A Novel Top-Down Proteomics Method Empowered by Photocleavable Surfactant for Comprehensive Analysis of Phospholamban Proteoforms

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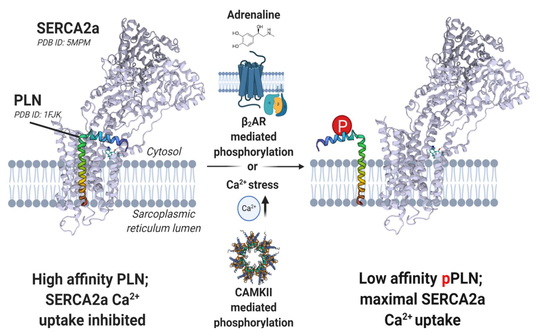
Overview

- **Phospholamban (PLN), a critical, cardiac membrane protein, was extracted from swine heart using Azo, a mass spectrometry (MS)-compatible, photocleavable surfactant [1].**
- **Hydrophilic interaction chromatography (HILIC) methods were developed for PLN analysis.**
- **HILIC sample preparation methods reproducibly detect PLN and limit the amount of TFA adducts.**

Introduction

Phospholamban (PLN) is an important Ca^{2+} handling protein regulating sarco(endo)plasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a), responsible for 70% of cytosolic Ca^{2+} handling [2]. As some heart disease is currently hypothesized to be a result of aberrant Ca^{2+} handling [2], methods for studying the major Ca^{2+}-handling proteins are critical. The regulatory role of PLN in heart disease is not fully understood, due to its hydrophobicity and lack of antibodies against rare PLN proteoforms. Herein, we have developed an unbiased, top-down MS method for characterization of PLN proteoforms in cardiac disease model enabled by Azo and HILIC.

Phosopholamban phosphorylation pathways



Methods

- Membrane proteins were extracted with a photocleavable surfactant, Azo. [1]
- RPLC was performed with polymeric reversed-phase material (PLRP)
- HILIC column: underivatized silica (Restek, Bellefonte, PA) 3 µm particle size, 300 Å pore size, 500 µm I.D., 10 cm column length. Samples precipitated and resuspended in formic acid and acetonitrile prior to HILIC.
- Data analyzed with MASH Explorer [6], Bruker DataAnalysis, and R studio.

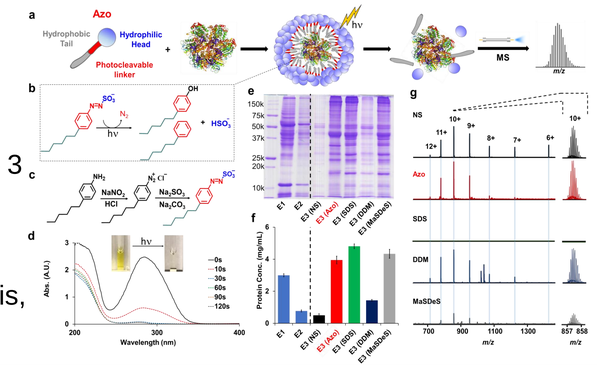


Figure 1. (A) Workflow (B) Azo degradation mechanism (C) Azo synthesis (D) Degradation kinetics (E-F) MS-compatibility. (From reference [3])

Results

1. TFA Improves HILIC Peak Shape

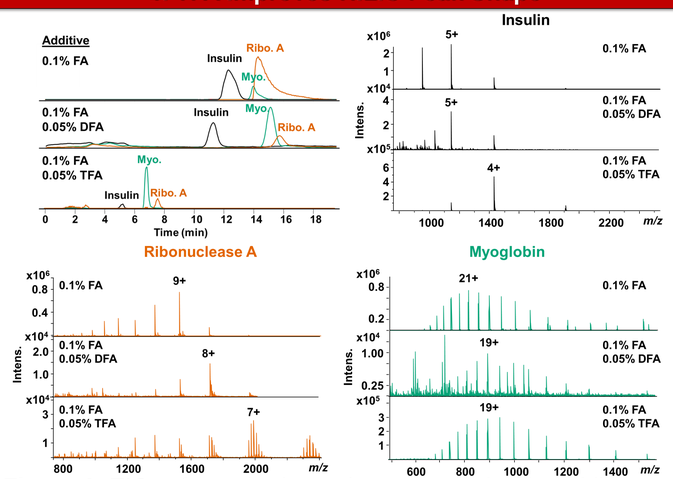


Figure 1. EICs of standard proteins separated with mobile phases containing different concentrations of TFA. Improved peak shape observed with 0.05% TFA.

