Thesis Title	Purification and Characterization of Liver-Fluke *Fasciola gigantica* Antigens
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Date of Graduation	16 May B.E. 2538 (1995)

ABSTRACT

Liver-fluke *Fasciola gigantica* is a veterinary important parasite in cattle industry causes tremendous economic damage. The disease is currently diagnosed by the conventional stool examination to observe the parasite eggs. To overcome some drawbacks of the current method, the present study was intended to isolate and characterize protein antigen(s) from the parasite aiming for serodiagnostic development of fascioliasis.

Main components of the excretory-secretory (ES) extract from the adult worm *in vitro* culture was demonstrated by immunoblotting to contain parasite antigens reactive against naturally infected cattle immune sera. The extract also exhibited azocollytic activity which was markedly stimulated by dithiothreitol as well as inhibited by iodoacetamide, leupeptin and specific cysteine protease inhibitor E-64 but not by

metallo-, aspartate and serine protease inhibitors suggesting the activity derived from cysteine protease.

Neither proteolytic nor immunogenic component was bound to the activated thiol-Sepharose 4B chromatography. However, purification was reproducibly achieved by using MonoS-FPLC and chromatofocusing. Both proteolytic and immuno-activities were coeluted in a protein peak from the MonoS-FPLC but subsequently separated by chromatofocusing into two components at pI 6.4 and 6.0 each appeared as a single protein band in SDS-PAGE at 27 and 26 kDa respectively. In addition, each protein(antigen) band was also detectable by immunoblotting. The amino acid composition of 26 kDa and 27 kDa antigens were different in hydrophobic amino acids i.e. phenylalanine and tryptophan. 17 phenylalanine and 9 tryptophan reidues were observed in 26 kDa whereas only 10 phenylalanine and 2 tryptophan were in 27 kDa. Both antigens exhibited protease activity of cysteine (thiol) type with different optimum acidic pH. In situ immunostaining using monoclonal antibody to ES revealed that the cysteine proteases were localized predominantly in the intestinal epithelial cells and lumen.

Purified 27 kDa cysteine protease antigen was used in ELISA for monitoring antibody in cows and water-buffaloes experimentally infected by *F.gigantica* metacercaria. Antibody was detectable at 5 weeks after infection approaching maximum at 15 weeks whereas parallel stool examination for the parasite eggs was found after 14 weeks. These studies indicated the presence of *F. gigantica* antigens possibly secreted from the parasite gastrointestinal tract, which might serve as candidates for future diagnostic development of cattle fascioliasis.