CHAPTER I
INTRODUCTION

1.1 Rational and background

Opisthorchis viverrini is a digenetic trematode that dwelling in the biliary tract of vertebrates and causes the disease called opisthorchiasis in human. Opisthorchiasis is remained major public health problem in many parts of Southeast Asia including Thailand, Laos, Cambodia and some part of Vietnam (Kaewkes, 2003; Sripa and Kaewkes, 2000). In Thailand, the high prevalent occurred in northeastern provinces where people have raw fish as staple food (Sripa et al., 2007). People were infected this parasite by eating undercooked cyprinoids fishes that harbored the infective stage, metacercaria. Adult worm lives in the bile duct and has long life span for up to 25 years (Upatham and Viyanant, 2003).

The pathology of *O. viverrini* infection associates with many hepatobiliary abnormalities including cholangitis, obstructive jaundice, hepatomegaly, cholecystitis and cholelithiasis (Sripa, 2003). The mechanisms of biliary damage due to *O. viverrivi* infection were involved by several factors. In experimental hamster models showed that acute inflammatory of bile duct are induced by parasite (Sripa and Kaewkes, 2002). At the early stage of infection, worms use their oral and ventral sucker to attach the biliary epithelium and cause tissue damage. During the fluke growing up, they induce hyperplasia of bile duct epithelium and form adenoma. Then bile duct tissues are proliferating due to imflammation processes that associate with periductal fibrosis. This fibrosis links between portal areas with fibrous bands resulting in the appearance of multilobular cirrhosis. Bile duct obstruction is frequently found in heavy infection in both animal model and human case. However the obstruction of bile duct in human case is unlikely because the worms are comparatively smaller than the human bile duct lumen. The obstruction may lead to cholangitis with abscess formation (Sripa, 2003; Watanapa and Watanapa, 2002).

Moreover, long term infection with *O. viverrini* associated with cholangiocarcinoma (CCA), cancer of the bile duct (Sripa et al., 2007). From many experiments and epidemiology evidences, *O. viverrini* has been classified as a class 1 carcinogen that causes CCA (IARC, 1994; (Bouvard et al., 2009). The pathogenesis of *O. viverrini* infection associated with CCA is hypothesized to be a complex processes, involving several mechanisms. The molecular mechanisms by which *O. viverrini* induces CCA in humans are being explored. Syrian golden hamsters provide an ideal laboratory host for determining the nature of pathology – they are a fully permissive host, and infection results in CCA when is accompanied by excessive levels of dietary nitrosamines (Bhamarapravati, Thammavit, and Vajrasthira, 1978). The exposure of *O. viverrini* involve in carcinogenesis mechanism because it relate to hyperplasia of bile duct epithelium due to mechanical damage of bile duct epithelial by worm sucker and toxic by worm excretory-secretory (ES) product. From *in vivo* in hamsters and *in vitro* studies indicate that the fluke's ES products, metabolic products excrete and secrete into the external environment from the excretory openings and epithelial surface (tegument), can stimulate host immune response that toxic or interact with bile duct epithelium (Harinasuta, Riganti, and Bunnag, 1984). These metabolic products can act as mitogen and likely play a role in the initiation of CCA in naturally infected humans and experimentally infected hamsters (Sripa et al., 2007; Thuwajit et al., 2004). It was assumed that acute damage may be induced by parasite factors but the progressive change to CCA may be effected from immunopathologic mechanisms (Bhamarapravati, Thammavit, and Vajrasthira, 1978). In addition, bile duct damages were induced by nitric oxide synthase released from macrophage, mast cell and eosinophil in inflammatory area surrounding the bile ducts so called endogenous nitrosation. Moreover the N-nitroso compound commonly found in the fermented and preserved foods were determined as exogenous nitrosation. Both endogenous and exogenous nitrosation may induce DNA damage in cell cycle proliferation and then neoplastic changes occur.

