REVIEW ARTICLE

Serine protease inhibitors of parasitic helminths

ADEBAYO J. MOLEHIN[1,2]*, GEOFFREY N. GOBERT[1] *and* DONALD P. McMANUS[1]

[1] *Molecular Parasitology Laboratory, Department of Biology, Queensland Institute of Medical Research, 300 Herston Road, Herston, Queensland, Australia 4006*
[2] *School of Population Health, The University of Queensland, Herston Road, Herston, Queensland, Australia 4006*

(*Received 13 October 2011; revised 29 November 2011; accepted 1 December 2011; first published online 6 February 2012*)

SUMMARY

Serine protease inhibitors (serpins) are a superfamily of structurally conserved proteins that inhibit serine proteases and play key physiological roles in numerous biological systems such as blood coagulation, complement activation and inflammation. A number of serpins have now been identified in parasitic helminths with putative involvement in immune regulation and in parasite survival through interference with the host immune response. This review describes the serpins and smapins (small serine protease inhibitors) that have been identified in *Ascaris* spp., *Brugia malayi, Ancylostoma caninum Onchocerca volvulus, Haemonchus contortus, Trichinella spiralis, Trichostrongylus vitrinus, Anisakis simplex, Trichuris suis, Schistosoma* spp., *Clonorchis sinensis, Paragonimus westermani* and *Echinococcus* spp. and discusses their possible biological functions, including roles in host-parasite interplay and their evolutionary relationships.

Key words: serine protease inhibitors, serpins, small serine protease inhibitors, smapins biochemical characterization, parasitic helminths.

INTRODUCTION

Serine protease inhibitors are a superfamily of proteins that were first identified as a set of proteins able to inhibit proteases; they play key roles in a variety of physiological and cellular functions and are associated with the vertebrate blood coagulation cascade, complement activation, inflammation, programmed cell death, cell development, and fibrinolysis (Marshall, 1993; Carrell *et al*. 1994; Huber and Carrell, 1989; Huntington *et al*. 2000; Gettins, 2002). The acronym 'serpin' was originally coined because many serpins acted by inhibiting chymotrypsin-like serine proteases (**ser**ine **p**rotease **in**hibitors) (Huntington *et al*. 2000). Serpins range in size from 350–400 amino acids with corresponding molecular weights of 40–60 kDa (van Gent *et al*., 2003), and they fall within two basic categories, namely inhibitory and non-inhibitory.

Serpins are thought to have evolved through gene duplication and divergence events, giving rise to a large number of serpin genes within an organism, each encoding a protein with a unique reactive region and physiological function(s) (Hunt and Dayhoff, 1980). This broad family of proteins was initially identified through similarities between the primary structure of 3 human proteins; anti-thrombin, α_1-protease inhibitor and chicken egg white albumin (Hunt and Dayhoff, 1980). Over 1000 serpins have now been described in viruses, bacteria, archaea, fungi, plants, eukaryotes and include 36 human proteins; they represent the largest and most diverse family of protease inhibitors (Rawlings *et al*. 2004). Many additional serpins are likely to be identified as more sequenced genomes become available. All serpins so far described have been classified into one of 16 clades, designated A through P, with an additional 10 unclassified 'orphan' sequences, all based on phylogenetic relationships (Irving *et al*. 2000). This review discusses serpin structure and function generally, and then details those serpins described to date for helminth parasites, emphasizing their possible biological functions, including their roles in the host-parasite interplay.

* Corresponding author: Molecular Parasitology Laboratory, Department of Biology, Queensland Institute of Medical Research, 300 Herston Road, Herston, Queensland, Australia 4006. Tel: +61 73362 0405. Fax: +61 73362 0104. E-mail: adebayo.molehin@qimr.edu.au

SERPIN STRUCTURE AND FUNCTION

Serpin structure

The structural archetype of the serpin superfamily is the main human blood plasma anti-proteolytic inhibitor α_1-antitrypsin (Axelsson and Laurell, 1965). All members of the serpin superfamily have a single common core domain consisting of 3 β-sheets and 8–9 α-helices, and this is responsible for the highly

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doi:10.1017/S0031182011002435

https://doi.org/10.1017/S0031182011002435 Published online by Cambridge University Press

unusual structural and functional properties of these proteins (Gettins, 2002; van Gent *et al*. 2003; Silverman *et al*. 2010; Whisstock *et al*. 2010). Serpins are also characterized by the presence of a single protein motif called the reactive centre loop (RCL) (Fig. 1). The RCL (Fig. 1) contains a scissile bond between the P1 and P1′ residues, which is recognized and subsequently cleaved by a target protease. The P1 residue acts as the 'bait' amino acid presented in the reactive centre which is thought to mimic the normal substrate of the target enzyme. All amino acids towards the N-terminal side of the scissile bond are labelled in order P1, P2, P3 etc, while those on the C-terminal side are labelled P1′, P2′, P3′ etc (Lawrence *et al*., 1994). The selectivity of a serpin for a particular protease is determined absolutely by this RCL (Elliott *et al*. 1996; Irving *et al*. 2000). The functionality of serpin family members as either inhibitory or non-inhibitory also depends on the structure of the RCL, which is generally composed of approximately 20 amino acids near the C-terminus and is the fastest evolving region within the serpin nucleotide sequence (Graur and Li, 1988). Inhibitory serpins are generally recognized by a consensus pattern in their sequences in the hinge region P17 P16, P15, P14, P12-P9 (Hopkins *et al*. 1993). P15 is usually glycine, P14 threonine or serine and positions P12-P9 are occupied by residues with short side-chains such as alanine, glycine or serine (Irving *et al*. 2000). These consensus residues are thought to permit efficient and rapid insertion of the RCL into the A β-sheet whereas, the corresponding regions of non-inhibitory serpins deviate from the consensus.

