

CHAPTER V

CONCLUSION

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. Fibrinolytic protein from Malayan pit viper are very interesting, because it can be used as drug for thrombosis. In this research, the biological and chemical properties of fibrinolytic enzyme from this snake were reported. The first step, fibrinolytic enzyme from *C. rhodostoma* venom was purified in three steps 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 and used to determine the purity of enzyme. 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. Moreover, opossum serum could counteract toxin from this snake including fibrinolytic, hemorrhagic and gelatinase activity. As the result, 3.5 mg of serum could counteract 1 MFD of venom (13 µg), 0.88 mg of serum could counteract 1 MHD of venom (15 µg) and 0.22 mg of serum could counteract 1 MGD of venom (10 µg). The LD₅₀ of Malayan pit viper venom was 5.15 mg/kg which was lower toxicity than those of North American snake. Enzyme

characterization was determined by human fibrinogen digestion assay. This enzyme was characterized to be α fibrinogenase because it digests α chain of human fibrinogen. From Mass spectrometric analysis, this enzyme was composed of 236 amino acid residue with molecular weight of 26701.5 Da. Three dimensional model revealing active site and post translation modification were studied using swiss model program.