From transcriptome analysis of the adult *O. viverrini*, several secreted proteins including proteases were identified (Laha et al., 2007). These secreted proteases were detected as a components in excretory-secretory products of the adult *O.viverrini* (Laha et al., 2008); (Suttiprapa et al., 2009); (Pinlaor et al., 2009). As most parasite

deploy proteases to accomplish some of tasks for maintain their life. Parasite proteases facilitate migration, invasion of host tissue, allow parasite digest host blood protein for nutrition and help parasite evade the host immune response. Proteases can play variety of role an establishing and maintaining the infection (McKerrow et al., 2006). It was not surprised that proteases have been found to play critical role in pathogenesis of parasitic disease. To date, several proteases from many parasites have been characterized and functional investigated including blood flukes - *Schistosoma mansoni* (Delcroix et al., 2006), the ruminant parasite - *Haemonchus contortus* (Shompole and Jasmer, 2001), the blood feeding nematodes – hookworms (*Ancylostoma* spp. and *Necator americanus*) (Williamson et al., 2003; Williamson et al., 2004) and the malaria parasites – *Plasmodium falciparum* (Kumar et al., 2007). It was found that proteases involved in the catabolism of host hemoglobin. The end products of this process are amino acids that necessary for parasite growth, development and reproduction (Rosenthal, 2004). If the expression of hemoglobin degrading proteases in parasite was inhibited, it showed the decreased of fecundity and egg production (Caffrey et al., 2004). Parasite proteases have been proposed as potential target for immunotherapeutic and chemotherapeutic agents and in some cases, serodiagnostic reagent for detection of parasitic diseases.

To date, there is little known about proteases from *O. viverrini*. However, several proteases gene sequences have been characterized from EST project (Laha et al., 2007; Young et al., 2010). Many of these enzymes encoded endo- and exo-proteases belonging to established families (Merops classification). Of particular interest were members of the S1A serine protease family. Other proteases included homologue of enzymes that digest hemoglobin in blood-feeding helminthes, including cathepsin D-like aspartic and cathepsin B-like cysteine proteases as well as an asparaginyl endopeptidase, which is known to activate the gastrodermal cathepsin B enzyme. From these valuable data, we favor for further investigation to determine whether *O. viverrini* proteases play pivotal role for survival in parasite life cycle and might involve pathogenesis to their host. In particular, the cathepsin B shows highly expressed among other proteases and it has been identified as a major enzyme for food catabolism. In addition, *O. viverrini* proteases and other secreted or membrane bound proteases may be key mediators of hyper-proliferation of biliary epithelial cells

that may progress to CCA. Thereafter, it is important to clarify the biochemistry, the molecular biology and function of the cathepsin B in *O. viverrini* and the evolution of proteases in general for gaining insight the basic knowledge of the parasite. The knowledge from this study leading to answer the questions about the important of proteases roles in catabolism of essential nutrition for *O. viverrini* and might be useful information to understand the pathogenesis of *O. viverrini* infection. All these information could provide an opportunity for the development of novel anti-parasitic drug, vaccine or serodiagnostic agent for detection of parasitic disease.

1.2 Objectives of the study

The specific aims of this work are;

1. To isolate and characterize cathepsin B genes from *O. viverrini*.
2. To study protein profiles and produce recombinant protein of *O. viverrini* cathepsin B.
3. To investigate the function of recombinant cathepsin B of *O. viverrini* in the roles of food catabolism enzymes and as pathogenic molecule.
4. To investigate the potential of recombinant cathepsin B of *O. viverrini* as immunodiagnostic tool and vaccine candidate.

The thesis chapters were written to describe each objective of the study above in separate chapter. The chapters were divided related to the specific aims of the study and were written in the format for scientific journal publication. The body of thesis included 6 chapters. Chapter 1 is an introduction that includes rational, background and objectives of the study. Chapter 2, 3, 4 and 5 are the research study details that were written in scientific journal format. Chapter 6 is general discussion and chapter 7 is the conclusions of all experiments.