Five conformational states have been structurally characterized for serpins, differing primarily in their RCL structure (Fig. 1). These conformational states are referred to as native, cleaved, latent, δ and polymeric (van Gent *et al*. 2003). For inhibitory serpins, the native state is characterized by an exposed RCL that is accessible for interaction with a target protease. The transition from native to cleaved state is referred to as 'stressed to relaxed' (S−>R) because the cleaved state is generally associated with increased stability (Carrell and Owen, 1985). This S−>R transition is integral for serpin inhibitory function. The latent state is characterized by the insertion of an uncleaved RCL into the β-sheet-A, and was first described from the crystal structure of Plasminogen Activator Inhibitor-1 (PAI-1) (Mottonen *et al*. 1992). This latent state has also been described in human antithrombin (Carrell *et al*. 1994), α_1-antitrypsin (Lomas *et al*. 1995) and α_1-antichymotrypsin (Gooptu *et al*. 2000). The δ state represents an intermediate structural conformation between the native and latent states resulting from the oxidation of reactive centre residues as demonstrated with the crystal structure of δ-antichymotrypsin (Gooptu *et al*. 2000). Polymeric forms occur as a result of mutant serpins, aggregating together to form stable polymers. In humans, the aggregation of these mutant serpins in the organs where they are produced results in various human pathologies such as thrombosis, emphysema, cirrhosis and mental disorders (Gils and Declerck, 1998).

Serpin activity and stoichiometry of inhibition (SI)

As mentioned earlier, the structure of the RCL is a critical feature for serine protease inhibitors to undergo the conformational change necessary for inhibitory activity. Upon recognition and cleavage of the scissile bond between the PI and PI' residues by the target protease, the RCL forms an additional strand which inserts into the β-sheet A, effectively trapping the protease. This mechanism of inhibition involves the formation of a very stable complex between the cleaved inhibitor and the protease, similar in some respects to an enzyme-ligand complex (Irving *et al*. 2000). This tight association results in significant conformational changes in the serpin molecule, including the permanent loss of 37% of the structure and overall distortion of the protease (Huntington *et al*. 2000; Irving *et al*. 2000; van Gent *et al*. 2003). Huntington *et al*. (2000) showed that this permanent loss of the protease structure is a direct consequence of the limited length of the serpin RCL, which causes the 'plucking away' of the protease ester-linked serine from its catalytic partners, hence the name 'suicide' substrate inhibitors. The regions within the serpin molecule that are important in controlling and modulating its conformation change include the hinge, the breach, the shutter and the gate (Fig. 1a and 2). The basic mechanism of serpin inhibition is also known as the branched pathway suicide inhibition mechanism (Gettins, 2002). In this system, the protease recognizes and attacks the scissile bond of the reactive centre loop of the serpin thereby cleaving the bond.

There are 5 steps involved in the serpin inhibitory mechanism which are: (i) formation of an initial non-covalent Michaelis complex with the target protease; (ii) attack of the active-site serine on the peptide bond by the protease resulting in a tetrahedral intermediate (Peterson *et al*. 2000); (iii) cleavage of the peptide bond of the serpin to give a covalent acyl ester intermediate with the release of the first product, the free amino group of the peptide bond; (iv) formation of the second tetrahedral intermediate through attack of water; and then (v) departure of the second product (Gettins, 2002). In non-serpin inhibitors, the only step involved in inhibition is the initial recognition, with the specificity and stability of the complex being dependent on the nature and extent of interactions between the two proteins (Gettins, 2002). In contrast, the formation of the initial non-covalent complex between the inhibitory serpins and their target proteases influences the specificity and the rate of

