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Molecular and biochemical characterization of stefin-2 from the liver fluke Fasciola gigantica

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Cystatins are the inhibitors of papain-like cysteine proteases and serve various important physiological functions including modulation of normal proteolytic processes, balance of the host-parasite interaction and defense against pathogens. This research was conducted to characterize the molecular and biochemical properties of a type I cystatin (Stefin-2) in the liver fluke *F. gigantica*. The cDNA encoding FgStefin-2 had a size of 887 bp and contained a 351 bp open reading frame. The bacterial expression and purification resulted in highly pure rFgStefin-2 with an expected molecular mass of 13-14 kDa. The rFgStefin-2 was used for cysteine protease inhibition assays and polyclonal antibody production. The polyclonal antibody was used to study the distribution of native FgStefin-2 in 2 and 4-week-old juveniles and used in immunoblots. Immunohistochemical analysis showed that FgStefin-2 is located in several tissues of the parasite including the prostate gland, gut epithelium and intrauterine eggs. The polyclonal antibody reacted with rFgStefin-2, CW extract and ES product of the adult parasite but did not cross-react with CW extracts of other trematodes. Mouse antisera raised against rFgStefin-2, rFgMDCd10 and rFgStefin-1 showed no cross-reactivity in immunoblots to the different recombinant cystatins. The purified rFgStefin-2 exhibited inhibitory activity against cysteine proteases (cathepsins B and L) and the proteolytic activity of ES product, CW extracts from metacercariae and adult parasites. We have demonstrated that FgStefin-2 is able to inhibit cysteine proteases (cathepsins B and L). In further analysis we would like to investigate the potential host-affecting functions such as immunomodulation.

Biography

Sinee Siricoon has completed her PhD in Molecular Parasitology Unit and Graduate Program in Biomedical Sciences from Faculty of Allied Health Sciences, Thammasat University, Thailand. She has published 3 papers in reputed journals.

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