Size Dependence for Protein Precipitation: Optimized Conditions for Efficient Recovery of Low-Mass Proteins in the ProTrap XG

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OBJECTIVES

- Determine which variables influence the precipitation of peptides.
- Optimize conditions for efficient recovery of low-mass proteins.
- Evaluate the properties of low molecular weight peptides following precipitation.

INTRODUCTION

An essential consideration of any protein sample cleanup approach is maintaining high recovery of all sample components during purification. With organic solvent precipitation, low molecular weight proteins are generally not susceptible to aggregation in organic solvent.
Our group previously demonstrated the importance of salt in maximizing recovery of proteins during solvent precipitation [1].
We also demonstrated that aggregated proteins could be rapidly isolated in a two-stage filtration cartridge called the ProTrap XG [2].

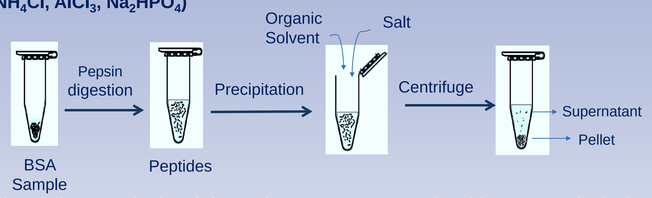
We herein extend our previous work with an objective to establish precipitation protocols for the purification and recovery of low molecular weight proteins and peptides, suitable for both top-down and/or middle-down proteomic applications.

METHODS

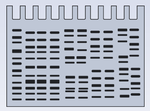
Vial Precipitation & Analysis

1. **Pepsin-digested BSA was used to generate a mixture of low molecular weight protein fragments (<5 kDa).**

2. **The sample was precipitated by addition of organic solvent (acetone, acetonitrile) with inclusion of various salts (NaCl, $ZnSO_4$, Na_2SO_4, $(NH_4)_2SO_4$, NH_4Cl, $AlCl_3$, Na_2HPO_4)**

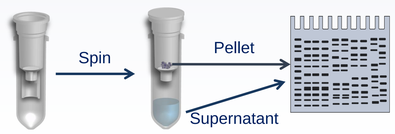


3. **The pellet was isolated from the supernatant and subject to analysis by HPLC with UV quantitation, as well as SDS PAGE and LC-MS/MS to identify proteins/ peptides recovered in the resulting fractions.**


Precipitation in the ProTrap XG

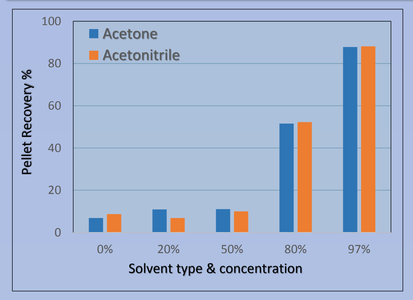
1. Precipitate pepsin-digested BSA without using salt as well as with 200 mM Na_2SO_4 and $ZnSO_4$ and 3 volumes acetone



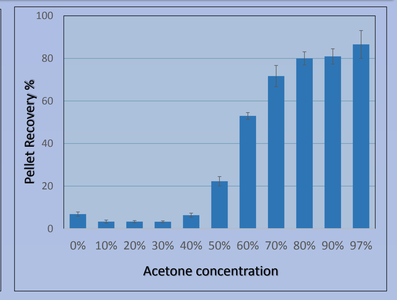
2. Analyze pellet and supernatant by SDS PAGE

RESULTS

Organic Solvent Type & Concentration



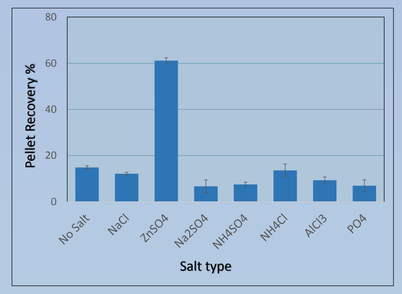
10 µg BSA-pepsin digested – precipitated with different concentration of organic solvent (Acetone, Acetonitlie)



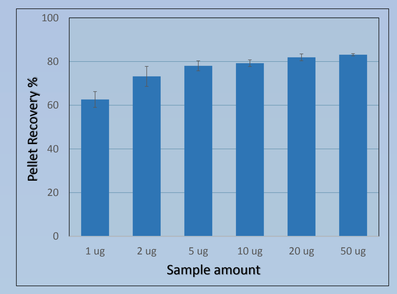
10 µg BSA-pepsin digested – precipitated with different concentration of Acetone

Precipitation recovery is improved with greater organic solvent concentration, but there is minimal difference in recovery between acetone and acetonitrile precipitations.

