การพิสูจน์เอกลักษณ์โปรตีนจากพิษงูเขียวหางไหม้ตาโต *Trimeresurus macrops*

นางสาวนฤมล สวัสดิพฤกษา

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PROTEIN IDENTIFICATION OF *Trimeresurus macrops* VENOM

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โปรตีนซึ่งเป็นองค์ประกอบในพิษงูเขียวหางไหม้ตาโต *(Trimeresurus macrops)* แยกโดยอาศัยเทคนิคทางเจลอิเลกโทรโฟเรซีส 2 เทคนิค ได้แก่ SDS-PAGE และ เจลอิเลกโทรโฟเรซีสใน 2 มิติ (2-D) และเทคนิคเจลฟิลเทรชัน โครมาโทกราฟี จากการวิเคราะห์ด้วยเทคนิคทางเจลอิเลกโทรโฟเรซีสทั้ง 2 เทคนิคทำให้ทราบถึงรูปแบบของโปรตีนทั้งหมดที่เป็นองค์ประกอบในพิษงูชนิดนี้ และจากการแยกโปรตีนด้วยเทคนิคเจลฟิลเทรชันโครมาโทกราฟีได้โปรตีนบริสุทธิ์ 3 ชนิด โดยอาศัยเครื่องแมสสเปกโทรมิเตอร์แบบ Matrix assisted laser desorption ionization/time of flight (MALDI/Tof) สามารถวิเคราะห์หามวลโมเลกุลได้เท่ากับ 13731.02 ดาลตัน, 13254.68 ดาลตัน และ 13340.09 ดาลตัน ลำดับกรดอะมิโนจากปลายด้าน N-terminal วิเคราะห์ด้วยเทคนิค Edman degradation ของโปรตีนในส่วนแยก X จากเจลฟิลเทรชันที่มีมวลโมเลกุลเท่ากับ 13340.09 ดาลตัน คือ HVLQLGLYIL ศึกษาลำดับกรดอะมิโนบางส่วนของโปรตีนในส่วนแยก VI ด้วยเทคนิคแทนเดมแมสสเปกโทรเมทรีแบบ Electrospray ionization quadrupole/time of flight จากสเปคตรัมการแตกตัวของ precursor ion ที่ *m/z* 659.3 และ 844.3 สามารถแปรผลได้เป็นลำดับกรดอะมิโนของเปปไทด์สองเปปไทด์คือ WAVQCSQQPNR และ EAVGEDPWYNHQ(I/L)K นอกจากนั้นโปรตีนในส่วนแยก II และ III แสดง proteolytic activity ให้ค่า specific activity เป็น 0.18 และ 0.15 unit/ml ตามลำดับ และพบ phospholipase A activity ในโปรตีนในส่วนแยก VI

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NARUMON SAWASDIPUKSA : PROTEIN IDENTIFICATION OF *Trimeresurus macrops* VENOM

THESIS ADVISOR : ASSOCIATE PROFESSOR AMORN PETSOM, Ph.D.

THESIS CO-ADVISOR : ASSISTANT PROFESSOR POLKIT SANGVANICH, Ph.D., 65 pp. ISBN 974-17-4589-3

The protein components from the venom of *Trimeresurus macrops* have been separated by sodium-dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE), two-dimensional electrophoresis (2-D electrophoresis), and gel filtration chromatography (GF). The protein profiles SDS-PAGE and 2-D electrophoresis of *Trimeresurus macrops* venom were obtained. The three molecular masses proteins, which were purified from GF, were determined using matrix assisted laser desorption ionization/time of flight (MALDI/Tof) mass spectrometer; 13732.02 *m/z*, 13255.68 *m/z*, and 13341.09 *m/z*, $[M+H]^+$. The ten amino acids from N-terminal of fraction X protein (13340.09 Da), which was analyzed using Edman degradation, is HVLQLGLYIL. The partial sequence of fraction VI protein was determined using electrospray ionization quadrupole/time of flight (ESI-Q/Tof) tandem mass spectrometer. The two product ion spectra of precursor at *m/z* of 659.3 and 844.3 could be manually interpreted. The first peptide sequence is WAVQCSQQPNR. The second peptide sequence is EAVGEDPWYNHQ(I/L)K. The protein fraction of II and III showed proteolytic activity with specific activity 0.18 and 0.15 unit/ml, respectively. Moreover, the phospholipase A activity was found in the fraction VI protein.

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Field of study....Organic Chemistry... **Advisor's signature.....**

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LIST OF ABBREVIATIONS

1-D electrophoresis	One-dimensional electrophoresis
2-D electrophoresis	Two-dimensional electrophoresis
µg	microgram
µl	microliter
ACN	Acetronitrile
APS	Ammonium persulfate
Bis	*N,N'*-methylenebisacrylamide
°C	degree Celsius
C	Crosslinking factor [%]
CAD	Collision activated dissociation
CCA	α-Cyano-4-hydroxycinnamic acid
CHAPS	3-(3-cholamidopropyl)dimethylammonio-1-propane sulfonate
CID	Collision induced dissociation
Da	Dalton
DC	Direct current
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionization
ESI-Q/Tof	Electrospray ionization Quadrupole time-of-flight
fmol	femtomole
g	gram
GF	Gel filtration chromatography
IEF	Isoelectric focusing
IPG	Immobilized pH gradients
kDa	Kilodalton
kVh	kilovolt-hour
LMW	Low molecular weight
nM	Nanomolar
mA	Milliampere
MALDI	Matrix Assisted Laser Desorption Ionization

MALDI/Tof	Matrix Assisted Laser Desorption Ionization/Time of flight
mg	Milligram
mg/ml	Milligram per milliliter
ml	Milliliter
min	Minute
mm	Millimeter
mM	Millimolar
MS	Mass spectrometry
MS/MS	Tandem Mass spectrometry
m/z	Mass per charge
PAGE	polyacrylamide-gel electrophoresis
pI	Isoelectric point
PMM	Peptide mass mapping
pmol	picomole
ppm	parts per million
PSD	Post source decay
Q/Tof	Quadrupole Time of flight
RF	Radiofrequency
rpm	Revolutions per minute
SDS	Sodium-dodecyl sulfate
SDS-PAGE	Sodium-dodecyl sulfate polyacrylamide-gel electrophoresis
T	Total acrylamide concertration [%]
TCA	Trichloroacetic acid
TEMED	*N,N,,N',N'*-tetramethylethylenediamine
TFA	Trifluoroacetic acid
Tof	Time of flight
Tris	Tris(hydroxymethyl)-aminoethane
UV	Ultraviolet spectroscopy
V	Volt
W	Watt