CHAPTER 2
LITERATURE REVIEW

1. Biology and epidemiology of *O. viverrini*

Opisthorchis viverrini is the parasitic worm belonging to the phylum "Plathyhelminthes", class "Trematoda", subclass "Digenea", family "Opisthorchidae" (King and Scholz, 2001). The adult stage of *O. viverrini* dwells in the biliary tract of vertebrates including humans, dogs, and cats. In adult worms of *O. viverrini* are monoecious, the body is dorso-ventrally flattened, lancet-shaped, thin, and transparent with a reddish-bile color.

Life cycles of *O. viverrini* are involved in many hosts and environments. Adult worms live in intra- and extra-hepatic bile ducts, gall bladders, and rarely in the pancreatic ducts of their definitive hosts. Humans have been known to be definitive hosts while dogs and cats serve as natural reservoir hosts. Adult worms use oral and ventral suckers to attach to the wall of bile ducts. Embryonated eggs containing ciliated miracidium laid from adult worms are passed out of the bile duct into the duodenum and excreted with feces into the external environment. Eggs ingested by *Bithynia* snails then hatch as miracidia in a snail's digestive tract before transformation to sporocysts, rediae, and cercariae respectively. Free-living cercariae, after exiting the snail, attach, penetrate, and transform to metacercariae encysted mainly in the muscle of about 18 susceptible species of fish in the family Cyprinidae (Waikagul, 1998). Metacercariae infect the final host when they ingest raw or inadequately cooked fish. After ingestion, the metacercaria cyst wall was digested by gastric and intestinal juices and juvenile flukes were excysted at the duodenum, and then migrate up through the Ampulla of Vater to the common bile duct into the intra-hepatic bile duct where they mature and fertilize (Figure 1) (Kaewkes, 2003). The life span of the adult *O. viverrini* in humans has been reported to be 25 to 30 years (Watanapa and Watanapa, 2002).

2. Proteases

Proteases are the enzyme that catalyzes the cleavage of amide linkage in macromolecular peptide and oligomeric peptides. Proteases are divided into two major groups, endoproteases and exoproteases, according to the site of hydrolysis on the peptide chains. The hydrolysis occurs within a polypeptide chain (endoprotease activity) or occurs from the amino or carboxyl end of peptide substrates (exoprotease activity). Proteases arise from a single evolutionary origin so they are also divided into groups or clans on the basis of the evolutionary relationship. From the peptidase database MEROPS (http://merops.sanger.ac.uk/), the available amino acid sequences of proteases have been

classified as clan, family, and subfamily. If a clan contains one or more families that represent the similarity in their tertiary structure, in case of the structure not available, the similarity in the order of catalytic-site residues in the polypeptide chain and conserve of the motif sequence around the catalytic site residues need to be considered. Proteases can be classified into 8 clans, aspartic peptidases, cysteine peptidases, glutamic peptidases, metallo peptidases, serine peptidases, threonine peptidases, mixed catalytic type (serine, threonine, cysteine), and unknown catalytic type. Each clan is identified aby two letters, the first letter representing the catalytic type of the families included in the clan. Some clans are divided into subclans because there is a very ancient divergence within the clan (http://merops.sanger.ac.uk).

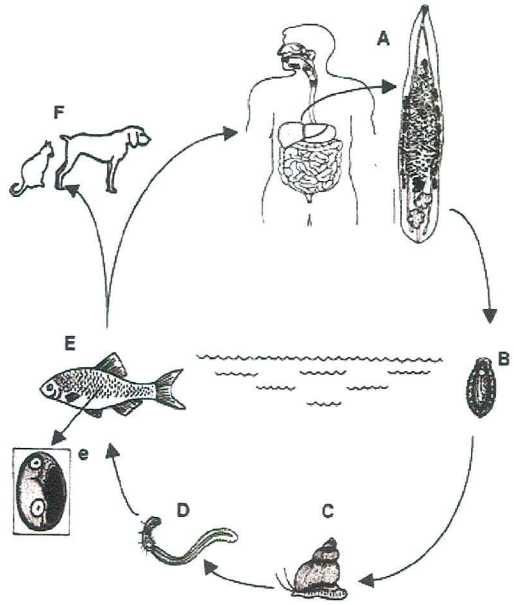

Fig. 1 Life cycle of *O. viverrini*: (A) adult worm in bile duct of humans; (B) embryonated egg reaches into fresh water; (C) eggs were eaten by first intermediate host; *Bithynia* snail; (D) cercariae released from snail; (E) encysted to metacercaria in second intermediate host (cyprinoids fish) and (e) metacercaria; (F) reservoir host, dog and cat (Kaewkes, 2003).

