Improved Preparation of Membrane Proteins Ahead of Mass Spectrometry

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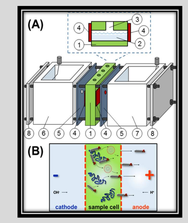


OBJECTIVES

To accelerate the rate of SDS depletion from membrane proteins by transmembrane electrophoresis (TME).

To improve the recovery of membrane proteins throughout the depletion process.

INTRODUCTION

Membrane proteins have multiple critical physiological roles including signal transduction, membrane trafficking, subcellular compartmentalization, and protein secretion. These functions lend membrane proteins as attractive targets for drug therapeutics. Despite their clinical significance, membrane proteins are notoriously underrepresented in proteomics workflows due to their poor solubility.

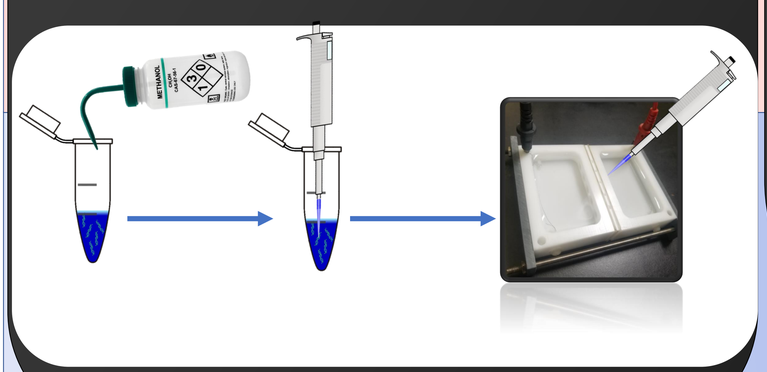
To improve the solubility of membrane proteins, previous groups have employed mass spectrometry (MS) compatible surfactant, as well as methanol (60%). Despite these approaches, sodium dodecyl sulfate (SDS) is still favored for the solubilization of membrane proteins. The addition of SDS however is known to impose challenges in downstream processing, as it deteriorates reverse-phase separation and suppresses MS ionization. Our group has developed an electrophoretic approach known as transmembrane electrophoresis (TME) to deplete SDS while maintaining a high soluble protein yield (>95%) in 5 minutes [1,2]. However, The recovery of insoluble membrane protein remains challenging

METHOD

Methanol and SDS were added to an *S. cerevisiae* membrane proteome extract in varying quantities. Samples were then depleted of SDS in a custom-built TME device 2.

BCA assay was used to quantify proteins recovered after a TME run, while MBAS assay was used to quantify the residual SDS in the sample. SDS PAGE was also used to further verify the quantity of proteins recovered.

Samples were then subjected to an orbitrap MS for profiling. The resultant spectrum was searched against a uniport database for protein count and identification.



RESULTS for SDS depletion and protein recovery

Organic solvent that caused the highest SDS depletion rate in TME.

Different organic solvents were tested for the highest SDS depletion rate. Methanol was responsible for the fastest SDS depletion (Figure 1A). The decay constant (λ) is the negative slop of a logarithmic plot that describes the exponential decay of SDS. The higher the decay constant the faster the SDS depletion.
The voltage drop for the different organic solvents was also measured in (Figure 1B). The drop of voltage throughout the experiment is due to the decrease in resistance of the TME to keep the constant current as per ohm's law (V=IR). The percentage of organic solvent were chosen based on the highest amount of solvent added without causing the proteins to precipitate.

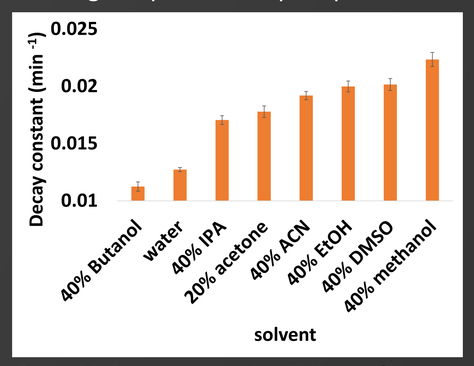


Figure 1A. Decay constant in a TME run of different organic solvents. Methanol had the highest SDS depletion.

