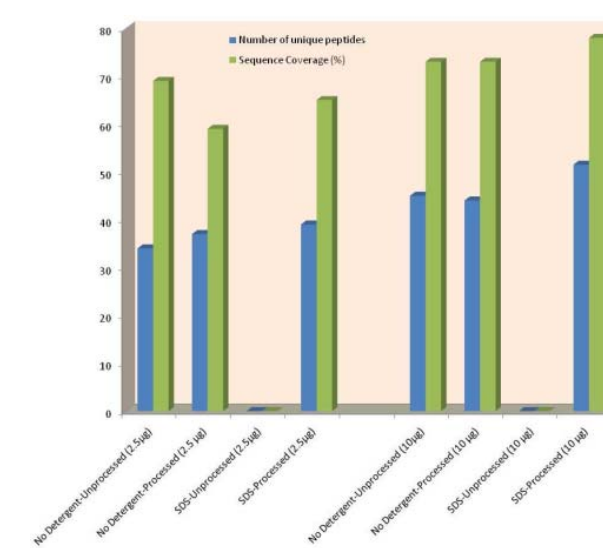
BSA (0.1 mg/mL) was digested in the presence and absence of detergents and the samples were processed for LC-MS/MS analysis as described in Methods. Detergents were effectively removed from the samples, eliminating the interference in MS analysis (Figure 3).

FIGURE 3. LC-MS/MS analysis of enzymatically digested BSA. Base peak LC chromatograms before and after detergent removal.



BSA (25 and 100 µg/mL) was digested in the presence and absence of SDS and the samples were processed for LC-MS/MS analysis as described in Methods. Effective detergent removal resulted in greater peptide identification and high Mascot scores (Figure 4).

FIGURE 4. Number of unique peptides and sequence coverage per sample



Conclusion

The HiPPR Detergent Removal Resin:

- Efficiently removes detergents (>95%) from low concentration (15–100 µg/mL) protein and peptide samples
- Provides high recovery of proteins and peptides
- Removes detergents in sample volumes as small as 25 µL
- Provides simple, efficient, and fast removal with batch and 96-well plate formats that take less than 15 minutes for the entire process
- Eliminates detergent interference in downstream applications like ELISA, isoelectric focusing and mass spectrometry

References

- R. Aebersold, M. Mann. Mass spectrometry –based proteomics. *Nature*, 422 (2003) 198–207.
- B. Canas, C. Pineiro, E. Calvo, D. Lopez-Ferrer, J. Gallardo. Trends in sample preparation for classical and second generation proteomics. *J. Chromatogr. A*. 1153 (2007) 235–258.
- R.R. Loo, N. Dales, P.C. Andrews. The effect of detergents on proteins analyzed by electrospray ionization. *Methods Mol.Biol.* 61 (1996) 141–160.
- B.S Antharavally, K.A. Mallia, M.M. Rosenblatt, A.M. Salunkhe, J.C. Rogers, P.J. Haney, N. Hagdoost. Efficient removal of detergents from proteins and peptides in a spin column format. *Anal. Biochem.* 416 (2011) 39–44.
- F. Rusconi, E. Valton, R. Nguyen, E. Dufourc. Quantitation of sodium dodecyl sulfate in microliter-volume biochemical samples by visible light spectroscopy. *Anal. Biochem.* 295 (2001) 31–37.
- A. Urbani, T. Worme. A colorimetric determination for glycosidic and bile salt-based detergents: applications in membrane protein research. *Anal. Biochem.* 336 (2005) 117–124.

Mascot is a trademark of Matrix Science Ltd. Triton is a registered trademark of The Dow Chemical Company. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.