Rhodamine B-based Labeling for Oligosaccharide and Glycan Analysis by CE/LIF

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Introduction

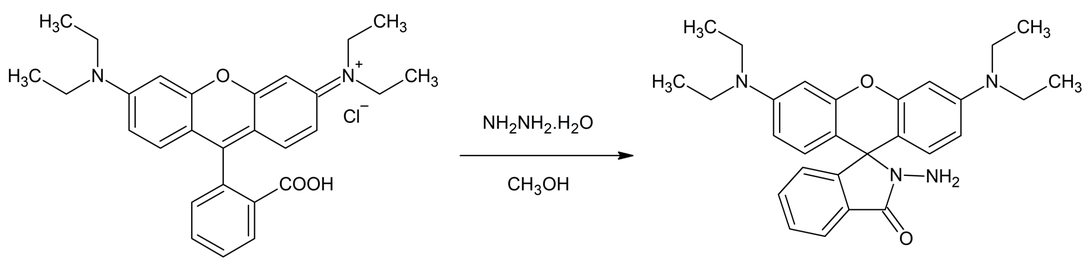
Derivatization of oligosaccharides and glycans released from glycoproteins represents a crucial step of their analysis by capillary electrophoresis (CE), liquid chromatography (LC), and mass spectrometry (MS). Since saccharides usually do not contain any chromophore or fluorophore in the structure, labeling is required for detection by optical methods such as UV/Vis absorption or fluorescence.

In this work, we present a synthesis of the rhodamine B-based fluorescent tag modified by a hydrazide functionality for saccharide derivatization via hydrazone formation chemistry.

Synthesis of rhodamine B hydrazide

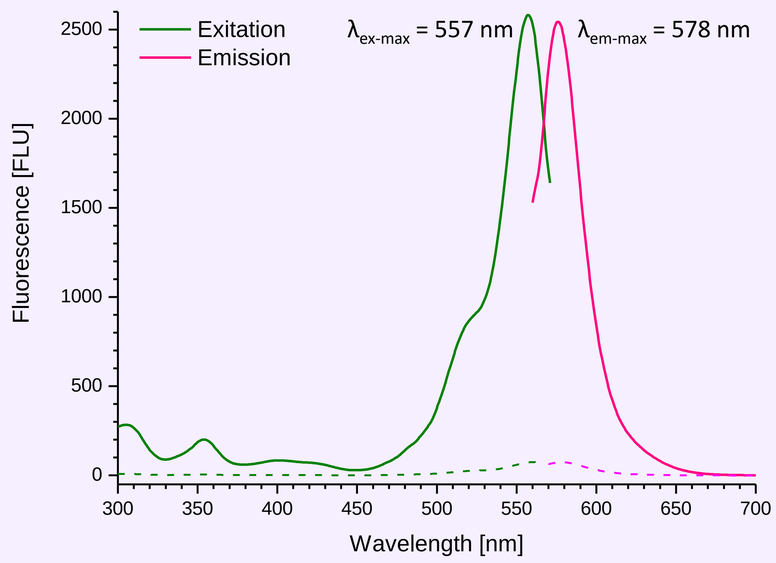
Rhodamine B hydrazide was synthesized by the hydrazinolysis of rhodamine B according to the method reported in *Yang XF, Liu P, Guo XQ, Zhao YB (2002) Development of a novel rhodamine-type fluorescent probe to determine peroxynitrite. Talanta 57:883–890*.

In brief, to a rhodamine B (0.5 g) solution in methanol (20 ml), hydrazine hydrate (1 ml) was added dropwise. Then reaction mixture was refluxed for 24 hrs. After that, the cooled solution was poured into water (100 ml) and 3 times extracted with 50 ml of ethyl acetate. The combined extracts were dried with anhydrous sodium sulfate, filtered, and then the solvent was evaporated.



Label characterization

Fluorescence excitation and emission spectra of rhodamine B hydrazide



Full lines – measured in 1M formic acid in **80% MeOH**

35 times increase in fluorescence signal

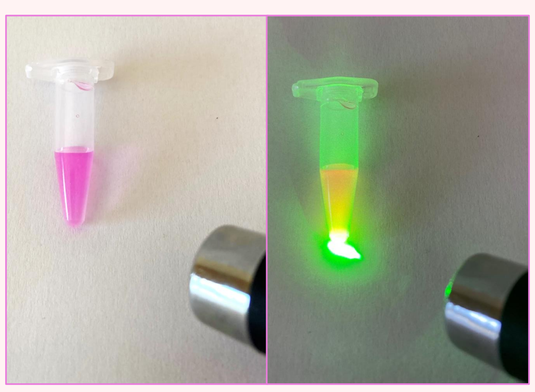
Dashed lines – measured in 1M formic acid in **20% MeOH**

