

ไฟบริโนไลติกโปรตีนจากพิษของงูกะปะ *Calloselasma rhodostoma*

นาย ไพบูลย์ พรหมณี

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FIBRINOLYTIC PROTEIN FROM THE VENOM OF *Calloselasma rhodostoma*

Mr. Piboon Pornmanee

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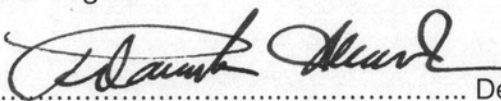
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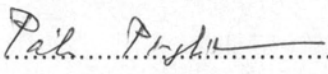
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By Mr. Piboon Pornmanee
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Thesis Advisor Associate professor Amorn Petsom, Ph.D.
Thesis Co-advisor -

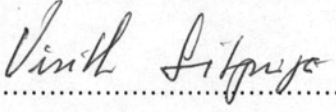
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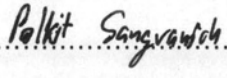

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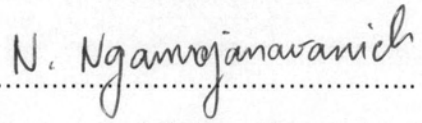
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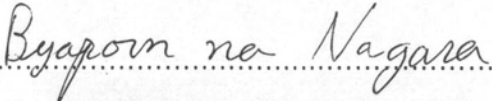

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ไพบลูย์ พรหมณี : ไฟบริโนไลติกโปรตีนจากพิษของงูกระจับ *Calloselasma rhodostoma*.
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งูกระจับ (*Calloselasma rhodostoma*) กัด เป็นปัญหาสาธารณสุขที่สำคัญของประเทศในภูมิภาคเอเชียตะวันออกเฉียงใต้ พิษของงูกระจับประกอบด้วยเอนไซม์ที่มีผลกระทบต่อระบบหมุนเวียนโลหิต โดยเอนไซม์บางชนิดจะมีความสามารถในการละลายลิ่มเลือด (fibrinolytic) สูง ในงานวิจัยครั้งนี้ได้สกัดแยกเอนไซม์ที่แสดงคุณสมบัติละลายลิ่มเลือดจากพิษของงูชนิดนี้ โดยใช้เทคนิคโครมาโทกราฟี ที่ประกอบด้วย 3 ขั้นตอนคือ hydrophobic interaction chromatography (HIC) และ size exclusion chromatography ซึ่งประกอบด้วย protein-pak™ 125 column และ protein-pak™ 60 column นอกจากนี้ยังได้ศึกษาผลทาง Electrophoresis titration curve ของพิษงูชนิดนี้ ด้วยการทดสอบคุณสมบัติทางชีววิทยาของเอนไซม์นี้ ได้ทดสอบคุณสมบัติ fibrinolytic hemorrhagic disintegrin ภายย่อย gelatin และ การย่อย insulin b chain พบว่าเอนไซม์ที่ละลายลิ่มเลือดจากพิษของงูกระจับแสดงคุณสมบัติ ย่อย gelatin และย่อย insulin b chain โดยไม่แสดงคุณสมบัติ hemorrhagic และ disintegrin สำหรับคุณสมบัติการย่อย insulin b chain ถูกยับยั้งด้วยเซรั่มของ Virginia opossum (*Didelphis virginiana*) ในการจัดจำแนกชนิดของเอนไซม์ พบว่าเอนไซม์นี้จัดอยู่ในกลุ่ม α fibrinogenase เพราะมีคุณสมบัติย่อยเฉพาะ α chain ของไฟบริโนเจน จากคน จากการวิเคราะห์ด้วยเทคนิคทาง แมส สเปคโตรเมทรี พบว่าเอนไซม์นี้ประกอบด้วย กรดอะมิโน 236 ตำแหน่ง มีน้ำหนักโมเลกุล 26701.5 ดาลตัน โครงสร้างสามมิติของเอนไซม์แสดงตำแหน่ง active site และ post translation modification คำนวณด้วยโปรแกรม swiss model

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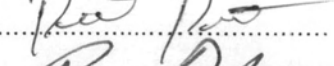
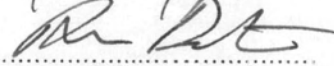
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Malayan pit viper (*Calloselasma rhodostoma*) envenomation is a major health problem in south east Asian countries. During envenomation, the venom component mainly effects the hemostatic system and the venom also exhibits strong fibrinolytic activity. In this research, fibrinolytic enzyme from *C. rhodostoma* venom was purified in three step including hydrophobic interaction chromatography (HIC), size exclusion chromatography with protein-pak™ 125 column and finally with protein-pak™ 60 column. Electrophoresis titration curve of protein was investigated. The biological activities were determined including fibrinolytic and hemorrhagic, disintegrin and gelatinase activities. Purified fibrinolytic enzyme showed gelatinase and insulin b chain digestion without hemorrhagic and disintegrin activities. In additionally, insulin b chain digestion was inhibited by Virginia opossum (*Didelphis virginiana*) serum. This enzyme was characterized to be α fibrinogenase because it digest α chain of human fibrinogen. From Mass spectrometry analysis, This enzyme was composed of 236 amino acid residue with molecular weigh of 26701.5 Da. Three dimensional model revealing active site and post translation modification were calculated using swiss model program.

Field of study.....Biotechnology.....Student's signature.....
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ABBREVIATIONS

μ	:	Microliter
ml	:	Milliliters
h	:	Hours
M	:	Molar
MFD	:	Minimum fibrinolytic dose
MHD	:	Minimum hemorrhagic dose
MGD	:	Minimum gelatinase dose
TPA	:	Tissue plasminogen activator
PAI-1	:	Tissue-type plasminogen inhibitor
MMP	:	Matrix metalloproteinase.
HPLC	:	High performance liquid chromatography
HIC	:	Hydrophobic interactive column
TFA	:	Trifluoroacetic Acid
N.D.	:	Not Determined
ET	:	Electrophoretic Titration