3. Parasite proteases

Proteases encompass a broad class of hydrolytic enzymes that play an essential role in cellular, developmental, and digestive processes, blood coagulation, inflammation, wound healing, and immune processing in parasitic organisms. Proteases have been identified in many organisms from viruses to vertebrates. In vertebrates, serine proteases play a large role,

unlike in invertebrates, and the cysteine proteases of the papain family and aspartic proteases are assumed to play the major role in intestinal protein digestion (Delcroix et al., 2006). Recently, leucine aminopeptidase (LAP), the exopeptidase in family M17 of metallo proteases have been characterized in many protozoa and helminth organisms. LAP was shown to be a crucial enzyme in parasite biology since it localized in gastrodermis of adult *Fasciola* spp. (Acosta et al., 2008) and was involved in the egg hatching process of *S. mansoni* (Xu and Dresden, 1986). Moreover, a model for the catabolic process of hemoglobin in *Schistosome* spp. proposed that exopeptidase is most likely to be the enzyme that cleaves short peptides to free amino acids before absorption into parasite tissue (Delcroix et al., 2006).

Proteases have different roles in different organisms but they have the same purpose in maintaining life. In parasitic organisms, proteases are supposed to accomplish a parasitic lifestyle, such as facilitate the tissue penetration, assist digestion of host tissue/protein for nutrition, help parasitic evasion of host immune responses, and mediate molting in parasitic nematodes. Some characterized proteases showed a strong immune response in infected humans and experimental animals that had the potential value as the target for immunotherapeutic and chemotherapeutic agents and, in some cases, serodiagnostic reagents for the detection of parasitic diseases (McKerrow, 1989). Thus, proteases are concerned as the target for the development of novel chemotherapeutic, immunotherapeutic, and serodiagnostic agents for the next generation of anti-parasitic interventions (Williamson et al., 2003).

4. Leucine aminopeptidase

Leusyl or leucine aminopeptidases (LAP) are members of M17 family in clan metallo proteases. LAP are the exopeptidases that catalyze the removal of amino acids from the unblocked N-termini of peptides and proteins. These peptidases are widely distributed and have been found in many tissues and cells, as either membrane-associated or soluble forms. Studies of parasitic protozoa and helminthes indicate a crucial role of LAP in parasite biology.

LAP consist of a two-domain structure. NH_2-terminal domain is a less conserved domain amongst LAP, varies in length among members, and does not exhibit identity with other proteins. In contrast, the COOH-terminal domain presents as a more conserved domain containing catalytic residues and zinc-binding residues. Searches of public databases found the peptide sequence NTDAEGRL that represents the conserved active site region of aminopeptidases in the C-terminal domain. Thus the phylogenetic relationship between

selected LAP of the M17 family of aminopeptidases was examined using selected C-terminal domains only.

LAPs are metallo-proteases and require the binding of two zinc ions that are pentahedrally coordinated within each active site. These metal ions act as nucleophiles and are essential for enzymatic activity. The residues that bind these zinc ions are highly conserved between all members of the M17 family of LAP enzymes. Residues Asp 289, Asp 367, and Glu 369 bind zinc 1, while Asp 289, Lys 284, Asp 307 and Glu 369 bind zinc 2. The residues Lys 296 and Arg 371 are also involved in the catalytic mechanism by acting as an electrophile and proton donor respectively, and are thus also conserved in all LAP (McCarthy et al., 2004). It was found that LAP activity displays a preference for a neutral/slightly alkaline (pH 6.5–9.4) environment. Enzyme activity is optimal at pH 8.25 and is not detectable below pH 6.5. LAP members of the M17 family are metalloenzymes and thus require the presence of metal cations to maintain enzyme activity and stability. These metal cations, such as manganese and magnesium ions, enhance the activity of LAP in contrast with zinc ions that reduce the activity of LAP. The metal chelators EDTA, 1,10-phenanthroline and the aminopeptidase inhibitor, bestatin, are all inhibitory for LAP. LAP prefers to cleave substrate at Leu residue keeping with its classification as a M17 LAP (McCarthy et al., 2004; Song et al., 2008).

MaCarthy et al., 2004 found that LAP is localized in gastrodermal cells lining caecum and sub-tegumental tissues of *S. mansoni* and *P. weatermani* (Song et al., 2008). This indicated that LAP or intestinal aminopeptidases play an important role in the enzymatic digestion of host macromolecules such as hemoglobin in the intestinal tract. However, LAP do not function efficiently in the slightly acidic environment of the gut lumen and this enzyme lack of signal peptide that assumes the function of LAP may occur intra-cellularly and indicate that generated small peptides may diffuse through the gut epithelium and hydrolyze to free amino acids. Moreover, LAP were localized at surface tegument of *S. mansoni*, indicating that it may have an additional role in surface membrane remodeling (McCarthy et al., 2004). *S. mansoni* LAP activity was reported in all developmental stages of *S. mansoni* (Xu and Dresden, 1986). In eggs, this enzyme plays a role in the hatching process, possibly through the degradation of the eggshell (Cesari, Auriault, and Capron, 1983); (Xu and Dresden, 1986). It was suggested that secreted schistosome LAP is involved in the penetration of host tissue and protection from immune attack by degradation of host immunoglobulin molecules on the surface of the parasite (Piacenza et al., 1999).

LAPs are attractive as a vaccine candidates since they have crucial roles in parasite nutrition and maintenance of tegumental structure, and expression in the early invasive stages of the parasite. In *F. hepatica*, native *Fh*LAP can induce strong IgG response in vaccinated sheep that inhibited LAP enzymatic activity. This property was proposed as the basis for the very high level (89%) of protection obtained (Piacenza et al., 1999).