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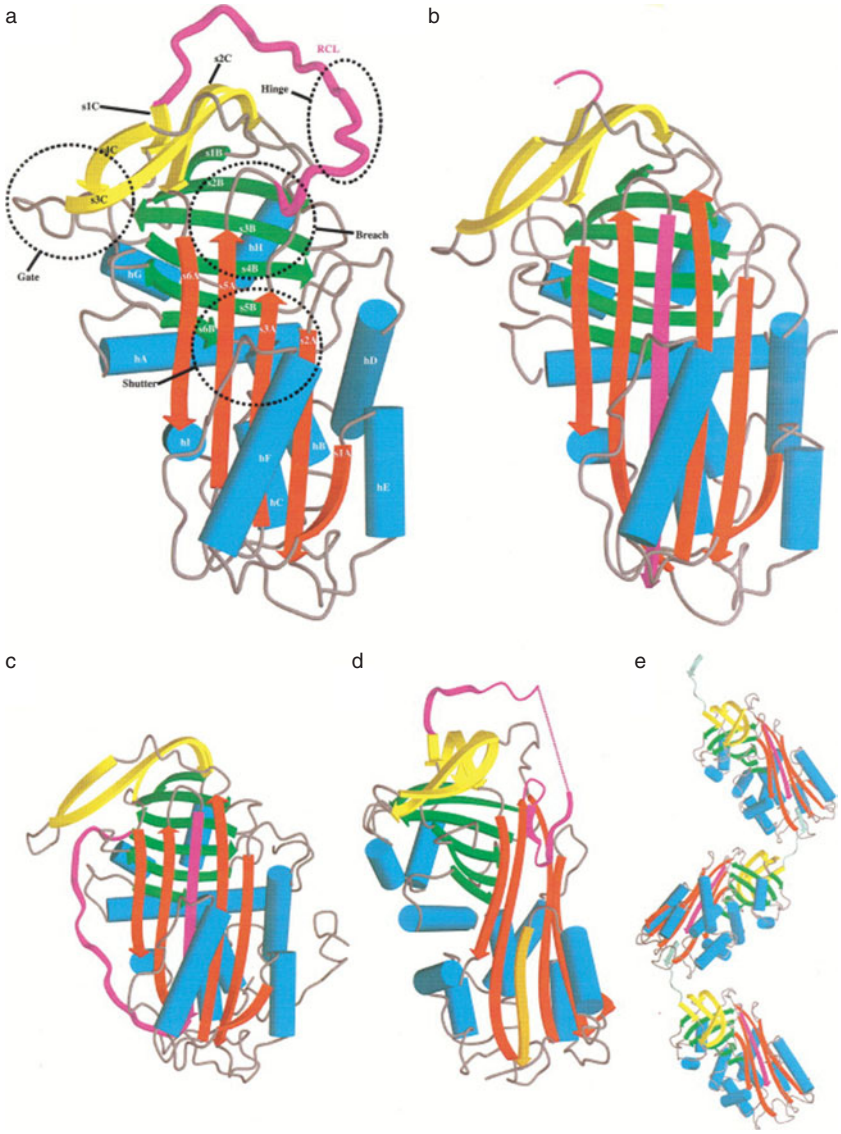

Fig. 1. Conformational states of serpins as differentiated by the reactive centre loop (RCL) structures (shown in magenta). (a) Native α1AT (adapted from Elliot *et al*. (2000)); (b) cleaved α1AT (adapted from Engh *et al*. (1989)); (c) latent anti-thrombin (d) the δ conformation of a variant of α_1-antichymotrypsin. Part of the F-helix is unwound and inserted into the bottom of the A β sheet (orange) (adapted from Gooptu *et al*. (2000)); (e) polymer of cleaved antitrypsin. In all parts of Fig. 1, the A β sheet is in red, the B β sheet in green and the C β sheet in yellow. The α helices are represented by cylinders coloured blue while the important breach, shutter, gate and hinge regions are shown by the broken circles. Adapted from Irving *et al*. (2000) with permission from Elsevier.

reaction since serpin inhibition goes beyond the formation of this non-covalent Michaelis complex.

In a 2D-Nuclear Magnetic Resonance (2D-NMR) study of the complex between S195A trypsin and α_1-PI-Pittsburgh (P1 Met – Arg), Peterson *et al*. (2000) found that, despite the extreme sensitivity of the serpin to conformational changes, as demonstrated by significant shifts of all alanine resonances, the conformation of the serpin body in the complex was still identical to that of the native serpin and no loop insertion of any RCL residue occurred into the β-sheet-A. This observation was later confirmed by Ye *et al*. (2001), who elucidated the X-ray crystal structure of the complex formed between the protease S195A trypsin and a different serpin (Serpin 1 K). The study by Ye *et al*. (2001) clearly showed there was no insertion of the RCL into the β-sheet-A and that there was considerable structural similarity between the body of the serpin in the complex and the native serpin.

The major molecular or functional consequence of the first serpin-serine protease inhibition pathway is the continuation of the proteolysis reaction and subsequent release of the cleaved form of the serpin. The second pathway involves the trapping of the acyl intermediate by disrupting the effectiveness of the protease to complete the proteolytic reaction as a result of the conformational change within the serpin and consequent distortion of the protease active site. Regions in the serine protease inhibitor that are

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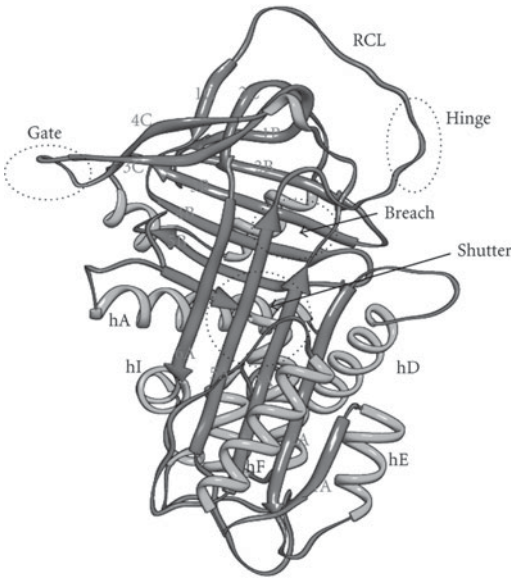

Fig. 2. Important domains in serpin conformations. Several regions are important in controlling and modulating serpin conformational changes. The *Reactive Centre Loop* is involved in protease recognition and conformational transformation as strand 4A after inhibition. The P15–P9 portion of the RCL is called the *hinge* region. The point of initial insertion of the RCL which is the *breach* region, located at the top of the A β-sheet. Near the center of A β-sheet is the *shutter domain*. The breach and shutter are 2 major regions that assist sheet opening and accept the conserved hinge of the RCL when it inserts. The *gate region* is composed of s3C and s4C strands which has been primarily observed by studies of the transition latency. The image was drawn in chimera using the PDB file of native antitrypsin conformation. Adapted from Khan *et al*. (2011) with permission from the *Journal of Amino Acids*.

crucial for controlling and modulating the conformational change are shown in Fig. 2.