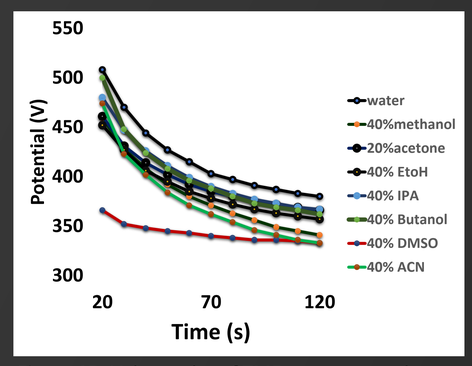


Figure 1B. Voltage plot of TME runs operated at constant current of 250mA, the run lasted for 2 minutes.

Improving membrane protein recovery.

BCA assay results demonstrate that methanol improves the solubility of membrane proteins. 40% methanol have solubilized 82% of membrane proteins compared to conventional addition of 0.5% SDS (Figure 2A).The addition of methanol to 0.5% SDS prior to the TME run did not affect the solubilization of membrane proteins (Figure 2B), and after TME run of samples containing 0.5% SDS and 40% methanol, higher proteins were recovered with the sample containing 40% methanol compared to the absence of methanol (Figure 2B and C). Moreover, 40% Methanol still improved the SDS decay (Figure 2D).

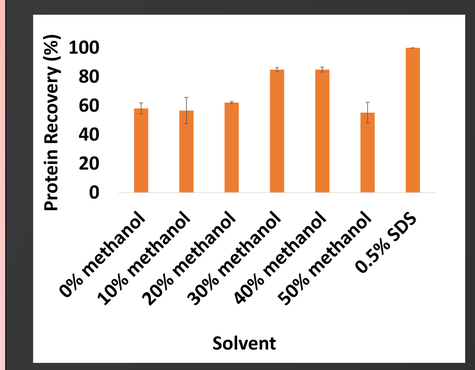


Figure 2A. Protein recovery of membrane proteins in methanol vs the addition of 0.5% SDS.

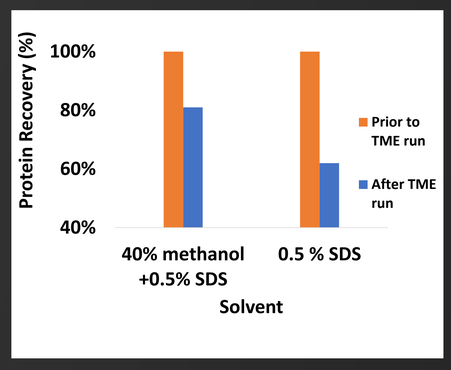


Figure 2B. Protein recovery of membrane proteins prior and after TME runs. Each sample started with 0.5% SDS.

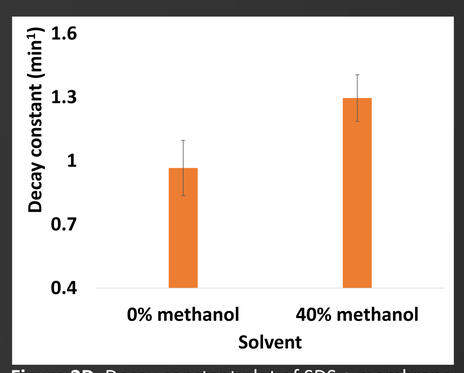


Figure 2D. Decay constant plot of SDS + membrane proteins, the run was done for 2 minutes at 250mA.

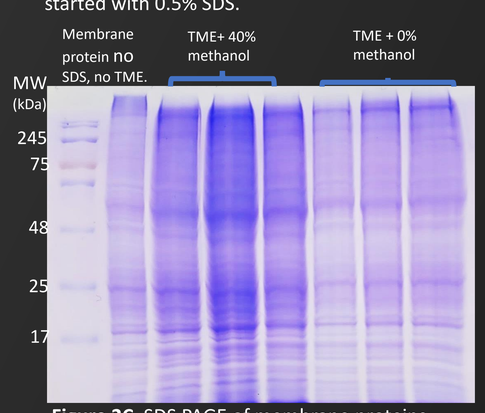


Figure 2C. SDS PAGE of membrane proteins.

