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Low abundance protein detection after acetone precipitation using the ProTrap XG

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Introduction

Sodium dodecyl sulphate is commonly used during protein extraction before mass spectrometry. Efficient removal of the detergent must be achieved to obtain clean data as residual detergent competes with peptides for ionization.

Result

Residual SDS level in the standard protein averaged 30.03 +/- 13.99 ppm. 150 ng of the mixture was analyzed, with all three proteins (4 orders of magnitude) positively identified. Coverage ranged from 90 to 60 %. To further determine limits of the salt effect, 50 µg samples of BSA were precipitated in the presence of 0.5 to 5% SDS with variable NaCl concentrations, increasing up to 300 mM NaCl. At higher SDS concentrations and NaCl at 300 mM, increased variability in SDS removal was observed. Optimal results were obtained at 2% or lower SDS in the presence of 150 mM or lower NaCl. The use of the ProTrap XG decreases the variability observed during precipitation.

Method

A standard protein mix of 3 purified proteins (beta-galactosidase (50 ug), cytochrome c (50 ng) and alpha-enolase (1ng)) was precipitated in 50 mM Tris pH 8.0, 50 mM NaCl in the presence or absence of 2% SDS in the ProTrap XG with four volumes of acetone, digested after reduction and alkylation. The final digest was desalted using the integrated SPE cartridge. Samples were analyzed on an Orbitrap Fusion Lumos Tribrid mass spectrometer using an EACY-nLC and a 60-minute gradient. Residual SDS level was measured with the MBAS assay. The effect of SDS on acetone precipitation efficiency was explored by precipitation of a bovine liver extract in the presence of 0.5 to 5% SDS, with residual SDS measured by MBAS assay.

Conclusion

Precipitation can be used to reproducibly and reliably remove detergent contamination. Precipitation can be used with both abundant and rare proteins successfully. The ionic strength of the starting matrix influences precipitation reproducibility.

Method

SDS is a traditional anionic detergent used for protein extraction and solubilization. Although useful for those purposes, the presence of SDS interferes with mass spec analysis and must be removed. Precipitation with organic solvents is one method of removal.

To explore the effect on both the following proteins were precipitated in the ProTrap XG: 50 µg β-galactosidase (*e. coli*, 116.3 kDa), 50 ng cytochrome C (bovine, 11.6 kDa and 5 ng of enolase-1 (yeast, 46.7 kDa). 50 µg of the mixture (in 100 µL of 2% SDS, 50 mM Tris pH 8, 50 mM NaCl) was precipitated in the ProTrap XG by addition of 4 volumes of acetone incubated for 30 minutes. The proteins were pelleted by centrifugation, then the plug was removed, and the acetone decanted by centrifugation through the filter. The pellet was washed with acetone, then subjected to reduction, alkylation and digestion after solubilization in urea. Resulting peptides were desalted with the SPE cartridge, eluted in 50% acetonitrile and dried. A similar sample without SDS was processed in a microfuge tube as a control.

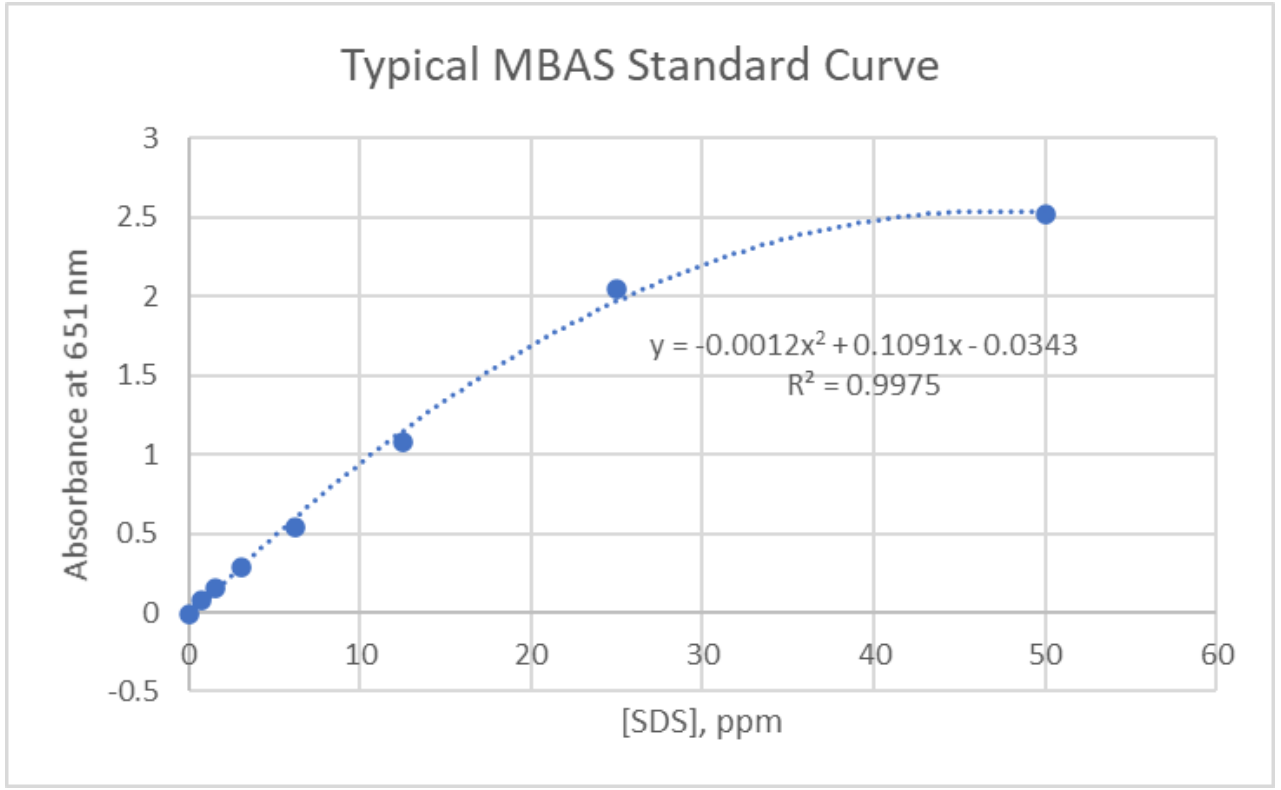
150 ng of the resulting peptides was injected onto an Orbitrap Fusion Lumos Tribrid mass spectrometer using an EACY-nLC and a 60-minute gradient.

