

CHAPTER 1

INTRODUCTION

1. Rationale and background

Opisthorchis viverrini infection remains a major public health problem in Southeast Asia, especially in Thailand and Lao People's Democratic Republic (PDR), affecting at least 8 million and 2 million people respectively. In Thailand, 9.6 % of the population is infected with *O. viverrini*, mainly in the North-East region where the prevalence is 16.6% due to occurrence of uncooked cyprinoid fish as a staple part of the diet. The high prevalence of *O. viverrini* infection is responsible for the high incidence of cholangiocarcinoma (CCA) and the high mortality rate due to liver cancer in this region (Andrews, Sithithaworn, and Petney, 2008); (Sripa and Pairojkul, 2008).

Poor sanitation practices, inadequate sewerage infrastructure, and the consumption of traditional dishes prepared with raw and/or insufficiently cooked fish promote opisthorchiasis outbreaks in North-East Thailand. Although *O. viverrini* infection in human is often asymptomatic, this parasite can be pathogenic in its own right and a direct risk factor for CCA of the bile duct. The World Health Organization and the International Agency for Research on Cancer reported that *O. viverrini* is carcinogenic to humans (1994). The infection is not immediately life-threatening, it taking 30 to 40 years after infection to develop into CCA, with death occurring within 3 to 6 months of diagnosis (Sripa et al., 2007).

The mechanisms by which *O. viverrini* induces CCA in humans are being explored and hypothesized to be a complex processes, involving several mechanisms. The exposure of *O. viverrini* involved in carcinogenesis is by concernment with hyperplasia of bile duct epithelium. Chronic irritation from mechanisms in the activities of feeding and migrating of flukes results in tissue damage. Even early infection is one cause of bile duct epithelium hyperplasia. Moreover, the metabolic products that secrete or are excreted from open glands or teguments of flukes, which are highly immunogenic, may be toxic to or interact with the biliary epithelium and induce biliary epithelial cells proliferation before progressive change to neoplasia, and develop to CCA (Sripa et al., 2007). *In vivo* studies in hamsters and *in vitro* studies indicate that the fluke's metabolic products excreted and secreted into the external environment can stimulate host immune response and promote immunopathologic processes mediating hepatobiliary damage in opisthorchiasis (Harinasuta, Riganti, and Bunnag, 1984).

It is of interest that these secreted proteins can promote cell proliferation and eventually cancer, since the benefit to the parasite is unclear.

There are many secreted and/or membrane proteins abundantly expressed in *O. viverrini* adult worms. Some of these secreted proteins are proteases that are involved in many aspect of parasitism, such as food digestion, immune invasion, and tissue invasion. In the function of food digestion, many proteases encoded endo- and exo-proteases are abundantly represented in *O. viverrini* adult stages (Laha et al., 2007). Some of these enzymes are characterized and found that they are highly immunogenic and attractive for serodiagnostic agents and vaccine candidates for opisthorchiasis. These proteases, including cathepsin D-like aspartic (Suttiprapa et al., 2009), cathepsin B-like cysteine proteases (Sripa et al., 2010), cathepsin F-like cysteine proteases (Pinlaor et al., 2009) and an asparaginyl endopeptidase (Laha et al., 2008). From these previous studies, we found that *O. viverrini* proteases play a pivotal role in survival in the parasite life cycle and might involve pathogenesis to their host. In addition, due to the high immunogenicity of these molecules, we use its potential as immunodiagnostic agents for the diagnosis of human opisthorchiasis (Laha et al., 2008); (Sripa et al., 2010).

Of particular interest is a member of the M17 family, Clan MF of metallo proteases, leucine aminopeptidase (LAP) that has been identified from adult stages of *O. viverrini* (Young et al., 2010). LAP is known as exopeptidases that play a crucial role in the parasite of protozoa and helminth with broader amidolytic activity beyond leucine hydrolysis. LAP participates in processing/maturation/activation or degradation of substrates that support their participation in vital processes in the parasite life cycle. In *Schistosoma* spp., LAP is a function in hydrolyzing short peptides to free amino acids before subsequently absorption and distribution to various parasite tissues by simple diffusion or via specific amino acid permeases (Delcroix et al., 2006). This is like the function in *Paragonimus westermani* (Song et al., 2008) and *Ceanorhabditis elegans* that showed LAP as a digestive enzyme with localization in the gastrodermis of adult worms. LAP is also required for hatching of *Schistosoma mansoni* eggs (Xu and Dresden, 1986) suggesting that aminopeptidases may also play a role in facilitating transition between developmental stages in other helminth phyla. In *F. hepatica*, vaccination trials using the recombinant protein of LAP *F. hepatica* (rFhLAP) for rabbit immunization showed a strong IgG response and a highly significant level of protection after experimental infection with metacercariae (Acosta et al., 2008). This indicated that LAP could be a potential molecule to use as a candidate for vaccine development against parasite infection and use in serologic diagnosis.

O. viverrini have been extensively studied in endopeptidases due to their prominent participation in fluke's biology but there have any studied in exopeptidases. Thus there is little known about this molecule regarding the benefits of *O. viverrini* and harmful effects on their host. It is important to clarify the biochemistry, the molecular biology, and function of the LAP in *O. viverrini* and the evolution of this molecule in general for to gain an insight of the basic knowledge of the parasite. This study seeks answers to questions about the importance of the roles of proteases in catabolism of essential nutrition for *O. viverrini* and may be useful in the understanding of the pathogenesis of *O. viverrini* infection. This information could provide an opportunity for the development of novel anti-parasitic drugs, vaccines, or serodiagnostic agents for the detection of opisthorchiasis.

2. Research question

What are the properties, roles, and effects of *O. viverrini* leucine aminopeptidase (OvLAP) in human hosts?

3. Objectives of the study

1. To isolate, characterize, and produce recombinant protein of OvLAP.
2. To study protein profiles, stages of expression, and localization of OvLAP.
3. To investigate the functions of recombinant leucine aminopeptidase of *O. viverrini* in the roles of food catabolism, enzymes, and pathogenic molecules.