Reference

1- Kachuk, C.; Doucette, A. A. J. Proteomics 2018, 175, 75–86.
2- Jakubec, P.; Doucette, A. Automated Electrokinetic Platform for High-Throughput Sodium Dodecyl Sulfate Depletion Ahead of Proteome Analysis by Mass Spectrometry. Anal. Chem. 2021, 93(42), 14042–14047.

Results of MS analysis

MS analysis results show that 1919 proteins were identified in the membrane sample extracted with the 40% methanol and 1792 were identified in. 1728 of the membrane proteins were found in both conditions with 191 unique to the 40% methanol and only 64 proteins unique to the absence of methanol (Figure 3A).

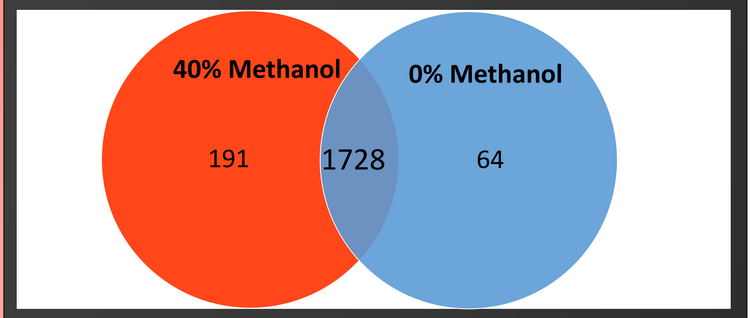


Figure 3A. Venn diagram comparing the identified *S. cerevisiae* membrane proteins found in both 40% methanol and 0% methanol after a TME run.

Next, we compared the proteins which are found significantly in higher amounts in both conditions across all MW and PI with p=0.1 (Figure 3B and C). 40% methanol extracted proteins across all PI and MW

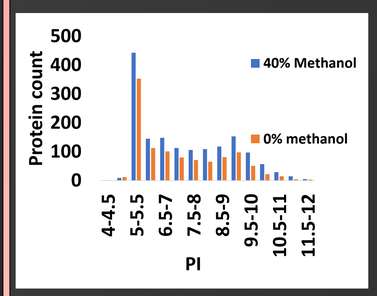


Figure 3B. PI distribution of proteins recovered in both solvent conditions.

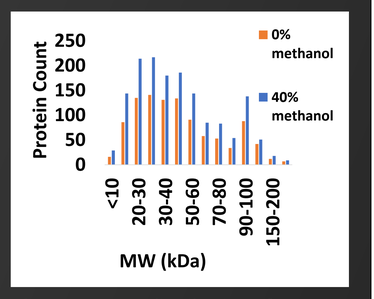


Figure 3C. MW distribution of proteins recovered in both solvent conditions.

Finally, we identified the cellular localization of proteins recovered in both conditions and found that higher cellular membrane proteins were recovered in 40% methanol.

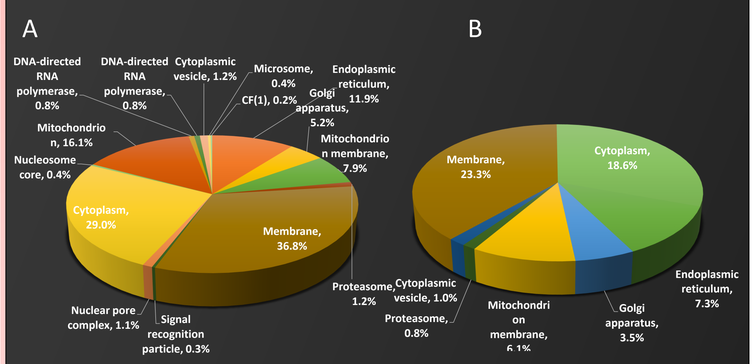


Figure 3D. A) Pie chart representing subcellular localization of proteins recovered in 40% methanol. B) subcellular localization of proteins recovered 0% methanol.

Conclusion

The addition of 40% methanol have improved the rate of SDS depletion in TME and improved the recovery of membrane proteins.

Acknowledge

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