HELMINTH SERPINS AND SMAPINS

A summary of the serpins and small serine protease inhibitors (smapins) identified in parasitic nematodes, trematodes and cestodes is provided in Tables 1 and 2. A description of their individual characteristics now follows.

PARASITIC NEMATODE SERPINS AND SMAPINS

Nematodes occupy a relatively low place in invertebrate evolution (Aguinaldo *et al*. 1997) but many of their parasitic representatives are of significant veterinary and medical importance, particularly as over two billion people are infected in tropical countries (Michael *et al*. 1996; Zang *et al*. 1999; de Silva *et al*. 2003; Meeusen *et al*. 2005). Useful reviews of parasitic nematode serpins are available (Zang and Maizels, 2001; Knox, 2007). Nematode serpins have limited sequence homology to their mammalian counterparts, although the key amino acid residues required for tertiary structure and functionality are well conserved, with the commonality of hypervariability restricted to the RCL (Zang and Maizels, 2001). This hypervariability is thought to result from the unusually high rates of non-synonymous substitutions occurring within the reactive site loops (Hill and Hastie, 1987; Goodwin *et al*. 1996). Through the analyses of nucleotide sequences, Zang and Maizels (2001) were able to clarify many evolutionary aspects of nematode serpins, specifically by comparing the genomic sequences of 8 genes encoding *Caenorhabditis elegans* serpins and a novel *Brugia malayi* serpin gene. The intron map of the 3′ end of the genes showed varied patterns with no single conserved position between the two organisms, and not even within *C. elegans* itself, suggesting that the extreme divergence in the position of introns may be indicative of the functional constraint for the C-terminus of the protein.

Two distinct nematode serpin families have been identified by this gene sequence database mining (Zang and Maizels, 2001). One family exhibits particular homology to mammalian serpins in terms of predicted structure based on nucleotide sequence, and a second encodes a totally different novel group of small proteins of less than 100 amino acids. Members of the latter family have been termed smapins (small serine protease inhibitors) and appear unique to parasitic nematodes, with no relatives from free-living nematodes or any other taxa evident (Zang and Maizels, 2001). Smapins have been identified in *Ascaris suum* (Grasberger *et al*. 1994), *Anisakis simplex* (Kobayashi *et al*. 2007*a*), *Onchocerca volvulus* (Ford *et al*. 2005), *Trichuris suis* (Rhoads *et al*. 2000*a*, *b*)), *Ancylostoma caninum* (Duggan *et al*. 1999). The major distinguishing feature of the smapin family is the presence of 10 cysteine residues that form 5 disulphide bonds at the protein core. Structural studies of smapins from *A. suum* (Grasberger *et al*. 1994) and *A. caninum* (Duggan *et al*. 1999), using nuclear magnetic resonance (NMR), identified 2 anti-parallel strands of β-sheets with the remainder of the tertiary structure consisting of extended loops and turns.

Serpins from Brugia malayi

Brugia malayi is one of the causative agents of human lymphatic filariasis. Its life cycle involves mosquitoes and humans and a correspondingly complex set of interactions with the human host immune system (Maizels *et al*. 1993). Only 2 serpin genes, designated *Bm-SPN-1* (Yenbutr and Scott, 1995) and *Bm-SPN-2* (Zang *et al*. 1999), have been characterised so far from *B. malayi*. Yenbutr and Scott (1995) employed reverse transcription PCR-based technology, which exploited the presence of a conserved

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Table 1. Characteristics of serpins from parasitic helminths

Serpin	Molecular size	Type	Species	Stage and/or localization	Target protease	Reference
Bm-SPN-1	44 kDa	Secretory	*B.malayi*	All life cycle stages	Unknown	Yenburt and Scott (1995)
Bm-SPN-2	47·5 kDa	Secretory	*B.malayi*	Microfilariae	Neutrophil elastase, cathepsin G	Stanley and Stein (2003) Zang *et al*. (1999); Zang *et al*. (2000)
Hc-serpin	63 kDa	Intracellular	*H. contortus*	Adult gastrointestinal tract epithelial cells	Anticoagulant/ trypsin	Yi *et al*. (2010)
Ts11 – 1	42 kDa	Intracellular	*T. spiralis*	Muscle larvae	Trypsin	Nagano *et al*. (2001)
TvSERP	42 kDa	Intracellular	*T. vitrinus*	All life cycle stages	Elastases, trypsin, cathepsin G, mast cell proteases	MacLennan *et al*. (2005)
SH serpin	46·2 kDa	Surface	*S. haematobium*	Adult worm tegument	Unknown	Blanton *et al*. (1994); Li *et al*. (1995)
Smpi56	56 kDa		*S. mansoni*	Adult worms	Neutrophil and pancreatic elastase	Ghendler *et al*. (1994)
Contrapsin	68 kDa	Surface and intracellular	*S. mansoni*	Male adult worms	Trypsin	Modha *et al*. (1994)
Sj serpin	45·2 kDa	Surface	*S. japonicum*	Intestinal epithelium of adult worms and cercariae	Unknown	Yan *et al*. (2005)
CsproSERPIN	42·2 kDa	Intracellular	*C. sinensis*	Metacercariae	Unknown	Yang *et al*. (2009)
CsSERPIN	44 kDa	Intracellular	*C. sinensis*	All life cycle stages	Chymotrypsin	Kang *et al*. (2010)
PwSERPIN	43 kDa	Intracellular	*P. westermani*	All life cycle stages	Human neutrophil cathepsin G, human and porcine elastases	Hwang *et al*. (2009)
Serpin^{Emu}	45 kDa	Intracellular	*E. multilocularis*	Oncospheres	Trypsin and elastase	Merckelbach and Ruppel (2007)
Antigen B C-terminal	12 kDa	Secretory	*E. granulosus*	Protoscoleces/ germinal layer	Porcine elastase	(Shepherd *et al*. 1991)

