University of Victoria | Graduate Studies

Notice of the Final Oral Examination
for the Degree of Doctor of Philosophy

of

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BSc (University of Victoria, 2014)

"Advancing mechanistic understanding of glycosyltransferases"

Department of Biochemistry and Microbiology

Tuesday, April 9, 2019
9:00 A.M.
Clearihue Building
Room B007

Supervisory Committee:
Dr. Stephen Evans, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Alisdair Boraston, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Monica Palcic, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Rodney Herring, Department of Electrical and Computer Engineering, UVic (Outside Member)

External Examiner:
Dr. Kenneth Ng, Department of Biochemistry, University of Calgary

Chair of Oral Examination:
Dr. Justin Albert, Department of Physics and Astronomy, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Glycosyltransferase enzymes synthesize glycosidic linkages, generating carbohydrates and carbohydrate-linked entities ranging from cellulose, starch, and chitin to glycolipids, glycopeptides, and natural product antibiotics. These syntheses involve stereo- and regio-specific sugar transfer from an activated donor molecule, often a UDP-sugar, to an acceptor molecule. Functionally, glycosyltransferases are classified as either “retaining” or “inverting” enzymes depending on whether the stereochemical linkage of the donor substrate is conserved in the product. While inverting glycosyltransfer is mechanistically straightforward, the retaining mechanism remains poorly understood. For retaining glycosyltransferases, the central question is whether transfer occurs via a front-face “S_Ni-like” mechanism or through a ‘double displacement’ mechanism that invokes a glycosyl-enzyme covalent intermediate.

GTA and GTB are retaining enzymes that catalyze the final step in human ABO(H) blood group A and B antigen synthesis through UDP-GalNAc or UDP-Gal transfer, respectively, to the Hantigen disaccharide acceptor. Although they have been intensively characterized, the processes of substrate recognition, mobile loop organization, and product release in GTA and GTB has long resisted explanation. Further, the question of the retaining enzyme mechanism persists, though the covalent intermediate of the proposed double displacement mechanism has been detected via mass spectrometry experiments with GTA/GTB mutants.

Building on previous investigations, we have aimed to characterize and have uncovered details of mechanism, substrate binding, loop organization, and product release using a combined kinetic and structural approach. These investigations are essential not only for understanding GTA, GTB, and retaining glycosyltransferases as a whole, but also for the rational design of inhibitors. Such inhibitors could selectively target, for example, bacterial glycosyltransferases and thus would represent a new class of antimicrobials.