Procainamide labelling as part of a sensitive workflow for the identification and quantitation of potential glycan drug targets in parasite cell membrane, insect vector saliva and host tissue models of infection by HILIC-HPLC-ESI-MS/MS

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Introduction

Changes in glycosylation have been associated with many states of health and disease and have the potential to provide diagnostic information and act as drug targets. In parasitological studies however, investigations into glycosylation changes are hampered by the availability of small sample amounts. This problem is particularly challenging in analyses that focus on characterisation of plasma membrane glyco-conjugates. These glycans offer the most obvious choice of glycan-based drug targets due to their position at the interface between a cell and its environment. In such workflows however the enrichment of plasma membranes from an already small starting amount further decreases the amount of glycoprotein available.

Currently, the most widely used glycan fluorescence label in the analysis of mammalian glycosylation systems is 2-aminobenzamide (2-AB) which is compatible for use in HILIC-HPLC-FLR coupled with ESI-MS/MS. However 2-AB suffers from relatively poor MS signal intensity when analysing small sample amounts. For the analysis of novel or non-mammalian parasite glycans, MS signal intensity is an important factor in acquiring good quality, data-rich MS/MS fragmentation information. Here we evaluate the use of procainamide as an alternative to 2-AB in HILIC-HPLC-ESI-MS/MS glycomic analyses.

Aims:

1. To determine a suitable glycomics workflow coupling HPLC with ESI-MS/MS to combat the problem of small sample amounts. Specifically, to compare and evaluate the use of two different labels (**2-AB and procainamide**) for released *N*-glycans from a standard glycoprotein (bovine fetuin) to ensure highly sensitive and reliable MS and FLR detection
2. To investigate the role of *N*-glycosylation in various parasite, vector and host models of parasitological infection

Methods & Results

1. Comparison of 2-AB and procainamide labelled bovine fetuin *N*-glycans

50 µg Bovine Fetuin standard glycoprotein

N-glycans were released in-solution enzymatically with PNGase F *[E-PNG01]* using a Hamilton Robotics StarLet high-throughput liquid-handling platform

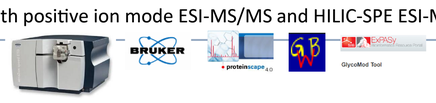
Labelled with either:

- Released *N*-glycan labelling via reductive amination with **2-Aminobenzamide (2-AB)** using LudgerTag™ 2-AB Glycan Labelling Kit *[LT-KAB-VP24]*
- 2-AB labelled *N*-glycan clean up using LudgerClean™ T1 Cartridges *[LC-T1-A6]*

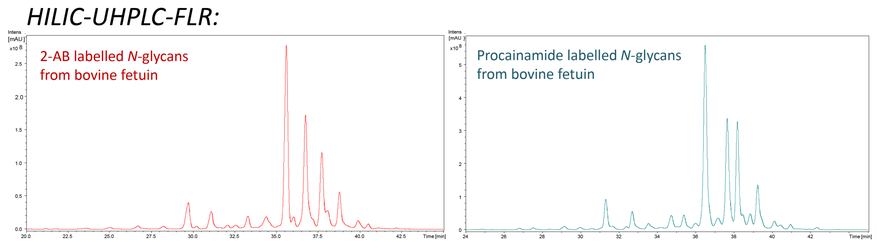
- Released *N*-glycan labelling via reductive amination with **Procainamide** using Ludger Procainamide Glycan Labelling Kit *[LT-KPROC-VP24]*
- Procainamide labelled *N*-glycan clean up using LudgerClean™ S Cartridges *[LC-S-A6]*

Analysed using:

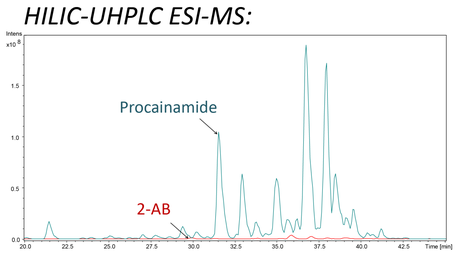
HILIC-UHPLC-FLR coupled online with positive ion mode ESI-MS/MS and HILIC-SPE ESI-MS using a Bruker amaZon speed ion trap



HILIC-UHPLC-FLR:

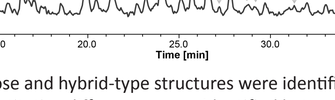
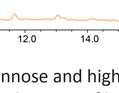
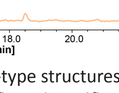
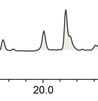
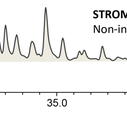


HILIC-UHPLC ESI-MS:



Procainamide and 2-AB showed good comparability however procainamide showed 2x higher fluorescence and **40x higher MS signal** intensity compared to 2-AB labelled glycans.

