CHAPTER I
INTRODUCTION

Fasciola gigantica and *Fasciola hepatica* are obligate parasitic trematodes and major pathogens of cattle and sheep. Humans are accidental hosts. Fasciolosis causes a decrease of milk and meat production of the livestocks and thereby, economic loss to farmers. *Fasciola* spp. can be found worldwide but the two mentioned species are endemic in different regions. *F. gigantica* is the major parasite causing fasciolosis in Southeast Asia, including Thailand. Sukhapesna *et al*. (1990) reported a 47.1% prevalence of buffalos infected with *F. gigantica* in Thailand.

To control *F. gigantica*, effective strategies such as anthelmintic treatment and many alternative control programmes have been proposed (Roberts and Suhardono, 1996). However, treatment of the infected host with anthelmintics is the principal method employed to control *Fasciola*. Although triclabendazole is an effective drug for controlling *F. gigantica* (Boray *et al*., 1983; Estuningsih *et al*., 1990; Suhardono *et al*., 1991), the cost of treatment is a burden in developing countries. Moreover, resistance to triclabendazole has been reported (Overend and Bowen, 1995). Alternatively, vaccines have been tried as sustainable strategy for the control of fasciolosis.

In recent years, several defined antigens have been tried as vaccines against fasciolosis (Rickard and Howell, 1982; Haroun and Hillyer, 1986; Estuningsih *et al*., 1997). Antigens tried in *Fasciola* and/or *Schistosoma* include fatty acid binding protein (FABP), glutathione *S*-transferase (GST), cathepsin L (CatL), hemoglobin, paramyosin, Kunitz-type serine proteinase inhibitor (KTM), saposin-like proteins (SAPLIPs), leucyl aminopeptidase (LAP) and asparaginyl endopeptidase (Legumain).

Asparaginyl endopeptidases or legumains (LGMNs) are cysteine proteases that show strict specificity for cleavage at the C-terminus of asparaginyl residues. To date, LGMNs have been described from plants, mammals, and also trematodes, i.e. *Schistosoma* and *Fasciola*. Several studies indicate that the parasite LGMNs are essential enzymes for parasite survival (Dalton and Brindley, 1996; Manoury *et al*., 1998; Okamoto and Minamikawa, 1999; Shirahama-Noda *et al*., 2003; Sajid *et al*.,

2003).

Recently, the potential of LGMNs as a vaccine was demonstrated in mice immunized with a *S. mansoni* LGMN (*Sm*32)-encoding DNA construct (Chlichlia *et al*., 2002). A modest anti-fecundity effect was observed with a 37% decrease in egg production (Chlichlia *et al*., 2002). Chacón *et al*. (2003) immunized mice with discontinuous synthetic peptides, covering the entire sequence of *S. mansoni* LGMN. Peptides with high homology to host legumain were poorly immunogenic. However, other peptides with less homology and of greater hydrophobicity elicited antibodies that reacted with the intact antigen.

To understand the biological function of asparaginyl endopeptidase or legumain (LGMN) in *F. gigantica*, I have undertaken the molecular cloning and characterization of FgLGMN nucleic acids, including related genes in the family, mRNA transcripts, and tissue-specific localization of mRNA. Subsequently, recombinant FgLGMN proteins were expressed in prokaryotic expression systems, purified and used to immunize experimental animals to obtain antibodies. The immune sera were used for identification of native FgLGMNs in immunoblots of parasite antigen preparations and *in situ* by immunohistochemical detection in tissue sections. The legumain activity was analyzed in parasite extracts and the inhibitor sensitivity was studied to clarify the basic function of *F. gigantica* legumain. The molecular biological information and basic functional assay in our and other studies indicated the essential function of legumain in survival of many organisms. Probably, the legumain could be developed as a vaccine against fasciolosis in the future.