Glycan structural determinants and their role in microbial interaction

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A thesis submitted for the degree of

Doctor of Philosophy

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Macquarie University, Sydney, Australia

April 2013

Dedicated to the

memory of my wonderful mother

Remegiscal Everest

Declaration

I hereby certify that the work presented in this thesis titled, "Glycan structural determinants and their role in microbial interaction" is the result of my own work except where acknowledged and is not being submitted for higher degree to any other university or institution. I consent to a copy of this thesis being available in the University library for consultation, loan and photocopying forthwith.

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April 2013

Abstract

Microbial infection is initiated only after adherence to the host cell surface. In many cases the microbial interactions with the host surface are mediated between the glycans on the host cell surface and the carbohydrate binding proteins of the pathogen. Mucosal epithelial surfaces, such as coat the oral cavity, are potential sites for colonisation by oral microorganisms. Saliva constantly bathes the buccal epithelial cell (BEC) surface of the mouth and in this study we have used the oral cavity as a model system to demonstrate the innate immune protective role played by the glycan receptors on the proteins of saliva against the adhesion of the oral pathogen *C. albicans* to the BEC. Thereby, this work could help design glycan inhibitors similar to the host's evolved innate immune system to naturally evade pathogens and thus address the problem of increased microbial resistance to antibiotics.

In the first phase of this work (Chapter 2, Publication 1), a flow cytometry based adhesion assay was developed to quantify the interaction between buccal cells and the commensal oral pathogen *Candida albicans*. The structures of the *N*- and *O*- linked oligosaccharides on the glycoproteins of saliva and BEC membranes were analysed using capillary carbon negative ion LC-ESI MS/MS. A total of 190 glycan structures were characterised and found to be qualitatively similar between saliva and epithelial buccal cell membrane proteins, but differed quantitatively in their relative amounts. The similarity of the terminal glycan epitope structures on saliva and BEC membrane glycoproteins, and the fact that whole saliva and released glycans from salivary proteins inhibited the interaction of *C. albicans* with BEC, confirmed the protective role of the glycans on salivary glycoproteins against pathogen infection of the oral surface mucosa.

Further investigation of the glycan determinants identified on the terminal *N*- and *O*-glycan structures of BEC and saliva supported the proposed function of blood group antigens as an evolutionary selection against pathogen infection. The detailed mass spectrometric glycan characterisation and relative quantification of BEC membrane glycans (Chapter 3) was carried out on 19 individuals of various A, B, AB and O blood group types. The *N*-glycans of BEC were similar in all secretor individuals and did not display the A and B determinants; while non-secretors did not possess the O/H antigens. In contrast, the *O*-glycans on the membrane proteins of BEC from secretory individuals expressed the A, B and H antigens, while the non-secretors lacked any of these structures. The Lewis

x/a and Lewis y/b blood group antigens were observed on secretor individuals *N*- and *O*-glycans; in non-secretor individuals, as expected, only Lewis x/a antigens were present. Multivariate statistical analysis showed that *C. albicans* demonstrated a significantly ($p < 0.05$) higher preference to adhere to BEC of blood group O individuals.

The diagnostic and signature fragment ions produced by negative ion MS/MS fragmentation, together with the elution selectivity of PGC retention, were identified and applied to differentiate the *N*- and *O*-glycan isomer structures of the complex salivary glycans (Chapter 4, Publication 2). This labour-intensive approach led to the construction of a PGC-LC-ESI-IT-MS^2 tandem mass spectral repository on the online UniCarb-DB database which was further expanded by an online MS^2 fragment spectral library of 30 common glycan substructures that typically occur at the non-reducing terminus of glycoconjugates, fragmented in the positive and negative ion mode (Chapter 5, Publication 3). The substructure spectra were used to identify and confirm terminal glycan determinants from the multistage (MS^3) mass spectra of the salivary *N*- and *O*-glycans. These mass spectrometric insights will enable the easier identification and confirmation of glycan determinants on oligosaccharides released from glycoproteins in future analyses.

The work presented here applies negative ion PGC-LC-ESI-MS/MS analysis for the detailed characterisation of the *N*- and *O*-glycans on epithelial cell surface and secreted fluid proteins and demonstrates the role played by terminal glycan structural determinants as receptors for pathogen binding.

Acknowledgements

This thesis would not have been possible without the support and influence of family, friends and colleagues who have made this journey a memorable and fulfilling task.