Methods

The Methylene Blue Active Substances or MBAS assay is a sensitive method to determine the presence of anionic detergents in the presence of proteins, peptides and nucleic acids. It is most sensitive under 50 ppm or 0.005%.

The assay is simple to perform and can be used with dilute proteins and peptides.

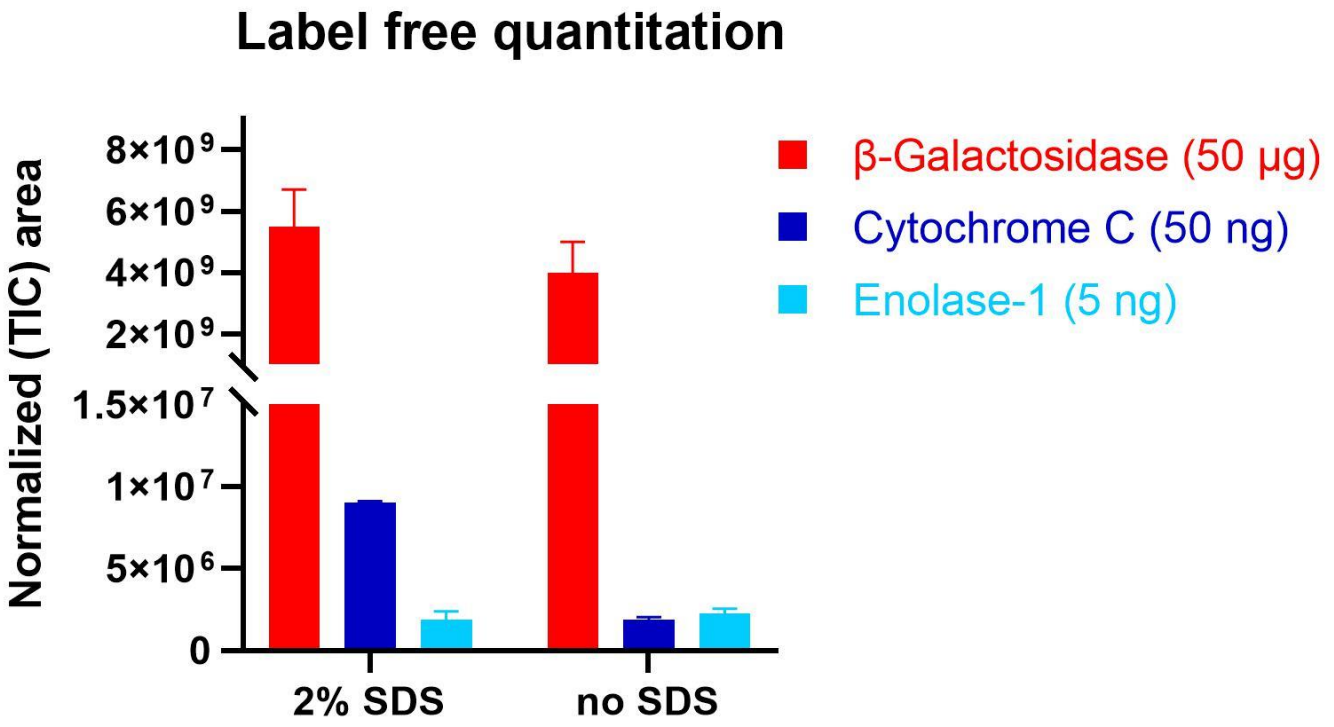
A typical standard curve is shown to the right.