22-nucleotide spliced sequence present at the 5′ end of a proportion of nematode transcripts, and cloned a PCR product that encoded the first described nematode serine protease inhibitor. Sequence analysis showed that the PCR product was 1287 bp long and the estimated molecular weight of the predicted protein was 44 kDa. Further reverse transcription PCR analysis showed that the protein – termed Bm-SPN-1- was expressed in all life stages of the parasite. These authors also demonstrated that Bm-SPN-1 was immunogenic in gerbils and that it was strongly recognized by sera from immunized animals suggesting that Bm-SPN-1 may play a role in the survival of the parasite during the early phase of its development in the vertebrate host.

In order to identify prominent antigens from blood-borne *B. malayi* microfilariae (mf) larvae that might be recognized by host T lymphocytes, Zang *et al*. (1999) identified a mf fraction containing proteins of 35-55 kDa in size that proved highly potent at inducing antigen-specific T-cell proliferation and cytokine production. Immunoscreening of an mf cDNA library isolated a clone encoding a native serpin protein termed Bm-SPN-2 with a molecular mass of 47·5 kDa. The expression of Bm-SPN-2 was highly stage-specific, being expressed only in the mf as one of the most abundant proteins of this life-cycle stage (Zang *et al*. 1999). Bm-SPN-2 was tested for its ability to inhibit a panel of mammalian serine proteases with differing substrate specificity and functions, but only neutrophil serine protease, elastase and cathepsin G were inhibited in a dose-dependent and highly specific manner. This high specificity of inhibition was confirmed by the fact that Bm-SPN-2 showed no cross reactivity with bovine pancreatic α-chymotrypsin or porcine

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Table 2. Characteristics of nematode smapins

Smapin	Molecular size	Type	Species	Stage and/or localization	Target protease	Reference
Chymotrypsin/ elastase isoinhibitors	60–66 amino acids	Intracellular	*A. suum*, *A. lumbricoides*	Egg, adult muscle, sperm and intestine	Chymotrypsin	Matzen *et al.* (1985), Matzen *et al.* (1986), Peanasky *et al.* (1984)
AcAPc5, 6	8·7 kDa	Secretory	*A. caninum*	Cephalic/amphidial glands	fXa	Cappello *et al.* (1995), Stanssens *et al.* (1996)
AcAPc2	8·7 kDa	Secretory	*A. caninum*	Oesophagus	fVIIa/TF	Stanssens *et al.* (1996)
Ac-AP-12	9·1 kDa	Secretory	*A. caninum*	Adult oesophagus	fXa	Jiang *et al.* (2011)
Ov-SPI-1, 2	7·5–11 kDa	Intracellular	*O. volvulus*	All life cycle stages	Elastase, chymotrypsin,	Ford *et al.* (2005)
Ani s 6	62 amino acids	Excretory/ Secretory	*A. simplex*	L3 larva	trypsin and cathepsin G. α-chymotrypsin	Kobayashi *et al.* (2007*a*)
TsCEI	6·4 kDa	Secretory	*T. suis*	Adult stage	Chymotrypsin, pancreatic	Rhoads *et al.* (2000*b*)
NAP5, NAP6 and NAPc2	75–84 amino acids	Secretory	*A. caninum*	Adult oesophagus	elastase, cathepsin G. factors VIIa and Xa	Duggan *et al.* (1999)

pancreatic elastase in a dose-specific manner, 2 enzymes with similar substrate specificity to neutrophil cathepsin G and elastase, respectively. It is noteworthy that neutrophil-derived cathepsin G is known to be an important chemoattractant for monocytes (Chertov *et al.* 1997). Mice infected with *B. malayi* mf mounted a strong, but short-lived Bm-SPN-2-specific Th1 response with significant increases in IFN-γ production (Zang *et al.* 1999, 2000). Filariasis patients elicited a potent Th2 immune response to Bm-SPN-2 in both IgG1 and IgG4 antibody subclasses (Zang *et al.* 2000). Overall, these studies suggested that Bm-SPN-2 functions by neutralizing the immunostimulatory properties of the host cathepsin G, thereby contributing to the longevity and pathogenicity of mf in the mammalian bloodstream.

However, the complete picture regarding the function of Bm-SPN-2 *in vivo* has yet to be determined, as a subsequent study by Stanley and Stein (2003) failed to repeat the earlier results of Zang *et al.* (1999). This group cloned the *Bm-SPN-2* gene from a different mf cDNA library, expressed the Bm-SPN-2 protein in *E. coli,* and characterized its structural and functional properties (Stanley and Stein, 2003). Sequence alignment, circular dichroism spectroscopy, and susceptibility to cleavage by proteases suggested that the Bm-SPN-2 shared the tertiary structure typical of the serpin family, including an accessible reactive centre loop (Irving *et al.* 2000). However, the protein had no effect on the activity of neutrophil elastase or cathepsin G, did not form SDS-stable complexes with these proteases, and did not undergo the characteristic stressed to relaxed transition required for protease inhibition by serpins. These authors concluded that Bm-SPN-2 was a new non-inhibitory serpin, in keeping with its sequence.