Effect of Salt Type & Peptide Concentration on Precipitation



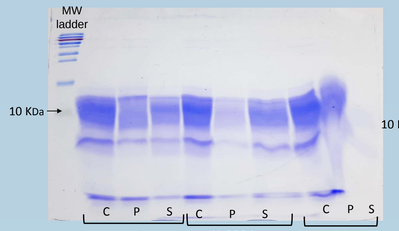
6.7µg BSA-pepsin digested – acetone precipitated with different salts



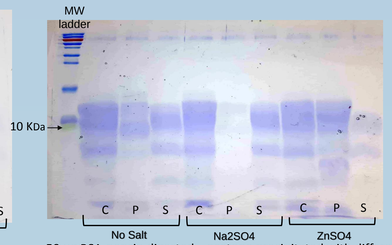
Different amount of BSA-pepsin digested sample– acetone precipitated with $ZnSO_4$

$ZnSO_4$ is the optimal salt for low-mass protein precipitation. Recovery is improved with greater protein concentration.

Precipitation in the ProTrap XG



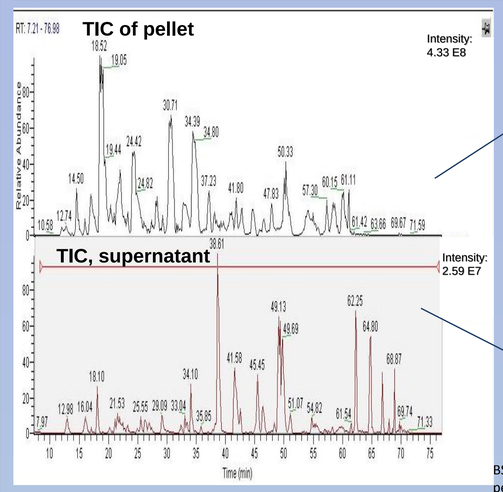
50 µg BSA-pepsin digested – acetone precipitated with different salts- Without clean up

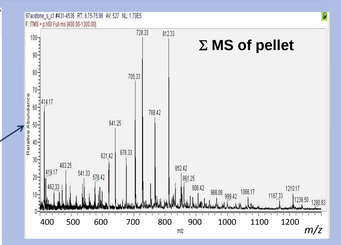


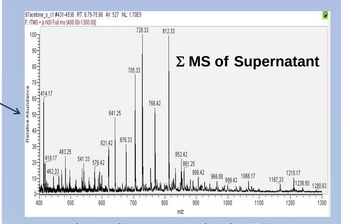
50 µg BSA-pepsin digested – acetone precipitated with different salts - cleaned up by ProTrap XG

The SPE cartridge within the ProTrap XG effectively cleaned the sample of ZnSO4 which improved the quality of SDS PAGE analysis.

TIC and MS Spectrum of Pellet & Supernatant



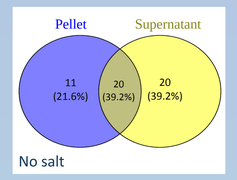


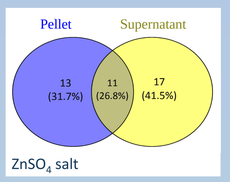


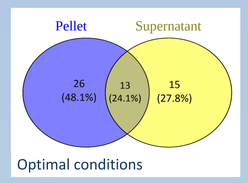
BSA-pepsin digested – precipitated with 97% acetone – pellet / Supernatant – chromatogram and MS spectrum

MS proves the presence of low Mw peptides in the pellet, and confirms that we can precipitate the peptides with high yield.

Venn Diagrams of Identified Peptides



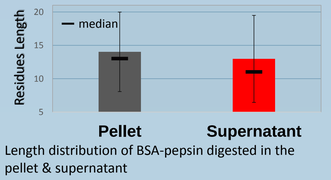




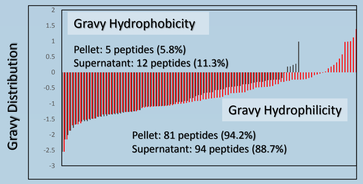
Venn diagram of identified recovered peptides in the resulting fractions

Venn diagram shows that adding $ZnSO_4$ improves the low-mass protein precipitation. Also it shows that in the optimal conditions we can precipitate most of the peptides.

Evaluation of Properties of Peptides in the Pellet & Supernatant



Length distribution of BSA-pepsin digested in the pellet & supernatant



	Average	Median	STD
Pellet	-0.879	-0.951	0.60
Supernatant	-0.736	-0.841	0.69

The average of residue result shows that the peptides in the pellet are slightly larger than the peptides in the supernatant.

The peptides in the pellet are more hydrophilic than the peptides in the supernatant.

CONCLUSIONS

- **Our findings indicate a strong dependence on the type of salt in the precipitation.**
- **The highest recoveries were determined by using ZnSO4.**
- **SDS PAGE confirms the recovery of low molecular weight peptides (<5 kDa) in the pellet fraction with intensity similar to HPLC_UV results.**
- **The SPE cartridge within the ProTrap XG effectively cleaned the sample of ZnSO4 which improved the quality of SDS PAGE analysis.**
- **Size trends and properties of peptides recovered in the pellet vs. supernatant by MS shows that protein precipitation in organic solvent remains an active approach for sample purification.**

REFERENCES

1. Crowell A.M.J., Wall M.J., Doucette A.A., Analytica Chimica Acta, 796 (2013) 48–54.
2. Crowell A.M.J., MacLellan D.L., Doucette A.A., Journal of proteomics, 118 (2015) 140-150

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