Notice of the Final Oral Examination
for the Degree of Master of Science

of

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BSc (University of Victoria, 2012)

“Purification and Uptake Studies of Recombinant Human N-α-D-
Acetylglucosaminidase from Sf9 Insect Cells”

Department of Biology

Monday, August 24, 2015
10:00 A.M.
Hickman Building
Room 110

Supervisory Committee:
Dr. Francis Choy, Department of Biology, University of Victoria (Supervisor)
Dr. Raad Nashmi, Department of Biology, UVic (Member)
Dr. Robert Chow, Department of Biology, UVic (Member)

External Examiner:
Dr. Juan Ausio, Department of Biochemistry, UVic

Chair of Oral Examination:
Dr. Peter Wan, Department of Chemistry, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies
Abstract

Human α-N-acetylglucosaminidase (Naglu) is a lysosomal enzyme implicated in the rare metabolic storage disorder Mucopolysaccharidosis III type B (MPS IIIB). A deficiency in Naglu results in a buildup of heparan sulfate in lysosomes, which is most detrimental in the central nervous system, causing mental retardation and a shortened lifespan. Enzyme replacement therapy is currently ineffective in treating the neurological symptoms of MPS IIIB due to the inability of Naglu to cross the blood-brain barrier. This laboratory uses a Spodoptera frugiperda (Sf9) insect cell system to express recombinant Naglu conjugated to a synthetic protein transduction domain with the intent to allow Naglu to cross the BBB and treat the neurological symptoms.

In the present study, we aimed to purify a recombinant Naglu-PTD4 fusion protein in order to assess its capacity to cross cellular membranes. A three-step method involving multi-modal, hydrophobic interaction, and gel filtration chromatography was optimized to achieve pure Naglu-PTD4, in good yield. Cellular uptake by human MPSIIIB fibroblasts of Naglu-PTD4 was not detectable. It is hypothesized that additional amino acids, including a hexahistidine domain, following the PTD4 domain limited the fusion protein’s membrane transduction capacity. Future studies will focus on removing the additional amino acids and adjusting the purification method as necessary. The ultimate goal of this research is to develop a large-scale recombinant Naglu production protocol for enzyme replacement therapy of MPS IIIB.