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B. Pharm (Pharmacy), University of Dhaka, 2006
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DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

TITLE OF THESIS:	GLYCOSPHINGOLIPID METABOLISM AND THE GLYCOSPHINGOLIPID-METABOLIZING ENZYME BETA-GLUCOSIDASE-2: BIOCHEMICAL AND CELL BIOLOGICAL STUDIES
TIME/DATE:	1:30 pm, Monday, December 2, 2019
PLACE:	Room 3107, The Mona Campbell Building, 1459 LeMarchant Street

EXAMINING COMMITTEE:

Dr. Timothy E. Shutt, Department of Medical Genetics, University of Calgary (External Examiner)

Dr. Barbara Karten, Department of Biochemistry and Molecular Biology, Dalhousie University (Reader)

Dr. Stefan Krueger, Department of Physiology and Biophysics, Dalhousie University (Reader)

Dr. Petra Kienesberger, Department of Biochemistry and Molecular Biology, Dalhousie University (Reader)

Dr. Aarnoud C. van der Spoel, Department of Pediatrics/Biochemistry and Molecular Biology, Dalhousie University (Supervisor)

DEPARTMENTAL REPRESENTATIVE:	Dr. Jan Rainey, Department of Biochemistry and Molecular Biology, Dalhousie University
CHAIR:	Dr. Richard Nowakowski, PhD Defence Panel, Faculty of Graduate Studies

ABSTRACT

Glycosphingolipids (GSLs) are constituents of eukaryotic cell membranes and consist of a lipid (ceramide) linked to one or more sugar residues. GSLs occur in many structurally distinct forms, varying in both their lipid and oligosaccharide domains. Most cells contain multiple structurally different GSLs. Different cell types and animal tissues have distinct and consistent GSL complements, indicating that cellular GSL homeostasis is strictly regulated. Deviations in GSL homeostasis have pathological consequences. Our understanding of the regulation of GSL levels is very limited. In the first part of my project I attempted to assess the kinetics of GSL turnover by testing a mathematical model correlating the rates of GSL biosynthesis and degradation, using genetic and pharmacological approaches to target GSL-metabolizing enzymes. This project, however, produced inconsistent results.

In the second part of my project I studied the involvement of a GSL-metabolizing enzyme in a neurodegenerative disorder. The gene encoding the enzyme β-glucosidase 2 (GBA2) is mutated in patients affected with a form of hereditary spastic paraplegia, SPG46 (SPastic Gait locus #46). These patients present with a combination of spastic paraplegia and cerebellar ataxia and suffer from muscle weakness, and spasticity in the upper and lower limbs along with other neurological symptoms. Currently, the cascade of events leading from mutations in the *GBA2* gene to SPG46 is largely unexplored. I characterized five nonsense and five missense GBA2 mutants and found that all ten mutants are catalytically inactive. The lack of enzyme activity was not associated with decreased protein expression. Native gel electrophoresis showed that, relative to wild-type GBA2, all mutants fold into aberrant conformations, with the exception of the clinically mild Gly683Arg mutant. In addition, C-terminally truncated GBA2 mutants migrate to mitochondria, cause mitochondrial depolarization, and interfere with mitochondrial fusion, resulting in fragmentation of the mitochondrial network. I also identified an internal domain responsible for the mitochondrial import of the truncated GBA2 mutants. Altogether my research provides new insights into the biochemical and cell biological bases of the neurodegenerative disorder SPG46.