Smapins from Onchocerca volvulus

Onchocerca volvulus is another filarial nematode parasite of humans causing onchocerciasis. Ford and colleagues (2005) adopted a transcriptomics approach to identify novel proteins from *O. volvulus* involved in the parasite moulting process. Analysis of the datasets derived from expression sequence tags (ESTs) of cDNA libraries constructed from the infective third-stage larva (L3) and molting L3 (mL3) of *O. volvulus* identified novel cysteine proteases involved in the moulting process (Hashmi *et al.* 2002; Guiliano *et al.* 2004). In addition to these cysteine proteases, these authors also identified a novel family of small molecular weight serine protease inhibitor (*Ov*-SPI-1 and *Ov*-SPI-2) with structural similarity to smapins already identified in *A. suum* (Peanasky *et al.* 1984; Martzen *et al.* 1985, 1986) and hookworm (Stassens *et al.* 1996). The expression profile for *Ov-spi-1* and *Ov-spi-2* genes demonstrated that both genes were expressed in all life stages of the parasite with

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expression increasing during moulting larval stages and reproducing adult worms. Immunolocalization of the native *Ov*-SPI proteins carried out with specific antibodies raised against r*Ov*-SPI-1 showed that *Ov*-SPI-1 and -2 were endogenous proteins found within the body channels, multivesicular bodies and in the basal layer of the cuticle of the L3 larva. Protease inhibition assays carried out showed that *Ov*-SPI-1 reduced the enzymatic activity of a panel of serine proteases including elastase, chymotrypsin, trypsin and cathepsin G. However, although the specific endogenous target enzyme of the *Ov*-SPI-1 was not identified, the authors suggested that *Ov*-blisterase, a subtilisin-like serine protease (Poole *et al*. 2003), could be the potential target of the *Ov*-SPI proteins since *Ov*-blisterase was shown to co-localize with *Ov*-SPI proteins to the same regions of the curticle during moulting of the *O. volvulus* L3 s.

Indirect evidence for involvement of the Ov-SPIs in immune regulation was reported by Ford *et al*. (2005) who showed that these proteins are antigenic and strongly recognized by persons previously exposed to *O. volvulus,* suggesting that Ov-SPIs are released from the parasite during the early stages of the parasite establishment in the host. The mechanism(s) involved in the possible release of these endogenous Ov-SPIs remains unknown.

Serpins from Haemonchus contortus

Haemonchus contortus is an important parasitic nematode of veterinary importance that affects the gastrointestinal tract of ruminant animals, especially sheep, goats and cattle, in various regions of the world (Meeusen *et al*. 2005). In a recent study, a serpin from *H. contortus* termed Hc-Serpin was identified and its biological activities described. The rHc-Serpin inhibited trypsin activity effectively and prolonged the coagulation time of rabbit blood *in vivo*. Thermostability assays indicated that the rHc-Serpin was thermally inert, maintaining its proteolytic activity even at temperatures above 75 °C. Immunohistochemistry, using rat anti-rHc-Serpin antibodies, showed that native Hc-Serpin was localized exclusively to the epithelial cells of the gastrointestinal tract in adult worms (Yi *et al*. 2010). Analysis of its deduced amino acid sequence showed the serpin was devoid of a typical signal peptide cleavage site at its N-terminal end, suggesting an intra-cellular location. However, the rHc-Serpin was recognized by serum from goats naturally infected with *H. contortus* indicating exposure to the host immune system.

Three possible explanations were provided by Yi *et al*. (2010) the authors for the recognition of the Hc-Serpin protein by serum from naturally infected hosts. First, the protein may be exposed to the immune system on the death of the adult nematodes. Second, the larvae (L3, L4, and L5) of *H. contortus* are killed by the host immune response, and the killing process exposes many internal cytoplasmic components that are expressed by all life stages of the parasite. Third, some intracellular proteins from the L4/L5 larvae and adults of *H. contortus* may be excreted through undefined pathways and be recognized by the host immune system. It was further suggested that the internalization of the Hc-serpin by host tissues is an active process and that the targets of the serpin are the host proteases rather than endogenous parasite proteases (Yi *et al*. 2010). The release of intra-cellular proteins *in vitro* is thought to depend on the presence of secretory vesicles (Zhang *et al*. 2006; Merckelbach and Ruppel, 2007). However, the precise mechanism as to how the Hc-Serpin is shed into host tissue is still unknown, and the role it plays in the host-parasite interplay warrants further research.

A serpin from Trichinella spiralis

Trichinella spiralis infects the skeletal muscles of a wide variety of vertebrate hosts including pigs, rats, horses, wild animals and humans causing zoonotic trichinellosis (Nagano *et al*. 2001; Mitreva *et al*. 2011). Nagano *et al*. (2001) isolated a cDNA clone – Ts11-1 – from a cDNA library constructed from the muscle larvae of *T. spiralis* that encoded a recombinant protein with protease inhibitory activity. The 42 kDa recombinant protein encoded by the Ts11-1 clone was cloned, expressed in a prokaryotic system and purified. Multiple sequence alignment of the predicted amino acid sequence of the Ts11-1 clone with serpins from *Caenorhabditis elegans* serpin and *B. malayi* (Bmserp) indicated that Ts11-1 was a serpin because of its sequence homology to these proteins at the putative reactive region. This conclusion was further strengthened by the inhibition of trypsin activity *in vitro* when co-incubated with recombinant Ts11-1. Nagano *et al*. (2001) showed that Ts11-1 was expressed only in the early developmental stage of the muscle larvae of *T. spiralis*, but further studies are required to more fully understand its biochemical and biological functions.