2. Application of Procainamide labelling with HILIC-HPLC-FLR and ESI-MS/MS to the study of three model systems

PARASITE	INSECT VECTOR	HOST
What glycans are on the parasite?	*What glycans are present during parasite transmission?*	*Does glycosylation change upon infection?*
***T. brucei* Blood stream vs. Adipose Tissue forms**	**Sandfly and tsetse fly salivary glycoproteins**	**Naïve *vs. T. brucei*-infected mice**
 Blood Stream Forms (BSFs) ***vs.*** Adipose Tissue Forms (ATFs)	 Tsetse fly[1] ***vs.***  Sandfly	 Mouse[2] **+/-**  *Trypanosoma brucei*[3]
• BSFs and AFTs were collected from *T. brucei*-infected C57BL/6J mice (with pleomorphic clone AnTat1.1E) • Parasite membrane proteins were enriched by ultracentrifugation and Triton X-114 phase partitioning • Release of *N*-glycans with PNGase F *[E-PNG01 from Ludger]* from PVDF-immobilised *[LC-PBM-96]* glycoproteins	• Tsetse fly (unknown amount) and sandfly saliva (approx. 15 µg) • In-solution release of *N*-glycans from salivary glycoproteins with PNGase F *[E-PNG01 from Ludger]*	• Gonadal and retroperitoneal fat pads from naïve and *T. brucei*-infected C57BL/6J mice were collected and pooled • Adipose tissue was collagenase-digested: The resultant floating adipocytes and pelleted stromal vascular fraction were collected separately • Cell membrane proteins were enriched by ultracentrifugation and Triton X-114 phase partitioning • Release of *N*-glycans with PNGase F *[E-PNG01 from Ludger]* from PVDF-immobilised *[LC-PBM-96]* glycoproteins

• LABELLING: *N*-glycan labelling via reductive amination with Procainamide using Ludger Procainamide Glycan Labelling Kit *[LT-KPROC-VP24]*
• POST-LABELLING CLEAN-UP: LudgerClean™ Plate for Procainamide Clean up *[LC-PROC-96]*

Analysed using:

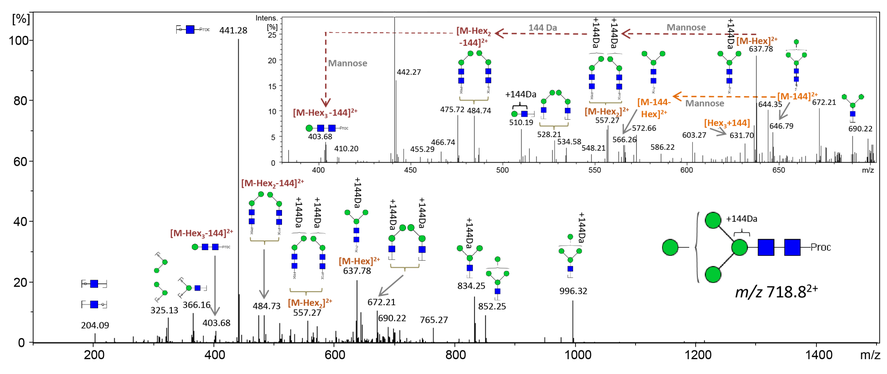
• HILIC-UHPLC-FLR coupled online with positive ion mode ESI-MS/MS and HILIC-SPE ESI-MS
• Manual data analysis

• High mannose and hybrid-type structures were identified in both stages
• Relative quantitative differences were identified between the blood stage and adipose tissue stage forms

• Pauci mannose and high mannose-type structures dominate the *N*-glycomes of both tsetse fly and sandfly saliva
• Low abundance hybrid-type glycans were detected in both samples with samples carrying a 144 Da modification identified only in the sandfly
• No detectable differences were identified between PNGase F and PNGase A released *N*-glycans in the sandfly

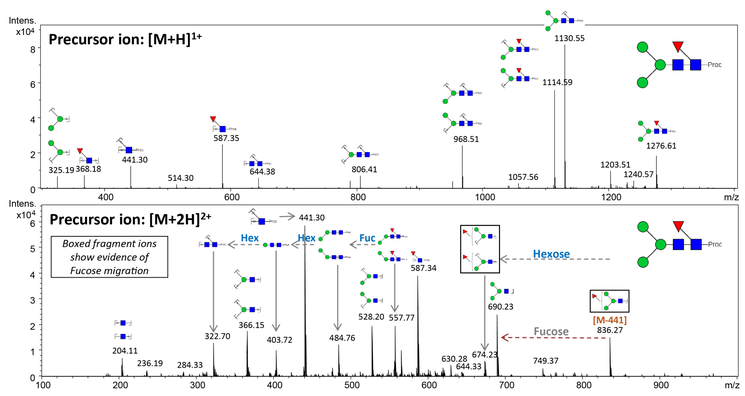
• A variety of structure types were identified including high mannose, hybrid and complex type
• Relative quantitative and qualitative differences were observed upon infection
• NeuGc carrying structures were also identified

3. Improved MS signal intensity provides high quality MS/MS fragmentation which contains diagnostic ions to aid in *N*-glycan structural characterisation



Representative MS/MS fragmentation profile of *m/z* 718.8^{2+} with composition $Hex_4HexNAc_2$144Da-Proc

• Structures carrying a 144 Da modification were detected in sandfly saliva



Representative MS/MS fragmentation profiles of *m/z* 638.79^{2+} and 1276.58^{1+} with composition $Hex_3HexNAc_2Fuc$-Proc

• Fucose migration was detected in positive ion mode MS/MS of in core fucosylated glycans where the precursor ion was doubly charged

Conclusions

- Here we present a procainamide labelling workflow suitable for HPLC-MS analyses
- The workflow offers a sensitive and robust way of characterising and quantifying mammalian and non-mammalian glycans from small starting sample amounts.
- This workflow can be applied to a wide range of studies and biological samples.

Acknowledgements

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References

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