NORGEN BIOTEK CORPORATION

Application Note ProteoSpin™ Silicon Carbide Technology

Efficient Removal of Triton X-100

Eric C. C. Yang, Ph.D., and K. W. Michael Siu, Ph.D.

York Universityís Department of Chemistry and Centre for Research in Mass Spectrometry, Toronto, Ontario, Canada

ProteoSpin Detergent Clean-up Micro Kit

Purpose

This application note evaluates the ProteoSpinô Deterg ent Clean-up Micro Kit (P/N 10500) for the removal of a biological detergent present in a protein solution, and demonstrates the Detergent Clean-up Micro Kitś efficiency in preparing a purified protein for downstream applications such as mass spectrometry. This evaluation also establishes that detergent removal is a necessary step for subsequent proteolytic digestion of the protein with trypsin, and that these results are more easily achieved with the ProteoSpin kit than by ultrafiltration with a molecular cut-off membrane.

Overview

The removal of detergents from protein samples is often a necessary step before many downstream applications, particularly those that involve structural analysis including mass spectrometry. A number of conventional methods for detergent removal are available, including dialysis, gel filtration chromatography, hydrophobic interaction chromatography and ultrafiltration. Recently, a limited number of kits for detergent removal have been introduced to the market.

Ultrafiltration devices remove detergent from protein samples through the process of size exclusion. Based on a particular molecular weight cut-off (MWCO), the larger protein is separated from smaller molecules by centrifugation. The PALL Microsep Omega 3K is an ultrafiltration device that can be used for detergent removal based on the principle of MWCO. The MWCO principle assumes that the detergent is free in solution and is not tightly bound to the protein sample.

The ProteoSpin Detergent Clean-up Micro Kit removes detergents from protein solutions using ion-exchange chromatography. Spin columns that contain silicon carbide (SiC) remove detergents from the protein sample. The sample is applied to the column, and the proteins bind while the detergent is washed off. Norgen claims that the Detergent Clean-up Micro Kit can easily and efficiently remove over 95% of detergents.

Triton X-100, a polymeric product with an average molecular weight around 800 Da, belongs to the family of non-ionic detergents and is commonly used for protein preparation. We investigated the efficacy of the Detergent Clean-up Micro Kit to remove Triton X-100 from a BSA solution.

Methods and Materials

The Detergent Clean-up Micro Kit was assessed in terms of protein recovery, removal of the non-ionic detergent Triton X-100, and the subsequent ability of the protein sample to be digested with trypsin. The use of an ultrafiltration membrane to achieve similar detergent clean-up effects was also investigated.

The protein sample used in both cases consisted of 30 µg bovine serum albumin (BSA) (Sigma A-4503) prepared in 1 mL of phosphate buffer (11 mM, pH 7.9) with or without 0.1% (w/v) Triton X-100.

For detergent clean-up using the Detergent Clean-up Micro Kit, 100 µL of acidic pH binding buffer and 1050 µL of emulsifier were added to the 1 mL BSA solution containing Triton X-100, resulting in a final volume of 2.15 mL. The pH, as verified by a pH stick, was approximately 4.5–5. The ProteoSpin column was activated with the supplied activation and wash buffer according to the Application Manual. The BSA solution was applied to the ProteoSpin column in 350 µL aliquots followed by centrifugation at 14,000 x *g* for one minute. A total of three sample applications and centrifugations were performed to load the entire 2.15 mL sample onto the ProteoSpin column. The bound protein was eluted into 50 µL of the supplied elution buffer (50 mM phosphate buffer, pH 12.5). The eluate was diluted to 1 mL with 950 µL phosphate buffer (11 mM, pH 7.9) for subsequent analysis.

To remove detergents by ultrafiltration, we used the PALL Microsep Omega 3K cut-off membrane. The 1 mL BSA solution was applied into the MWCO membrane and centrifuged at 7,500 x *g* for two hours at room temperature. After centrifugation, 320 µL of the protein solution remained, which was diluted to 1 mL with 680 µL of phosphate buffer (11 mM, pH 7.9) for subsequent analysis.

Protein quantitation was performed on the ProteoSpin processed sample using the BioRad protein assay (#500-0006). Trypsin digestion was performed by mixing 1 µL (out of 1 mL) of purified protein solution with 9 µL of 50 mM ammonium bicarbonate containing 10 ng of trypsin at 37°C for 16 hours. The resulting reaction mixtures were desalted by C18 Zip-tips (Millipore) and analyzed by a Voyager DE-STR MALDI-TOF. Subsequently, the digested samples were also run on SDS-PAGE.

Results and Discussion

The ProteoSpin procedure took approximately 20 minutes to complete. No clogging of the ProteoSpin columns was observed. The BioRad protein assay was used to determine protein recovery, and 21.5 µg of the original 30 µg input was recovered (71.6%). Any remaining trace detergent should not interfere with the protein assay since the detergent was depleted to an undetectable level, as shown by the MALDI-TOF (see Figure 1).

In contrast, the ultrafiltration procedure took two hours to complete. Evidence of membrane clogging was found in that the sample was concentrated to only 320 µL from the original 1 mL sample. Unfortunately, the percent recovery could not be determined for the ultrafiltration procedure as the residual detergent in the sample interferes with the BioRad protein assay.