A serpin from Trichostrongylus vitrinus

Trichostrongylus spp. are the cause of ovine parasitic gastroenteritis. MacLennan *et al*. (2005) isolated a novel serpin (TvSERP) cDNA from *Trichostrongylus vitrinus* following the screening of a cDNA library prepared from adult worms with rabbit antisera to adult excretory/secretory products. Sequence analysis of the predicted protein sequence for TvSERP indicated the absence of a signal sequence but the presence of 4 N-linked glycosylation sites. A phylogenetic comparison of the TvSERP sequence with serpins from other invertebrates and vertebrates

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showed that the protein was most closely related to *C. elegans* serpins. Immunoblot analysis showed that TvSERP was expressed in all life-cycle stages of the parasite and that it formed complexes with other *T. vitrinus* proteins suggesting a functional role in regulating its endogenous proteases. The serpin was recognized by antibodies in the serum and lymph of lambs immunized with recombinant TvSERP. Protease inhibition assays showed that TvSERP inhibited not only serine proteases of *T. vitrinus* origin but also those produced by the host, including those of potential importance for host anti-parasite immune responses such as mast cell proteases (Miller, 1984). Although these data did not prove a specific biological function for TvSERP, they did indicate possible roles in the regulation of *T. vitrinus* serine proteases as well as in modulation of the host immune response by inhibiting the activity of serine proteases released from host inflammatory cells (MacLennan *et al*. 2005). Additional studies are necessary to more fully understand the complete biological role of TvSERP and its possible function in worm survival in the host intestine.

Smapins from Ascaris *spp*.

Two early studies showed that the activities of host proteases such as trypsin and chymotrypsin, disappeared from the micro-environment of live *Ascaris suum* with a functioning gastrointestinal system (Juhasz and Nemeth, 1979; Hogan, 1980). Both studies revealed that the only proteases removed from the environment were those for which the parasite had developed inhibitors, although the mechanism involved with the disappeance of the proteases was not determined. A subsequent immunolabelling study by Martzen *et al*. (1985) showed that host chymotrypsin co-localized with *A. suum* chymotrypsin/elastase isoinhibitors in the muscle sarcolemma, in developing eggs and larvae, as well as at the epithelial surface of the gut of the adult parasite; inactive complexes were formed, indicating a possible role in protecting the parasite from host digestive attack. The serpin-host protease complex formation may also mask the surface of the developing migrating larvae and promote effective evasion from the host immune system (Martzen *et al*. 1985, 1986).

Five isoinhibitors (1–5) of chymotrypsin/elastase have been isolated and purified from *A. lumbricoides* by CM-Sephadex C-25 column affinity chromatography (Peanasky *et al*. 1984). They comprise 63–66 amino acids with 10 cysteine residues (Babin *et al*. 1984), the characteristic feature of smapins. Protease inhibition assays carried out with these 5 isolated isoinhibitors showed that each reacted more strongly with chymotrypsin than any other serine protease tested. The assays showed also that these isoinhibitors reacted very strongly with porcine elastase-1 suggesting that chymotrypsin and elastase may be the possible targets of these inhibitors. Nevertheless, the precise roles that these isoinhibitors might play in the survival of *A. lumbricoides* in the host intestine remain unknown.

Smapins from Anisakis *simplex*

Anisakis simplex is a marine nematode worm parasite of fish that frequently causes gastrointestinal symptoms in humans, which may be associated with mild to severe immunological, usually allergic-type, reactions (Audicana and Kennedy, 2008). To date, 8 *A. simplex* allergens have been described at the molecular level (Ani s 1 to Ani s 8) (Audicana and Kennedy, 2008). Of these, the ES-derived Ani s 6 is a smapin and the first identified nematode serpin causing allergy in humans; it was shown to inhibit α-chymotrypsin but not trypsin in a dose-dependent manner, and may act as a blood anticoagulant inhibiting the serine proteases, factors Xa and VIIa (Audicana and Kennedy, 2008; Kobayashi *et al*. 2007*a*,*b*). Earlier, Lu *et al*. (1998) isolated 3 elastase isoinhibitors from *A. simplex* and reported the presence of a hypervariable region within the reactive site centres; sharing 95–98% amino acid sequence identity, these serpins may be involved in reproduction although the serine proteases they inhibit have not been determined.

Smapins from Ancylostoma *caninum*

Hookworms cause anaemia in their mammalian hosts as they feed on blood from capillaries of the small intestine (Cappello *et al*. 1995). Like other haematophagous invertebrates, hookworms have evolved potent anti-clotting strategies to facilitate blood feeding. Three different smapins with anticoagulant properties (NAP5, NAP6 and NAPc2) were identified and characterized from the dog hookworm, *Ancylostoma caninum* by Duggan *et al*. (1999). These NAPs are 75–84 residues long and contain the 10 cysteine residues, paired into 5 disulfides, typical of smapins. Being highly potent and specific inhibitors of the serine proteases, factors VIIa and Xa, the key physiological initiators of blood coagulation, they have been targeted as novel anticoagulants for treatment of thrombotic disorders.