Labeling procedure

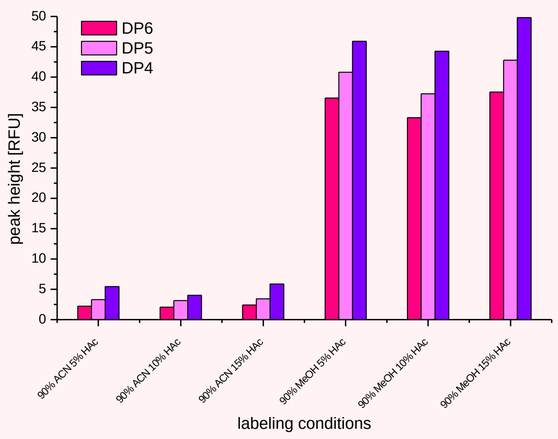
Oligosaccharides standards/glycans released from RNase B, IgG + rhodamine B-based label

Optimized conditions:

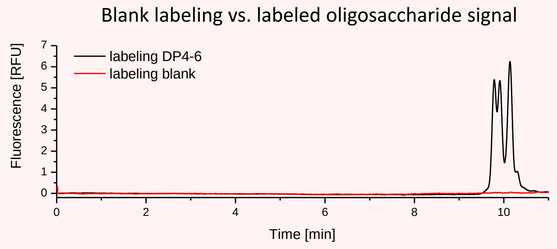
- oligosaccharide:label ratio, 1:10
- 90 % methanol, 10 % acetic acid
- 50 °C, 5 hrs
- no reducting agent



Labeling efficiency in different solvents and HAc concentrations



Blank labeling vs. labeled oligosaccharide signal



Acknowledgment

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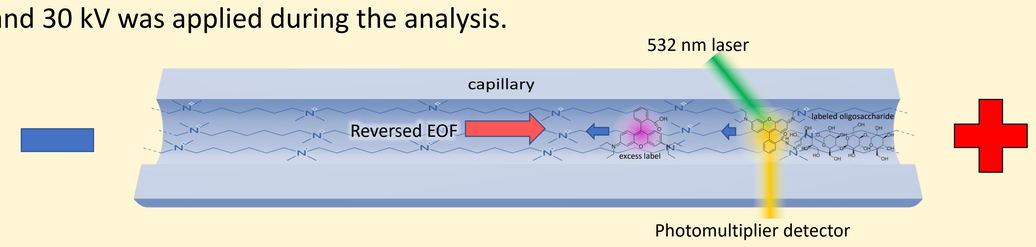
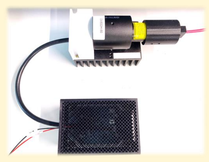
CE conditions

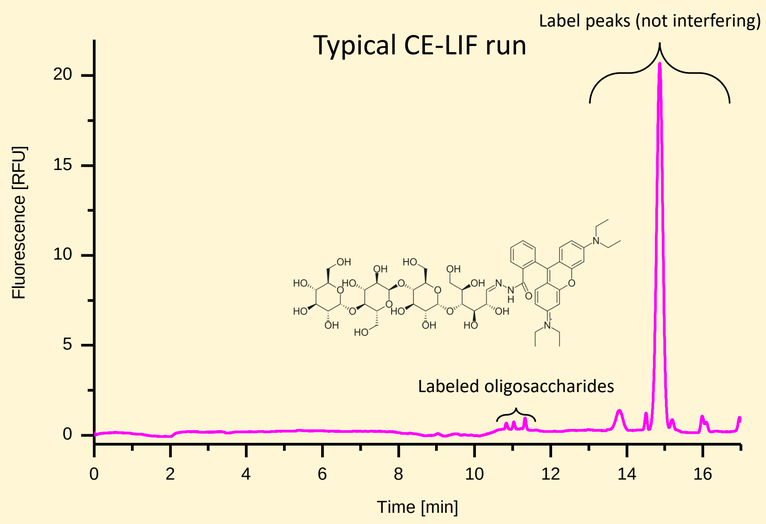
P/ACE MDQ plus (Sciex) instrument was used to perform the CE measurements. 50 µm i.d. fused silica capillary L_{tot}/L_{eff} 60/50 cm was coated using 5 % w/V solution of hexadimethrine bromide (polybrene) to achieve the reverse (positive) charge of the capillary wall. The coating procedure consisted of four subsequent rinses of H_2O/1M NaOH/H_2O/polybrene/H_2O/polybrene (1/15/3/3/3/3 min respectively). Finally, the coating was stabilized by application of 3 kV over the length of the capillary for 20 min. BGE containing 1M formic acid in 80% MeOH V/V was selected based on the dramatic increase of fluorescence in solutions containing high percentages of organic solvents (see the fluorescence spectra below). The capillary was rinsed with BGE for 3 min at 5.17 bar (75 PSI) prior to every analysis. Sample was injected using 35 mbar (0.5 PSI) for 10 s and 30 kV was applied during the analysis.