The efficiency of detergent clean-up was assessed by performing a trypsin digestion followed by MALDI-TOF analysis. As shown in Figure 1, unprocessed BSA and ultrafiltration-processed BSA contained a series of characteristic Triton X-100 peaks (m/z 581.4, 597.2, 625.4, 641.2, 669.4, 685.2., etc (Figure 1a, c, d, and f). This could possibly lead to competition of the ionization such that other peptide peaks were not observed, and/or inhibition of the trypsin digestion. On the other hand, the ProteoSpin Detergent Clean-up Micro Kit removed Triton effectively and Triton peaks were not observed in the MALDI-TOF spectra (Fig 1b and e). Moreover, the trypsin digestion was successful and resulted in putative tryptic BSA fingerprints (m/z 927.4, 1479.8, 1567.7, 1639.9, etc.)

The efficacy of Triton X-100 clean-up by the Detergent Clean-up Micro Kit was further demonstrated by the SDS-PAGE analysis of the tryptic BSA samples, based on the fact that Triton X-100 is inhibitory to trypsin digestion. As shown in Figure 2, the majority of BSA recovered was digested by trypsin (Fig 2, lane 4), whereas a substantial amount of BSA recovered from the cut-off membrane remained undigested (Fig 2, lane 5) indicating that the remaining detegent interfered with the trypsin digestion.

Conclusion

In this study, the ProteoSpin Detergent Clean-up Micro Kit proves to be a quick and effective tool in detergent clean-up. The detergent is depleted to a level that allows for subsequent trypsin digestion and MS analysis. The level of protein recovery was found to be 71% by the BioRad protein assay. The Detergent Clean-up Micro Kit provides a high quality, detergent-free protein sample that can be used in various downstream applications, including trypsin digestion and MALDI-TOF analysis.

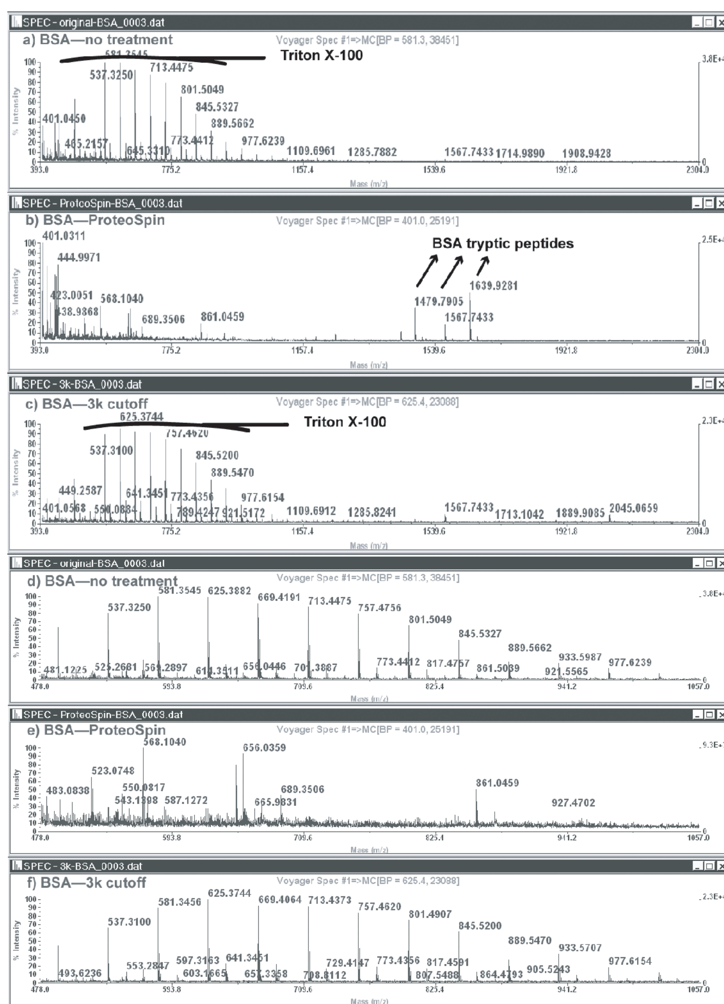

Figure 1: MALDI-TOF analysis of BSA samples after trypsin digestion. a) untreated BSA; b) ProteoSpin-treated BSA; c) cut-off membrane–treated BSA; d) and e) enlarged to show the region of m/z from ≈ 500–1000 corresponding to a)–c), respectively.

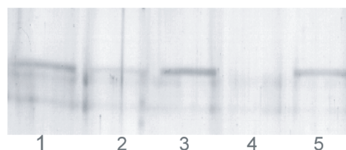

Figure 2: SDS-PAGE analysis of the BSA samples (silver stained).

Legend for Figure 2

Lane	
1	BSA + Triton (uncut)
2	BSA ➞ trypsin
3	BSA + Triton ➞ trypsin
4	BSA + Triton ➞ ProteoSpin ➞ trypsin
5	BSA + Triton ➞ cut-off membrane ➞ trypsin