GRADUATE AND POSTDOCTORAL STUDIES

McGILL UNIVERSITY

FINAL ORAL EXAMINATION
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

OF

NANA AKYAA ACKAAH-GYASI

DEPARTMENT FOOD SCIENCE AND AGRICULTURAL CHEMISTRY

NOVEL CHYMOTRYPSINS FROM *LOLIGO OPALESCENS* AND *SEPIOTEUTHIS LESSONIANA*: ISOLATION, PURIFICATION AND MOLECULAR CHARACTERIZATION

DATE: Friday, February 26, 2016
TIME: 1:15 p.m.
RAYMOND BUILDING, Room R2-013
McGill University, Macdonald Campus

COMMITTEE:
Dr. K. Wade (Pro-Dean) (Animal Science)
Dr. V. Yaylayan (Chair) (Food Science and Agricultural chemistry)
Dr. B. K. Simpson (Supervisor) (Food Science and Agricultural Chemistry)
Replaced by S. Kermasha (Member) (Food Science and Agricultural Chemistry)
Dr. T. Geary (Co-Supervisor) (Institute of Parasitology)
Dr. S. Karboune (Internal Examiner) (Food Science and Agricultural Chemistry)
Dr. M.J. Dumont (External Member) (Bioresource Engineering Dept.)

Dr. Josephine Nalbantoglu, Dean of Graduate and Postdoctoral Studies
Members of the Faculty and Graduate Students
are invited to attend

ABSTRACT

Chymotrypsins are widely distributed among living species and have found widespread use in different industrial applications. However, until the last two decades, most studies on chymotrypsin have been restricted to mammalian species with few reported works on marine invertebrates. The high catalytic activity of some aquatic enzymes at low temperatures, coupled with high pH and the relatively low thermal stability makes them robust in certain industrial applications where cold temperatures are preferred. In this study, chymotrypsin was purified to homogeneity and characterized from the viscera of two squid species (*Loligo opalescens*, cold water adapted and *Sepioteuthis lessoniana,* warm water adapted). Cold adapted chymotrypsin can transform substrates at low temperature thereby reducing loss of organoleptic properties and nutritional value of heat-sensitive substrates and products mostly found in industrial processing of food such as fish. They also provide economic benefit through energy savings during processing. This study also looks at the possibility of producing the enzymes using recombinant DNA technology, since it is currently not feasible or practical to extract these proteases from crude sources such as viscera for commercial application. The enzymes were purified about 300-fold, with a recovery of 44% and specific activities between 273.25 and 280 U/mg protein using ultrafiltration and affinity chromatography. Both enzymes migrated as single polypeptide chains with molecular masses of 22.0 ± 2.7 kDa and 18 ± 1.7 kDa, respectively, by SDS-polyacrylamide gel electrophoresis. The enzymes showed temperature and pH optima between 25°C - 35°C and 7.5 – 8.5, respectively. The kinetic constants K_m and k_{cat} for hydrolysis of benzoyl tyrosine ethyl ester (BTEE) were determined based on Hanes plots. K_m was 1.43 mM and k_{cat} was 103.43 sec^{-1} with a catalytic efficiency (k_{cat}/K_m) of 72.33 sec^{-1} mM^{-1} for *Sepioteuthis*; while for *Loligo*, K_m was 0.4 mM and k_{cat} was 349.21 sec^{-1} with a catalytic efficiency (k_{cat}/K_m) of 873.01 sec^{-1} mM^{-1}. *De novo* assembly from massively parallel sequencing data were generated from total RNA from *L. opalescens* (due to its biochemical characteristics) on an Illumina Genome Analyzer platform. The transcriptome was assembled on normalized reads using the Trinity assembler. Each component longest transcript was aligned against the uniprot_sprot_2013_11 protein database using the Blastx program from the NCBI BLAST family. Overall, 61661 transcripts were obtained with a total transcript length of 46232292 bp. Maximum transcript length obtained was 16932 bp, while the minimum length was 201 bp. A partial cDNA encoding *L. opalescens* chymotrypsin was identified from the *de novo* assembled transcripts using BLAST algorithms in NCBI. A full- length cDNA was obtained using nested PCR. The deduced sequence consists of 293 amino acid residues, being

