IMPROVING THE GLYCOPROTEOMIC WORKFLOW FOR THE ANALYSIS OF *N*-LINKED GLYCANS BY CE/LIF

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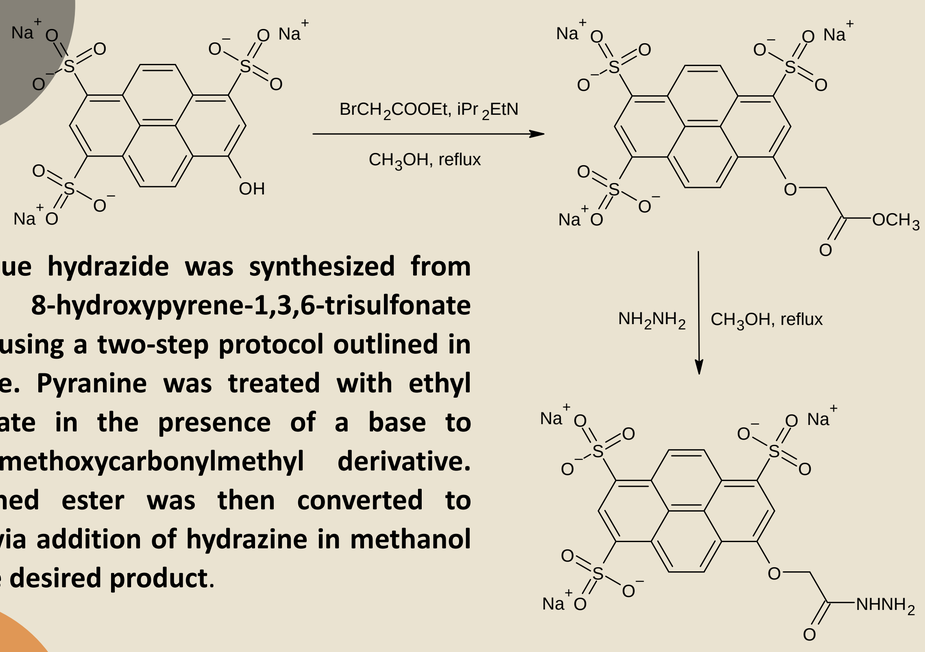
INTRODUCTION

Protein glycosylation is involved in the control of many important biological processes and structural changes in *N*-linked glycans are associated with various types of diseases. High-throughput profiling of *N*-glycans often contains a derivatization step in order to increase detection sensitivity by optical or mass spectrometric methods.

In this work, we report the effect of different proteomic protocols varying in enzyme or digestion procedures followed by derivatization by Cascade Blue hydrazide (8-(2-hydrazino-2-oxoethoxy)pyrene-1,3,6-trisulfonic acid trisodium salt, CBH) on the glycan detection in CE/LIF analysis. CBH represents a more reactive analog of 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt (APTS) widely used for glycan labeling before CE analysis.