LIF setup:

- 532 nm laser CW532H-050 from Roithner LaserTechnik
- Laser coupling by 400 µm i.d. lightguides with a 3D-printed adapter
- Detection
  - Notch filter OD4 532 nm
  - 560/10 nm bandpass filter
  - or 550 nm longpass

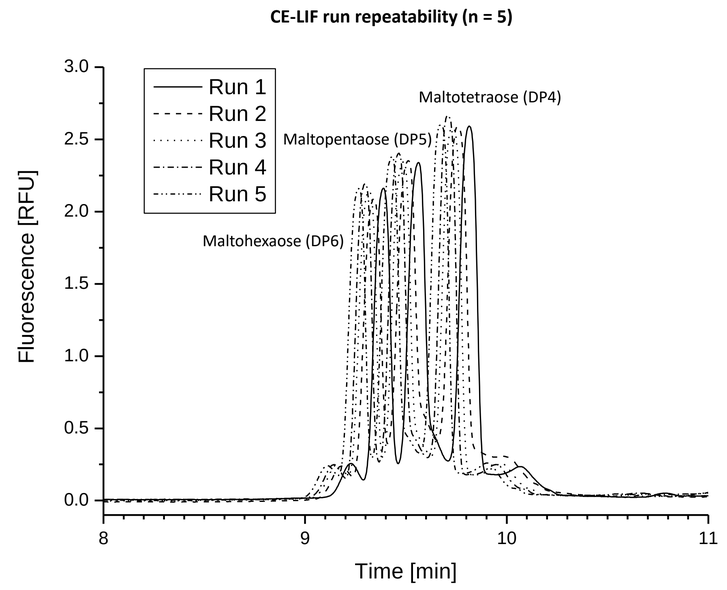




High organic-solvent CE

- Fast rate of BGE evaporation
- Fresh BGE every 5 to 10 runs (depending on the analysis length)
- MS-friendly BGE
- Repeatability evaluated on 5 subsequent runs of DP4-6 labeled saccharides

peak	DP6	DP5	DP4
	migration time (min)		
AVE	9.320	9.489	9.733
SD	0.046	0.049	0.054
RSD	0.50%	0.52%	0.56%
	peak height		
AVE	21287	23426	25814
SD	325	226	310
RSD	1.52%	0.97%	1.20%

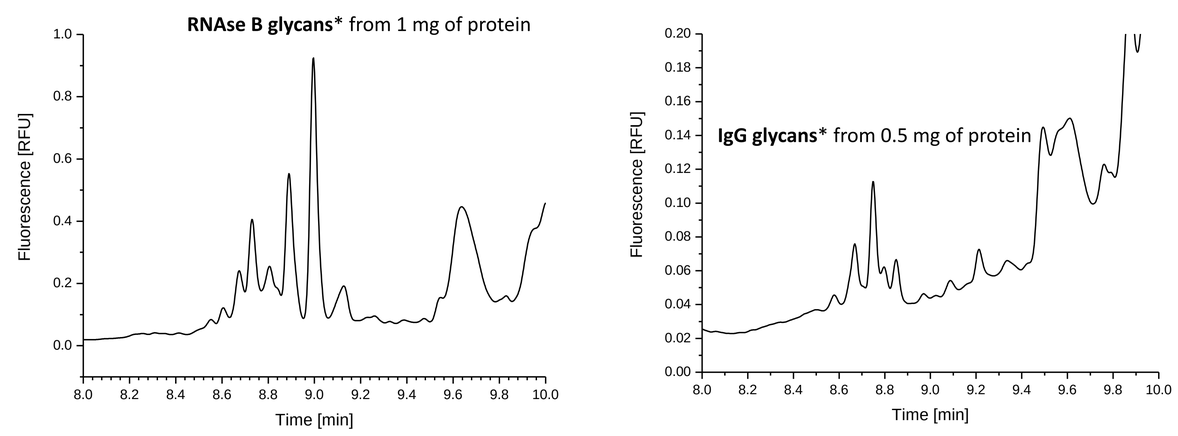


LOD* for oligosaccharides was 52 nmol·l^{-1} corresponding to 0.9 pg of the injected DP4

*determined as 3 times fluorescence signal noise

Glycan analysis

*glycans cleaved by PNGase glycosidase using standard protocol



Conclusion

A new fluorescent rhodamine B-based label for capillary electrophoresis analysis of oligosaccharides and glycans was successfully designed and synthesized. To achieve the most efficient derivatization, we optimized the labeling conditions such as temperature, time, solvent composition, and separation conditions. In addition, the labeling method by rhodamine B was used for *N*-linked glycan profiling of several glycoproteins (RNase B, IgG) by CE/LIF analysis.