Investigating the role of insect vector glycosylation in African sleeping sickness transmission: *Characterisation of procainamide-labelled tsetse fly saliva* N-*glycans*

Radoslaw P. Kozak[1], Katherine Wongtrakul-Kish[1], Christopher Williams[2], Samirah Perally[2], Claire Rose[2], Daniel I.R. Spencer[1], and Alvaro Acosta-Serrano[2]

[1] *Ludger Ltd, Culham Science Centre, Oxfordshire, UK;* [2] *Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK*

Introduction

African trypanosomiasis or sleeping sickness occurs in sub-Saharan African countries and is transmitted through the saliva of the haematophagus insect vector (tsetse fly) during feeding. The causative agents of this disease are trypanosome parasites of the species *Trypanosoma brucei*. Although sustained efforts to curb infection have resulted in a decrease of cases from 9,878 in 2009 to 7,216 in 2012[1], efforts to identify new drug targets to treat or prevent infection continue. Salivary glycoproteins have been reported to facilitate host infection through binding and transport of vector-borne diseases to host tissues, and also participate in host responses such as inflammation and immune response[2]. This role in infection presents new opportunities to identify the key mediators of transmission as well as to increase our understanding of the role of salivary glycans in haematophagus insects. The analysis of fly salivary glycoproteins however is challenging due to small sample volumes despite collection from several hundred flies, together with the need for high sensitivity.

Aims

1. To determine a suitable glycomics workflow coupling HPLC with mass spectrometry to combat the problem of small sample amounts in tsetse fly saliva. Specifically, to compare and evaluate the use of two different labels (2-AB and procainamide) for released *N*-glycans from a standard glycoprotein (IgG) to ensure highly sensitive and reliable mass spectrometric and fluorescence detection
2. To investigate the role of *N*-glycosylation in vector based trypanosome infection by characterising the *N*-glycome of *T. brucei*-infected and naïve tsetse salivary glycoproteins

Methods

TSETSE FLY SALIVA: Naïve *vs.* *T. brucei*-infected samples

Tsetse fly[3]

+/-

Trypanosoma brucei[4]

- Naïve (~5µg/µl) and *T. brucei*-infected (~1µg/µl) tsetse fly saliva collection
- In-gel release of *N*-glycans with PNGase F *[E-PNG01 from Ludger]*

Labelled with:

- 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:

Bovine Fetuin standard glycoprotein:
2-AB vs. Procainamide labelling of released *N*-glycans

- 50 µg fetuin was reduced and alkylated with DTT and IAA respectively
- *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]*

Analysed using:

- 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]*

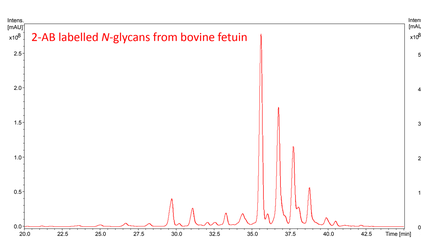
Analysed using:

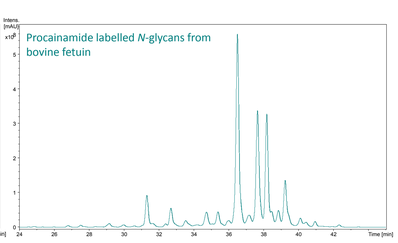
- HILIC-UHPLC-FLR coupled online with positive ion mode ESI-MS/MS and HILIC-SPE ESI-MS using a Bruker amaZon speed ion trap
- Manual data analysis

Results

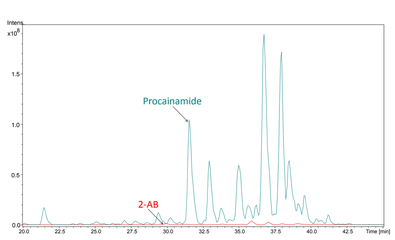
1. Comparison of 2-AB and procainamide labelled bovine fetuin released *N*-glycans:

HILIC-UHPLC-FLR:



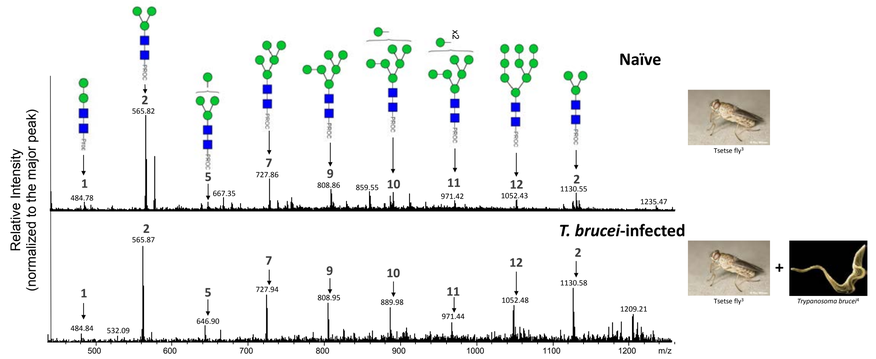


HILIC-UHPLC-ESI-MS:



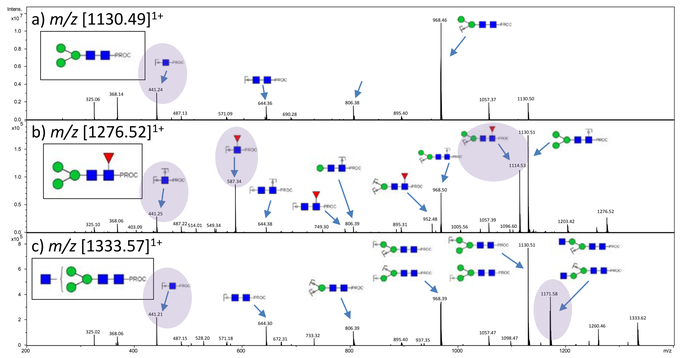
Procainamide and 2-AB showed good comparability however procainamide labelling displayed 2x higher fluorescence and 40x higher MS signal intensity compared to 2-AB labelled *N*-glycans.

2. Comparison of procainamide labelled *N*-glycans from naïve and *T. brucei*-infected tsetse fly saliva glycoproteins by HILIC-SPE ESI-MS/MS



Average MS *N*-glycan profiles
Structure numbers correspond to table below

- **Pauci mannose and high mannose-type structures showed high expression the *N*-glycomes of both naïve and *T. brucei*-infected tsetse fly saliva glycoproteins with (Man)3(GlcNAc)2-Proc the dominant structure in both**
- **Low abundance hybrid-type glycans were also detected in both samples (not visible in average MS profile)**



MS/MS fragmentation contains diagnostic ions that aid in *N*-glycan structural characterisation

3. Total structures identified in tsetse saliva glycoproteins by MS/MS fragmentation

HPLC Peak Id	Glucose Units	Tsetse Fly Saliva *N*-glycans Identified by HILIC-UHLPC ESI-MS/MS: Composition Hex	HexNAc	Fuc	Theoretical $[m/z]^{1+}$	Theoretical $[m/z]^{2+}$	Detected $[m/z]^{1+}$	Detected $[m/z]^{2+}$	Proposed Structure
1	3.21	2	2	0	968.46	484.73	968.47	n.d.	
2	4.17	3	2	0	1130.51	565.76	1130.49	565.74	
3	4.62	3	2	1	1276.57	638.79	1276.52	638.77	
4	4.76	3	3	0	1333.59	667.30	1333.57	667.29	
5	5.00	4	2	0	1292.56	646.78	1292.54	646.74	
6	5.54	4	3	0	1495.64	748.32	1495.65	748.30	
7	6.00	5	2	0	1454.61	727.81	1454.57	727.79	
8	6.46	5	3	0	1657.69	829.35	1657.64	829.33	

HPLC Peak Id	Glucose Units	Tsetse Fly Saliva *N*-glycans Identified by HILIC-UHLPC ESI-MS/MS (cont.): Composition Hex	HexNAc	Fuc	Theoretical $[m/z]^{1+}$	Theoretical $[m/z]^{2+}$	Detected $[m/z]^{1+}$	Detected $[m/z]^{2+}$	Proposed Structure
9	6.87	6	2	0	1616.67	808.84	1616.61	808.81	
10	7.79	7	2	0	1778.72	889.86	1778.68	889.84	
11	8.53	8	2	0	1940.77	970.89	n.d.	970.87	x2
12	8.66	8	2	0	1940.77	970.89	n.d.	970.87	x2
13	9.35	9	2	0	2102.83	1051.92	n.d.	1051.90	

n.d. = not detected

Conclusions

- Here we present a procainamide labelling system suitable for HPLC-MS based glycomic analysis of insect saliva *N*-glycans small sample amounts showing high sensitivity
- These results suggest that upon colonisation and maturation of trypanosomes in the tsetse salivary glands, there are no detectable changes in the glycosylation of tsetse salivary glycoproteins.
- The presence of high levels of mannosylated structures may influence the half-life in blood of pharmacologically active salivary components.

Acknowledgements

This work was partially supported by funding from the Wellcome Trust and GlycoPar EU FP7 Marie Curie Initial Training Network

References

1. http://www.who.int/mediacentre/factsheets/fs259/en/
2. R. G. Titus, J. V. Bishop, and J. S. Mejia, "The immunomodulatory factors of arthropod saliva and the potential for these factors to serve as vaccine targets to prevent pathogen transmission," Parasite Immunology, vol. 28, no. 4, pp. 131–141, 2006
3. http://www.raywilsonbirdphotography.co.uk/Galleries/Invertebrates/vectors/Tsetse_Fly.html
4. http://www.york.ac.uk/cii/media-library/