2. Highly reproducible detection of PLN

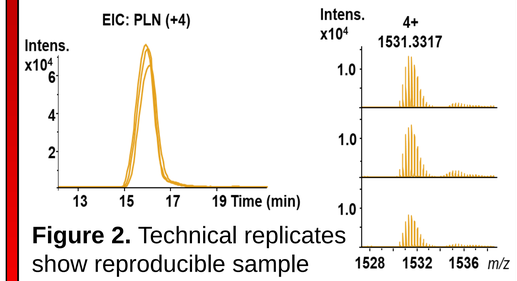


Figure 2. Technical replicates show reproducible sample preparation.

3. HILIC Separation of Swine LV Azo Extract

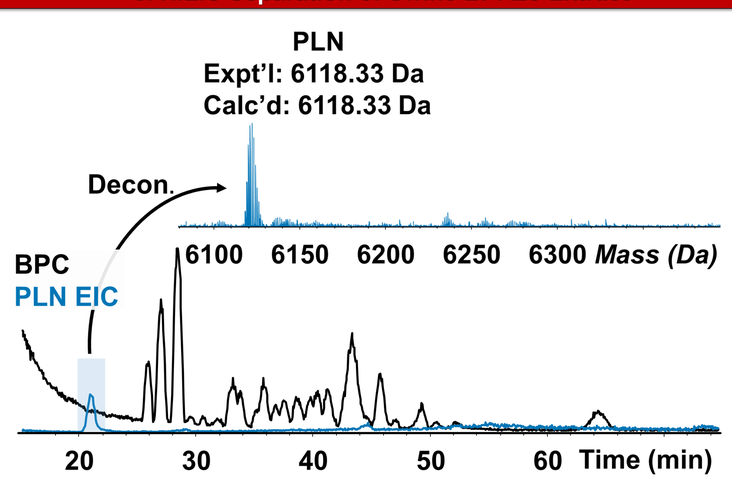


Figure 3. Swine left ventricular tissue extracted with Azo in ammonium bicarbonate buffer. Mobile phase A: ACN w/ 0.1% FA, 0.05% TFA. Mobile phase B: H_2O

4. PLN elution time ins HILIC vs RPLC

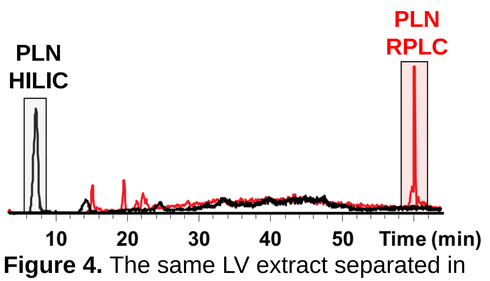


Figure 4. The same LV extract separated in HILIC mode versus RPLC mode.

5. PLN MS in HILIC vs RPLC Mode

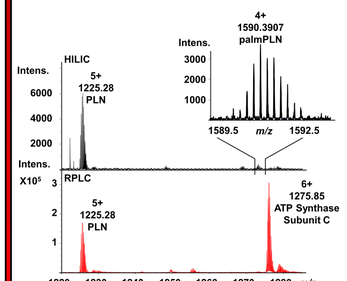


Figure 5. HILIC eliminates interference of co-eluting proteins with similar properties.

6. PLN Proteoforms

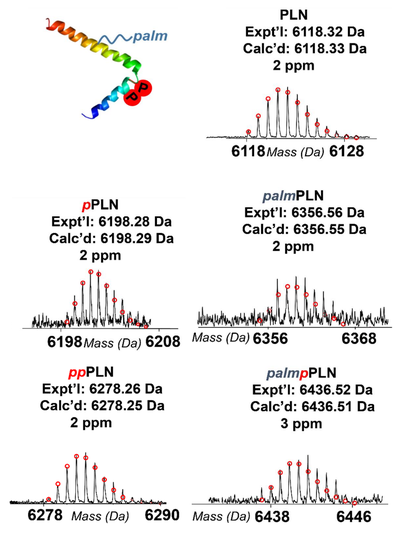


Figure 6. PLN proteoforms identified by LC-MS/MS.

References

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Acknowledgments

This research is supported by National Institutes of Health R01 GM117058 (to S.J. and Y.G.). Y.G. acknowledges R01 HL109810, R01 HL096971, R01 GM125085 and S10 OD018475.