Introduction

African trypanosomiasis or sleeping sickness occurs in sub-Saharan African countries and is transmitted through the saliva of the haematophagous 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, 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 response1. 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 glycoproteins in haematophagous 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 choose a glycomics workflow suitable for very small samples of complex biological fluids. Specifically, we aimed to develop a suitable glycomics workflow coupling UHPLC with ESI mass spectrometry that would work well with very small samples of tsetse fly saliva. To this end we evaluated two candidate glycan labelling systems - 2-aminobenzamide (2-AB) and procainamide - using N-glycans enzymatically released from two standard glycoproteins, IgG and bovine fetuin. Both labelling systems employed reductive amination with 2-aminobenzamide as the reductant. Our focus was on finding a method that worked in a highly sensitive and reliable way on with both mass spectrometric and fluorescence detection and that was suitable for future high throughput glycomics studies.

2. To apply the chosen glycomics workflow to infected and non-infected tsetse fly saliva samples. The emphasis was to investigate the role of N-glyclosylation in vector trypanosomal infection by characterising the N-glycome of T. brucei-infected and naïve tsetse fly salivary glycoproteins.

Methods

1. Comparison of glycomics workflows for 2-AB and procainamide labelled N-glycans

- **Results**

• 2-AB labelled glycans gave poor ESI-MS profiles with the amounts of samples tested
• Procainamide labelled glycans gave very good ESI-MS profiles with the amounts of samples tested
• Procainamide labelled glycans and 2-AB labelled glycans showed comparable separation on the HILIC UHPLC
• Low abundance hybrid-type glycans were also detected in both samples (not visible in average MS profile) - they look the same

- **Conclusion**

• The procainamide system suitable for detailed 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 saliva glycoproteins.
• The presence of high levels of mannosylated structures may influence the half-life in blood of pharmacologically active salivary components.
• To our knowledge this is the first study to characterise saliva glycosylation from any insect vector. We are planning further studies on the glycosidase of parasitic infections using the procainamide-UHPLC-ESI-MS system. These will include investigation into changes in the glycosylation patterns of tissues from the human host, insect vector and the parasites throughout the disease process.

References

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Contact for more information

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