It is with immense gratitude that I acknowledge my advisor Professor Nicki Packer for the continuous support of my PhD study and research, for her patience, enthusiasm, and immense knowledge. Thank you Nicki for your kind words and wisdom that always inspired me and encouraged me to draw views in a different perspective. I would like to thank you for your scientific advice and insightful discussions that have frequently led to key insights. I hope that I can someday pass on the research values and mentorship that you have imparted on me.

I would like to thank Professor Helena Nevalainen for her constructive criticism, advice and encouragement that have been instrumental to my progress as a researcher. I have been privileged to get to know and collaborate with many amazing people who became friends over the last several years. It has been an incredible learning experience working with Dr. Daniel Kolarich, you made glycan mass spectra look like a jigsaw puzzle, and solving glycan structures an art. I also want to thank you for your brilliant comments and suggestions. Dr. Matthew Campbell was highly influential in helping me crank out this thesis, thank you for your advice on scientific writing, proof reading and corrections of my written work, that were paramount to this thesis. I would also like to thank other postdoctoral fellows from Macquarie Liisa Kautto, Dayong Jin, Miyako Nakano, Jasmine Grinyer, Pia Jensen, Terry Nguyen, Dana Pascovici, Morten Andersen and Maja Christiansen for your technical assistance and valuable discussions.

My time at Macquarie was made enjoyable in large part due to the many friends and groups that became a part of my life. Thanks to all my fellow PhD students and friends at Biomarker and Edge Lab including Liisa, Siba, Albert, Nandan, Rajeev, Katherine & Kathrin,

Jodie, Sri, Vignesh, Merrina, Zeynep, Sarah, Clara, Jenny, Kunal, Suja, Mehdi, Naveid, Karlie, Shingo, Kate, Angela, Robyn and Yukie. I don't think this journey would have been fun without your presence. Furthermore, I also want to thank all other friends who put up with me through the whole PhD process and for their understanding, in particular Prasanna, Dileep, Prabhu, Molita, Joel and Rishi.

I gratefully acknowledge the funding sources that made my PhD work possible. I would like to thank Macquarie University for providing me with the International Macquarie University Research Scholarship (iMURS) for pursuing my research. I was also supported by the Cystic Fibrosis Federation Australia PhD top-up scholarship.

Words are inadequate to express my appreciation to my family. My parents raised me with a love of science and supported me in all my pursuits. Thank you for the tremendous sacrifices you both have made throughout my life and providing me with a care free environment so that I can concentrate on my studies. To my grandad who inspired me to dream and pursue, I would have loved to have you around to explain my fascination with sugars. Sandra and Danie, thanks to you both for taking care of mum and dad and my responsibilities back home. Your constant love, support and encouragement has made a difference to my life. A heartfelt thanks goes out to my girlfriend Richa for her love, devotion and understanding even during hard times. Your faithful support and encouragement during the final stages of this PhD is so highly appreciated. Thank you.

Publications

1. **Everest-Dass, A. V.**, Jin, D., Thaysen-Andersen, M., Nevalainen, H., Kolarich, D. & Packer, N. H. Comparative structural analysis of the glycosylation of salivary and buccal cell proteins: innate protection against infection by *C. albicans*. *Glycobiology* **22**, 1465-1479, (2012).
2. **Everest-Dass, A. V.**, Abrahams, J., Kolarich, D., Packer, N. H. & Campbell, M. Structural feature ions for distinguishing *N*- and *O*-linked glycan isomers by LC-ESI-IT MS/MS. *J Am Soc Mass Spectrom,* **24**, 895-906, (2013).
3. **Everest-Dass, A. V.**, Kolarich, D., Campbell, M., & Packer, N. H. Tandem mass spectra of glycan substructures enable the multistage mass spectrometric identification of determinants on oligosaccharides. *Rapid Commun. Mass Spectrom.* **27**, 931–939, 2013.

I, Arun Vijay Everest Dass was the lead author of these papers presented in this thesis as Chapter's 2, 4 and 5 respectively. All experimentals, sample processing, protocols and manuscript preparation were performed by myself. My supervisors, Prof. Packer and Prof. Nevalainen supervised, provided access, advice and edited the manuscripts. Dr. Kolarich gave technical support, conceptual advice and reviewed the manuscripts. In publication 1, Dr. Jin assisted with the flow cytometry setup and Dr. Thaysen-Andersen critically reviewed the manuscript. In publication 2, Dr. Campbell advised and edited the manuscript. Ms Abrahams assisted with the experimental setup of the exoglycosidase digestion and wrote the exoglycosidase method section. In publication 3, Dr. Campbell critically reviewed the manuscript.

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