longer than its vertebrate analogs. A search of the non-redundant protein database showed highest identity to a hypothetical protein from *Octopus bimaculoides* (69%), chymotrypsinogen A-like protein from *Lingular anatina* (45%) and a serine protease from *Aplysia californica* (45%). The catalytic triad involving histidine, aspartate and serine was conserved in *L. opalescens* chymotrypsin. The primary structure also contained higher content of methionine, arginine, histidine and glutamic acid relative to chymotrypsin from mammalian homologues. A substitution was observed in the *Loligo* chymotrypsin sequence corresponding to position 124 in bovine chymotrypsin sequence from proline to alanine. A maximum likelihood phylogenetic tree comparing chymotrypsin from *L. opalescens* with other vertebrate and invertebrate chymotrypsin sequences suggests the existence of two main groups representing chymotrypsin from vertebrates and invertebrates and shows that *L. opalescens* chymotrypsin shares a common ancestor with most insect chymotrypsins. A homology model of *L. opalescens* chymotrypsin was built using the crystal structure of bovine chymotrypsin (PDB ID: 1t8o) as a scaffold. The model was assessed for stereochemical quality and side chain environment. Sequence alignment shows that the target and the template (1t8o) share 36% sequence identity. The model had the characteristic 2 β-barrel domains typical of chymotrypsin. The Ramachandran plot using 258 residues gave a total stereochemistry of 88.5% with 8.3% in the additional allowed region. The percentage of residues found in the disallowed region was 0.9%. Based on crystallographic data, *Loligo* chymotrypsin and bovine chymotrypsin showed almost superimposable tertiary structures.

CURRICULUM VITAE

AWARDS

GRADUATE EXCELLENCE FELLOWSHIP

PUBLICATIONS

Nana Akyaa Ackaah-Gyasi, Timothy Geary and Benjamin Simpson (2015). Novel chymotrypsin-like enzymes from squid (*Sepioteuthis lessoniana* and *Loligo opalescens*) viscera; Purification, biochemical characterization and peptide identification using LC-MS/MS. Submitted to Journal of Food and Agricultural Chemistry

Nana Akyaa Ackaah-Gyasi, Timothy Geary and Benjamin Simpson (2015). Peptide sequencing and protein identification of a chymotrypsin-like enzyme from squid (*Loligo opalescens*). Presented at Biotechnology Industry Organization conference, July 22, 2015, Montreal, Canada

Nana Akyaa Ackaah-Gyasi, Priyanki Patel, Yi Zhang and Benjamin K. Simpson. 2014. Enzymes Current and Future Applications. In: Improving and tailoring enzymes for food quality and functionality. (Ed. R. Yada) pp.103-122.

Nana Akyaa Ackaah-Gyasi, Priyanki Patel, Julie Ducharme, Hui Yin Fan and Benjamin K. Simpson. 2014. Enzymes and Inhibitors in Food and Health. In: Functional Polymers in Food Science: From Technology to Biology. (Francesca Iemma, U.G. Spizzirri and G. Cirillo, Eds). Scrivener Publishing LLC in cooperation with John Wiley and Sons Ltd. pp. 289-328.

Nana Akyaa Ackaah-Gyasi, Yi Zhang and Benjamin K. Simpson. 2014. Enzymes Inhibitors: Food and Non-Food Impacts. In: Advances in Food Biotechnology. (Ravishankar Rai, Ed). John Wiley and Blackwell Publishers. In press.

Nana Akyaa Ackaah-Gyasi, Timothy Geary and Benjamin Simpson (2014). Purification and biochemical characterization of chymotrypsin from squid viscera. Presented at the 17th World Congress of Food Science and Technology, August 17 – 20, 2014. Montreal, Canada.