Original article **P59**

Molecular and biochemical characterization of type I cystatin (Stefin) of *Fasciola gigantica*

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Abstract

In the present study we describe type 1 cystatin, a cysteine protease inhibitor, as a major released antigen of the tropical liver fluke *Fasciola gigantica* (FgStefin-1). Immunohistochemical analysis showed that FgStefin-1 is abundant in tissue of tegumental type and the intestinal epithelium. Faint staining was observed in the epithelia of ovary and proximal uterus. Immunoblots showed the presence of FgStefin-1 in the parasite's excretion/secretion (ES) product. Sera of experimentally infected sheep reacted with recombinant FgStefin-1 starting 8 weeks postinfection. Activity analyses of recombinant FgStefin-1 showed nanomolar inhibition constants for mammalian host and parasite cysteine proteases. Our results suggest protective functions of FgStefin-1, regulating intracellular cysteine protease activity, and possibly protection against extracellular proteolytic damage to the parasite's intestinal and tegumental surface proteins.

Keywords**:** *Fasciola gigantica,* Cysteine protease inhibitor, Cystatin, Immunolocalization

Introduction

Cathepsin B and L cysteine proteases are established as important and abundantly produced antigens in the trematode genus *Fasciola* and are implicated in parasite nutrition, protection, and host invasion [1]. Expression patterns and biological functions of their counterparts, cysteine protease inhibitors of the cystatin family (MEROPS [2] inhibitor family I25, clan IH) have been less well researched in *Fasciola* and in trematodes in general. At present, neither immunohistochemical data demonstrating the distribution of cystatin in parasite tissues nor data regarding possible effects of trematode cystatins on the host immune system exist. In contrast, substantial immunomodulatory effects of type 2 cystatins from parasitic nematodes have been demonstrated in the last years [3-5]. They were found to decrease T cell responses by inhibition of proteases participating in MHC class II antigen processing and presentation and to increase interleukin 10 and nitric oxide production [6].

The purpose of this study was to characterize the molecular biological properties of a cysteine protease inhibitor from *F. gigantica* including its inhibition properties against mammalian and endogenous cysteine proteases. This work should lead to a better understanding of the biological function of type 1 cystatin in *Fasciola* and trematodes in general and in the future we will perform additional analyses to investigate whether FgStefin-1 has immunomodulatory properties as has been demonstrated for nematode cystatins.

Methods

A cDNA encoding a type 1 cystatin (FgStefin-1) was isolated from a *F. gigantica* cDNA library and used to produce recombinant FgStefin-1 (rFgStefin-1) in functional form

in *E. coli*. Polyclonal antibody against rFgStefin-1 was produced and used to study the distribution of native FgStefin-1 in *F. gigantica*. The inhibitory properties of rFgStefin-1 against cysteine proteases were characterized using fluorogenic substrates. The immune response of infected animals against FgStefin-1 was analyzed by ELISA.

Results

FgStefin-1 is present in the parasite from the metacercarial to the adult stage. It reacted with mouse anti-rFgStefin-1 antiserum as an 11 kDa antigen in immunoblotted crude worm (CW) extract, excretion/secretion (ES) product and tegumental antigen extract of the adult parasite. Immunohistochemical analysis showed that FgStefin-1 is abundant in tissue of tegumental type and the intestinal epithelium. Faint staining was observed in the epithelia of ovary and proximal uterus (Fig. 1). Purified rFgStefin-1 was tested for inhibitory activity against native human cathepsins B and L, recombinant *F. hepatica* cathepsin B and L. The protein was able to inhibit all tested proteases. FgStefin-1 was able to stimulate a host immune response in infected sheep before the parasites reached maturity.

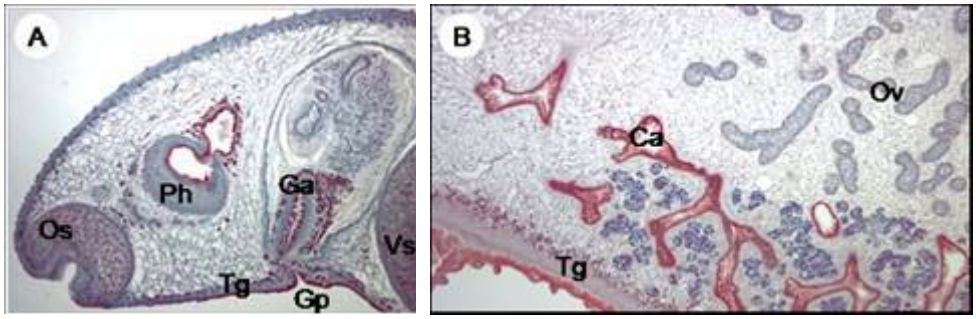

Figure 1. Immunohistochemical detection of FgStefin-1 in adult *F. gigantica* by mouse anti-rFgStefin-1 antiserum. (A) sagittal sections of the anterior region. (B) longitudinal section. Ca, caecum; Ga, genital atrium; Gp, genital pore; Os, oral sucker; Ov, ovary; Ph, pharynx; Tg, tegument; Vs, ventral sucker.

Discussion

FgStefin-1 has inhibitory properties comparable to homologs described from other organisms with higher activity against cathepsin L than cathepsin B. The occluding loop of cathepsin B might protect the active site of this protease from inhibition by cystatins. FgStefin-1 is present in tissues that constitute external and internal surfaces, tegument and gastrodermis. Cathepsin B is not only found in these tissues but also in vitelline cells through which it becomes incorporated in the parasite's eggs, Mehlis' gland, prostate gland, and male and female germ line cells while cathepsin L is a major gut-specific protease. An obvious function of cytoplasmic located FgStefin-1 is, therefore, inhibition of these proteases when leaking from their storage vesicles. The presence of FgStefin-1 in several tissues and its release in the ES product indicates that it has intracellular and extracellular functions. FgStefin-1 stimulates an immune response in *F. gigantica* infected sheep that could be detected 8 weeks postinfection. This finding suggests that FgStefin-1 cannot be applied for early diagnosis of fasciolosis.

Conclusion

We have demonstrated that FgStefin-1 is an abundantly expressed, highly stable protein in juvenile and adult *F. gigantica* that has intracellular and extracellular functions and is able to inhibit parasite and host cysteine proteases. Infected sheep showed an immune response against rFgStefin-1 when the juvenile parasites were still migrating through the liver

parenchyma. In the future we would like to investigate whether FgStefin-1 is active in the modulation of the host immune response.

Acknowledgements

This research was supported by a grant from Thammasat University and the Thailand Research Fund through a Royal Golden Jubilee Ph.D. scholarship to Mayuri Tarasuk.

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