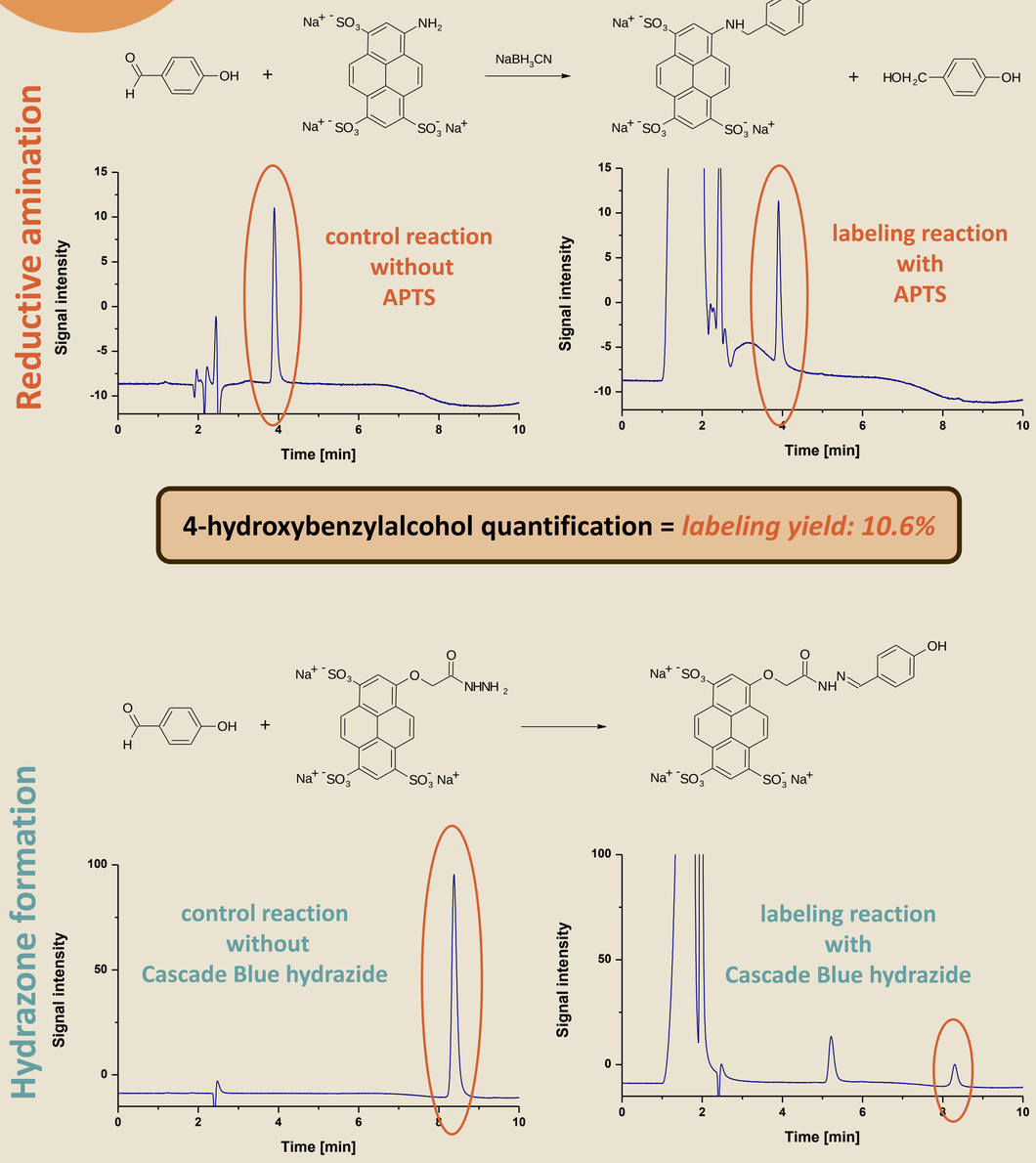
SYNTHESIS OF CASCADE BLUE HYDRAZIDE



Cascade Blue hydrazide was synthesized from trisodium 8-hydroxypyrene-1,3,6-trisulfonate (pyranine) using a two-step protocol outlined in the scheme. Pyranine was treated with ethyl bromoacetate in the presence of a base to afford *O*-methoxycarbonylmethyl derivative. The obtained ester was then converted to hydrazide via addition of hydrazine in methanol to yield the desired product.

COMPARISON OF LABELING CHEMISTRIES

HPLC/UV ANALYSIS



4-hydroxybenzylalcohol quantification = *labeling yield: 10.6%*

4-hydroxybenzaldehyde quantification = *labeling yield: 89.4%*

LC/UV

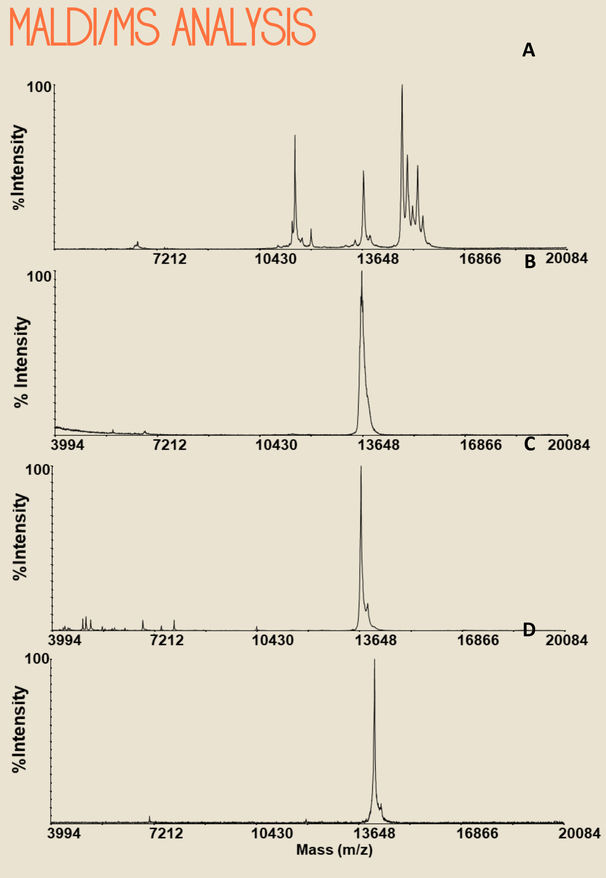
Agilent 1100 Series HPLC - Poroshell 120 SB-AQ (150 × 2.1 mm; 2.7 µm), 35 °C, 0.2 mL/min, gradient elution: 10%-70% acetonitrile in water/10 min, UV detection at 280 nm.

