EXECUTIVE SUMMARY

Opisthorchis viverrini infection remains a major public health problem in Southeast Asia. Due to, *O. viverrini* infection in human is often asymptomatic, but this parasite can be pathogenic in its own right and direct risk factor for CCA of the bile duct. The mechanisms by which *O. viverrini* induces CCA in humans are being explored and hypothesized to be a complex processes. The chronic irritation from mechanisms in activity of feeding and migrating of flukes resulting in tissue damage even early infection is the one cause of bile duct epithelium hyperplasia. There are many proteases that involved in many aspect of parasitism such as food digestion, immune invasion, tissue invasion etc. To the function of food digestion, many of proteases encoded endo- and exoproteases are abundantly represented in developmental stage of *O. viverrini*.

Leucine aminopeptidase (LAP), member of the M17 family, Clan MF of metallo proteases was known as exopeptidases that play crucial role in the parasite of protozoa and helminth with broader amidolytic activity beyone leucine hydrolysis. In *O. viverrini*, full-length of leucineaminopeptidase (*Ov*LAP) was identified. Two regions of metal-binding sites, IGKG and NTDAEGR, were examined in *Ov*LAP. Moreover, these regions were conserved throughout LAP from other organisms, including plant, helminth, protozoa, and vertebrate. The deduced amino acid sequence of *Ov*LAP was found to highly identity with closely related liver fluke, *C. sinensis* and shared more than 60% identity with lung fluke, *P. westermani* and blood fluke, *S. mansoni*. *Ov*LAP was predicted as non-secreted protein like other trematode LAP such as *C. sinensis*, *P. westermani*, *F. gigantica* and *S. mansoni* since it's absent of signal peptide. *Ov*LAP can function in a board pH range. The optimal pH for efficient activity of *Ov*LAP was found in slightly alkaline conditions (pH 8.0) similar with *P. westermani*, *F. gigantica* and *S. mansoni*. At this pH, *Ov*LAP can be activated by several inorganic cofactors such as Ca^{2+} and Co^{2+}, in contrast to Mg^{2+} that activates a lack of *Ov*LAP activity. From this study, the ordering of preference metal ions on *Ov*LAP active site was $Ca^{2+} > Co^{2+} > Mg^{2+}$. Moreover, fully occupied at metal-binding sites was elevated *Ov*LAP activity. Besides, bestatin; the specific inhibitor of leucine aminopeptidase and EDTA; the metal chelator showed a strong inhibitory effect on *Ov*LAP activity. The degree of inhibition of bestatin and EDTA against *Ov*LAP activity was dose-dependent, in contrast to E-64 that lacked an inhibitory effect on *Ov*LAP activity, similar with other known LAPs

OvLAP was observed in all developmental stages of *O. viverrini* including egg, metacercaria, juvenile and adult. High level of *Ov*LAP expression was found in juvenile. Localization to determine *Ov*LAP expression in adult *O. viverrini* using anti-*Ov*LAP antibody found the expression of *Ov*LAP in egg shell, tegument, sub-tegument, testis, ventral sucker, Melhis gland apical membrane of gut epithelial cell and parenchymal cell. Some cross reaction with host hepatocyte and epithelial cell of bile duct was observed. It is possible to have little cross reaction of anti-*Ov*LAP antibodies with the vertebrate host, especially the liver organ where normally found LAPs in cell.

From this study, *Ov*LAP was present as antigenic and essential molecule for maintain *O. viverrini* life cycle. *Ov*LAP was attractive for vaccine candidate and target for gene knock down by combine with other *O. viverrini* crucial molecules such as cathepsin B, cathepsin D, cathepsin F and asparaginyl enopeptidase. The specific combine inhibition for these multiple enzymes may impair these enzymes function and have an effect on systemic hierarchical events in host protein hydrolysis. The blocking host protein hydrolysis, nutrient uptake, protein synthesis and cytoplasmic metabolism may impair parasite survival.