Trends in Parasitology

CellPress

Review

Protease Inhibitors of Parasitic Flukes: Emerging Roles in Parasite Survival and Immune Defence

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Protease inhibitors play crucial roles in parasite development and survival, counteracting the potentially damaging immune responses of their vertebrate hosts. However, limited information is currently available on protease inhibitors from schistosomes and food-borne trematodes. Future characterization of these molecules is important not only to expand knowledge on parasitic fluke biology but also to determine whether they represent novel vaccine and/or drug targets. Moreover, protease inhibitors from flukes may represent lead compounds for the development of a new range of therapeutic agents against inflammatory disorders and cancer. This review discusses already identified protease inhibitors of fluke origin, emphasizing their biological function and their possible future development as new intervention targets.

Protease Inhibitors and Parasitic Flukes

The proteases of eukaryotes can be divided into serine (trypsin/chymotrypsin-like), cysteine (thiol), and aspartic (pepsin, cathepsin, rennin) proteases which are involved in multiple processes, including cell proliferation, homeostasis, inflammation, immune mechanisms generally, and apoptosis [1]. However, inhibitors of these proteases are equally important as they restrain potentially detrimental excess or ill-timed proteolytic activity. **Protease inhibitors** (see Glossary) therefore partner most of the known enzymes that cleave peptide bonds and include serine protease inhibitors (serpins, Kunitz-type, Kazal-type), cysteine protease inhibitors (cystatins), **metalloproteinase** inhibitors, and **alpha-2-macroglobulin** (α2M) [2]. According to function, protease inhibitors can be classified into four major groups: those that block the active site of a target protease (canonical inhibitors including serpins); those binding a region adjacent to the binding site, preventing substrate access (exosite-binding inhibitors like cystatins); a combination of canonical and exosite-binding mechanisms; and allosteric inhibitors such as caspase inhibitors [1,2].

This review considers the protease inhibitors of the **digenetic trematodes** (the flukes) (Table 1), which recent studies have shown are critical for worm survival and defence against the immune system of their vertebrate hosts. The majority of parasitic flukes inhabit the vertebrate gut and its associated organs, especially the lungs, bladder, ureter, pancreatic duct, liver, bile duct, and gall bladder. Many digenean species, including the *Schistosoma* blood flukes and food-borne intestinal and liver flukes, infect humans, and they use proteases to invade host tissues as well as in nutrition and development. Adult schistosomes live in the blood vessels of the mammalian hosts and survive in this hostile environment without triggering immune attack by immune cells, such as neutrophils, or initiating host blood coagulation or

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Protease inhibitors play major roles in infections, inflammatory disorders, and cancer.

Protease inhibitors of parasites create a safer environment in the host by inhibiting and regulating protease activity and immunomodulation.

Despite the increasing interest in developing parasite proteases as therapeutics and as vaccines, there are comparatively fewer available data on parasite-origin protease inhibitors.

Further understanding of protease inhibitors from parasitic flukes broadens knowledge of parasite biology and immunomodulatory mechanisms, and may lead to the discovery of novel antiparasite interventions and treatments against other diseases such as cancer.

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Table 1. Protease Inhibitors in Parasitic Flukes[a]

Protease inhibitor	Size kDa	Fluke species	Protease(s) inhibited	Stage/location of highest expression	Location/whether secreted	Refs
SmPi56	56	*Schistosoma mansoni*	*S. mansoni* 28 kDa serine protease, PE, NE	Adult worms	Secreted	[24]
SmSrpQ	47	*S. mansoni*	SmCE	Cercariae	Secreted	[25]
SmSPI	46	*S. mansoni*	Chymotrypsin, PE, NE	Head gland of schistosomules, spines of adults	Secreted	[26]
ShSPI	46	*Schistosoma haematobium*	Thrombin	Surface of adult worms	Partly membrane-bound	[27]
Sj serpin	45	*Schistosoma japonicum*	ND	Tegument, intestinal epithelium	ND	[30]
SjB10	45.7	*S. japonicum*	Trypsin, chymotrypsin, PE	Cercariae, schistosomula, eggs, adult male worms	Intracellular	[31]
SjB6	60	*S. japonicum*	Trypsin	Eggs	Secreted	[32]
PwSERPIN	43	*Paragonimus westermani*	Endogenous trypsin/ chymotrypsin, thrombin	Increased expression from metacercariae to adults	Intracellular	[34]
CsproSERPIN	44.7	*Clonorchis sinensis*	Trypsin, chymotrypsin, thrombin	Vitellarium and testis in adult worms, eggs, and uterus	Intracellular	[35]
CsSERPIN	42.2	*C. sinensis*	ND	Subtegument and oral sucker of metacercariae	Intracellular	[37]
CsSERPIN2	45.7	*C. sinensis*	Chymotrypsin	Vitellarium and testis in adult worms, eggs, and uterus	Intracellular	[35]
CsSERPIN3	43.	*C. sinensis*	ND	Tegument of metacercariae within cyst wall	Tegument-anchored	[37]
SmPI31	33	*S. mansoni*	Chymotrypsin-like activity of proteosome	All life cycle stages	Membrane associated	[42]
SjKI-1	8	*S. japonicum*	Trypsin, chymotrypsin, NE, FXa, PK	Eggs	Secreted (via nonconventional way)	[45]
SmKI-1	16	*S. mansoni*	Trypsin, chymotrypsin, NE, FXa, PK	Adults, schistosomula, eggs	Secreted	[46]
FhKTM	6.5	*Fasciola hepatica*	Trypsin	Gut, paranchymal tissue and tegument of adults, vomitus	Secreted	[48]
FhKTI	7	*F. hepatica*	FhCL1, FhCL2, human cathepsins L and K	Gut and paranchymal tissue of 24 h newly excysted juveniles	Secreted	[51]
SjIAP	45	*S. japonicum*	Human caspase 3 and schistosome caspase	Adult male worms	No signal peptide	[53]

Glossary

Alpha-2-macroglobulin: largest nonimmunoglobulin present in plasma which functions as an inhibitor of fibrinolysis and coagulation by inhibiting plasmin, kallikrein, and thrombin.
Complement proteins: these are distinct plasma proteins that include a number of proteases such as zymogens. At sites of infection the complement system is activated through a triggered-enzyme cascade.
Cytokines: a group of small proteins released from cells that include interleukins, interferons, and cell signal molecules which can modulate immune responses.
Digenetic trematodes: endoparasitic flatworms with complex life cycles involving one or more intermediate hosts with the majority inhabiting the alimentary canal and associated organs of vertebrates.
Extracellular vesicles (EVs): EVs are membrane-surrounded structures released by cells; major populations include exosomes, microvesicles, and apoptotic bodies.
Mass-spectrometry (MS): this is an analytical technique that helps identify the amount and type of chemicals present in a sample by measuring mass-to-charge ratio and abundance of gas-phase ions.
Metalloproteinases: a group of enzymes secreted into the space between cells in tissues that can digest proteins like collagen. These enzymes need zinc or calcium ions in the active site for their catalytic activity.
Protease inhibitors: these comprise a family of proteins which inhibit the function of proteases in biological systems.
RNA-seq: this is a next-generation sequencing technique that reveals the presence and quantity of RNA in a biological sample at a given time and can analyze the continually changing cellular transcriptome.
Secretome: organic molecules and inorganic elements secreted from biological cells, tissues and organisms.
Venom trypsin inhibitors: these proteins contain the basic pancreatic trypsin inhibitor (BPTI) domain and Kunitz inhibitor domain and have serine-type protease inhibitor activity.