OPTIMIZATION OF PROTEOMIC PROTOCOLS

LABELING

Glycans released from RNase B + Cascade blue hydrazide label
Optimized labeling conditions: 1:10 molar ratio, 7.5 % acetic acid, 50 °C, 20 h, no reduction agent

MALDI/MS ANALYSIS



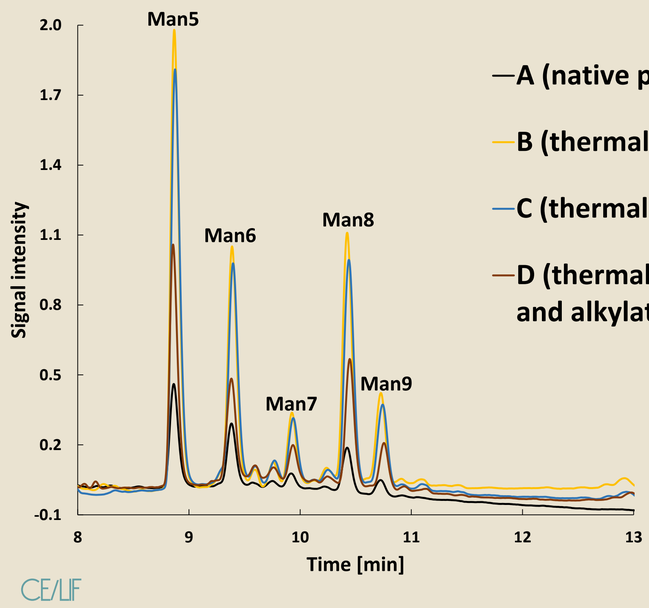
MALDI/MS spectra of intact RNase B after individual deglycosylation conditions:

A) native protein
B) thermally denatured protein
C) thermally denatured protein, reduced by DTT
D) thermally denatured protein, reduced by DTT, and alkylated by IAA

MALDI/MS

- **Individual RNase B samples (1mg.ml^{-1}) were purified using C18 Zip Tips before MALDI/MS analysis**
- **MS measurements were carried out using a 5800 Proteomics Analyzer (AB Sciex)**
- **Intact protein measurements were performed in the positive linear (+LIN) mode with SA as a matrix**
- **The reproducibility was proven using five independent MALDI-TOF MS measurements for each sample**

CE/LIF ANALYSIS



CE/LIF

Sciex P/ACE MDQ Plus CE instrument
CE: 50 µm ID NCHO-coated capillary (48.5/38.5 cm), carbohydrate separation gel buffer (Sciex), -25 kV
LIF: 405 nm laser/425 nm long pass filter

CONCLUSIONS

- **This work compares the efficiency of glycan labeling by reductive amination (APTS) and hydrazone formation (CBH) chemistries**
  - **CBH labeling provides: a) single product formation, b) higher labeling yield, c) no competitive reaction**
- ***N*-linked glycans were released from RNase B after individual deglycosylation protocols (denaturation, reduction, or alkylation steps)**
  - **the most effective approach: the protocol based on thermal denaturation (B) and DTT reduction (C)**
- **The effective hydrazone formation technique was applied for labeling of released *N*-linked glycans followed by CE/LIF analysis**
- **In the next step, the research will focus on the use of the optimized glycoproteomic protocol at different concentrations of glycoproteins and in-gel enzymatic treatment**

ACKNOWLEDGEMENT

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