Results – Coverage and Detection

All three proteins were detected in proportion to each starting concentration, whether precipitated in the presence or absence of SDS.

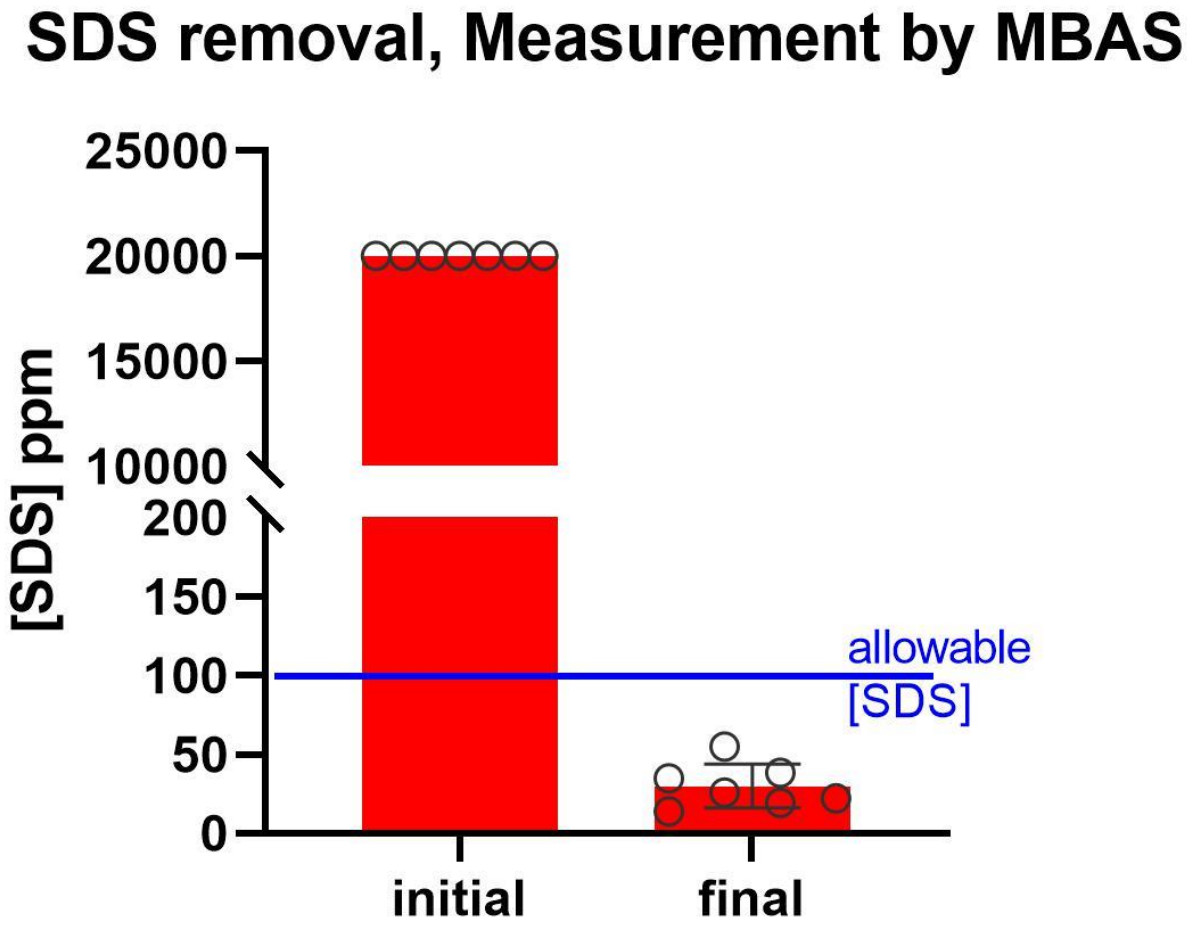
No difference in coverage detected, regardless of SDS presence. Overall coverage for β-galactosidase was 91%, for cytochrome c was 71% and enolase was 61%.



Results – Removal of 2% SDS

SDS was consistently reduced to less than 100 ppm by acetone precipitation in the ProTrap XG.

Average residual SDS concentration for 7 samples was 30.03 +/- 13.99 ppm.



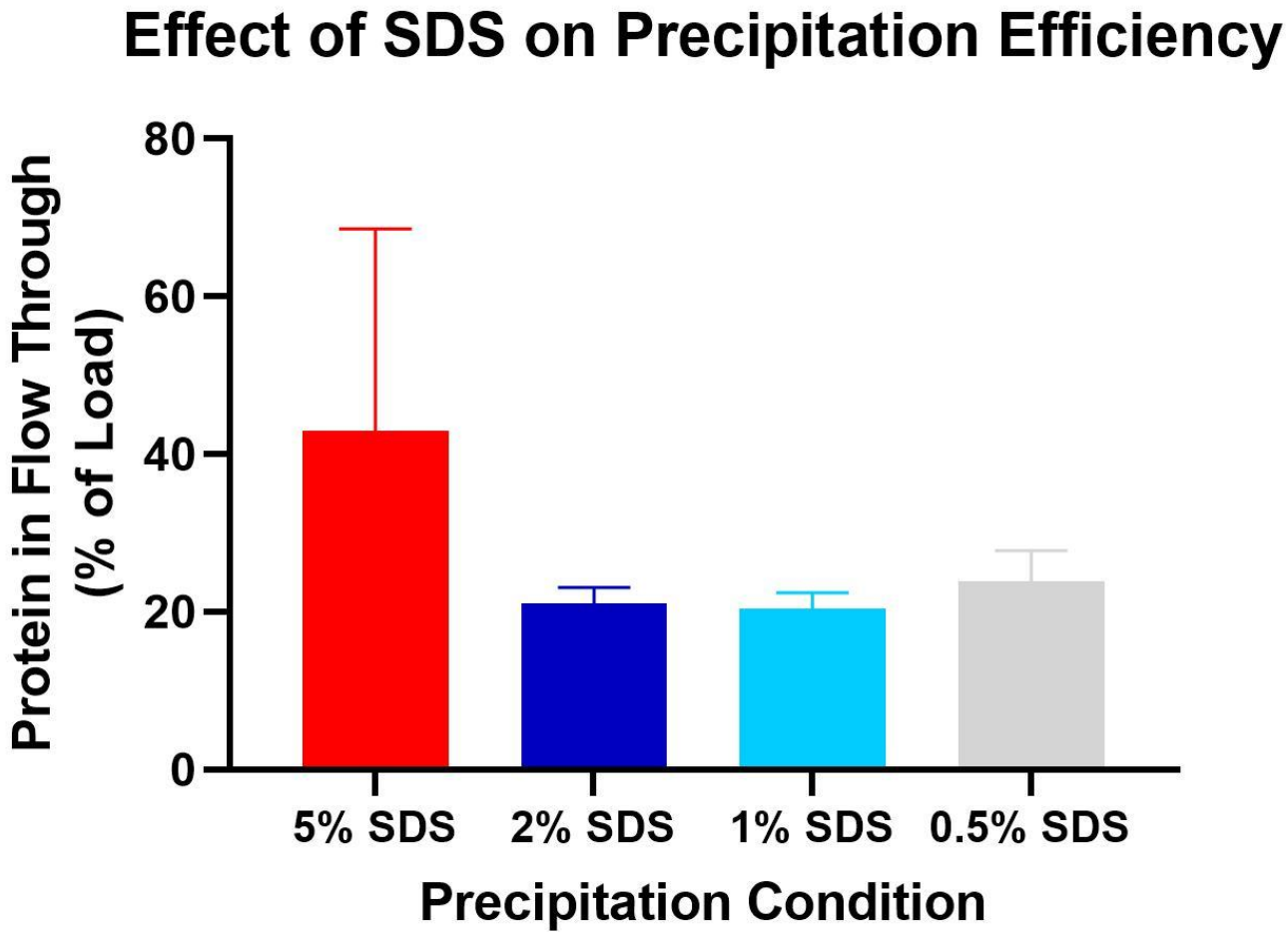
Results – Effect of SDS on Precipitation

Precipitation at 5% SDS results in variable precipitation.

Precipitation at > 5% SDS results in consistent precipitation.

Precipitation at > 5% SDS results in better recoveries.

Protein content of flow through fraction was measured by BCA assay and compared to load.



Discussion

Acetone precipitation of a standard mix of 3 purified proteins illustrates the utility of the method to reliably precipitate both abundant and low concentration proteins.

Acetone precipitation is a simple effective method for SDS removal prior to mass spec analysis. The ProTrap XG simplifies this method by removing the variability caused by pipetting. Instead of manual removal, organic solvent is removed from aggregated protein by centrifugation through a filter membrane. Precipitated proteins remain on top of the filter, ready for resolubilization and digestion.

Precipitation is affected by ionic strength of the protein solution. SDS is an anionic detergent and contributes to the overall ionic strength of the solution. Excess ionic strength can alter precipitation kinetics variably, resulting in variable recovery.

Conclusion

Acetone precipitation is an effective method to reduce SDS to under 100 ppm

Acetone precipitation can effectively precipitate both highly abundant and low abundant proteins (4 orders of magnitude illustrated here)

Choice of detergent and the concentration can impact recovery of protein

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References

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