Earlier, Cappello *et al*. (1995) purified and biochemically characterized another hookworm-derived blood-clotting inhibitor of human coagulation factor Xa, termed *A. caninum* anticoagulant peptide (AcAP). Amino acid analysis of the purified protein showed that this inhibitor was made up of 71 amino acids with a molecular weight of 16·5 kDa. Protease inhibition assays carried out with several serine proteases indicated that AcAP specifically inhibited factor Xa and not trypsin, chymotrypsin or thrombin. Pro-thrombin time (PT) and activated partial thromboplastin time (PTT) are standard

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blood-clotting time assays used to measure the time it takes for blood to clot and AcAP was shown to prolong both, suggesting that interfering with the ability of the adult worm to feed on host blood may lessen the morbidity of chronic hookworm infection. Determination of the first 30 amino acids of the recombinant AcAP revealed a unique partial sequence with heterogeneity at 2 distinct positions suggesting the presence of more than one protein responsible for the anticoagulant activity observed.

This hypothesis was subsequently confirmed by Stassens *et al*. (1996) who identified and characterized 3 homologous small protein anticoagulants from *A. caninum*, termed AcAPc2, AcAPc5 and AcAPc6; these authors showed that AcAPc5 and AcAPc6 directly inhibited factor Xa while AcAPc2 predominantly inhibited the catalytic activity of a complex composed of blood coagulation factor VIIa and tissue factor fVIIa/TF. Homologues of AcAPc2 (AcAPc3 and AcAPc4) with the same substrate specificity have also been characterised (Mieszczanek *et al*. 2004). Very recently, another novel small serine protease inhibitor anticoagulant peptide, designated Ac-AP-12, was identified and shown to be expressed exclusively in the adult stage of the parasite (Jiang *et al*. 2011). RT-PCR, Western blotting and immunolocalization studies with an anti-Ac-AP-12 rabbit anti-serum showed that the protein was expressed only in the adult stage of the parasite. Multiple sequence analysis of the predicted amino acid sequence of the protein showed 43–60% identity to the other anticoagulant peptides previously described in *A. caninum*. Phylogenetic analysis showed that Ac-Ap-12 belongs to the group of factor Xa inhibitors (Jiang *et al*. 2011) and, like the other *A. caninum* serpins, it may be suitable for development as a blood-clotting agent.

Serpins from Trichuris suis

The swine whipworm, *Trichuris suis*, inhabits the caecum and colon of infected pigs and can cause severe mucohaemorrhagic enteritis. Rhoads *et al*. (2000*a*) identified a trypsin inhibitor, termed TsTCI, in extracts of adult *T. suis* and culture fluid from a 24-h *in vitro* cultivation of adult parasites. Elastase, thrombin, and factor Xa were not inhibited. The cDNA-derived amino acid sequence of the mature TsTCI consisted of 61 residues including 8 cysteine residues with a molecular weight of 6·687 kDa.

The same group (Rhoads *et al*. 2000*b*) purified another serpin, termed TsCEI, with an estimated molecular weight of 6·437 kDa from adult *T. suis*. TsCEI potently inhibited both chymotrypsin and pancreatic elastase. Neutrophil elastase, chymase (mouse mast cell protease-1, mMCP-1) and cathepsin G were also inhibited by TsCEI, whereas trypsin, thrombin, and factor Xa were not. The cDNA-derived amino acid sequence of the mature TsCEI consisted of 58 residues including 9 cysteine residues with a molecular mass of 6·196 kDa. TsCEI displayed 48% sequence identity to TsTCI. These two smapins from *T. suis* may function as components of a parasite defence mechanism by modulating intestinal mucosal mast cell-associated, protease-mediated, host immune responses (Rhoads *et al*. 2000 *a*,*b*).

TREMATODE SERPINS

Serpins from Schistosoma *spp*.

Schistosomes have evolved highly efficient mechanisms, including the expression of serpins to counteract potentially damaging host proteases, which allow them to persist long term in their hosts (Blanton *et al*. 1994).

In an attempt to identify protein(s) in schistosomes that may be involved in inhibiting host clotting mechanisms, Blanton *et al*. (1994) screened a cDNA library constructed from *Schistosoma haematobium* with specific human antisera and identified a clone termed SHW 4-2, with a predicted amino acid sequence belonging to the serpin gene superfamily. Analysis of the cDNA clone showed that the sequence had 1 open reading frame predicting a 409 amino acid protein. Multiple sequence alignment revealed that the SHW 4-2 cDNA exhibited greatest sequence similarity to the glial-derived nexins and anti-thrombin whose specific targets are thrombin (Monard *et al*. 1990), indicating a possible role in inhibition of blood coagulation. Immunolocalization studies showed that the *S. haematobiun* serpin was present on the surface of the parasite and, therefore, able to interact with host cells and proteases. The serpin was species-specific being recognized only by sera from *S. haematobium*-infected individuals (Blanton *et al*. 1994). The species specificity of this serpin was subsequently confirmed by Li *et al*. (1995) who, additionally, characterized the human IgG4 and IgE antibody isotype responses to the molecule. The crystal structure of this *S. haematobium* serpin was obtained by Huang *et al*. (1999) who demonstrated that the protein formed a tight covalent complex with human trypsin *in vitro,* suggesting that the parasite might be using this serpin-trypsin complex to evade the host immune response by reducing the immunogenicity of the exposed serpin. Another possibility might be that the parasite uses this serpin-host trypsin complex to reduce the proteolytic activity of the host proteases.

Ghendler *et al*. (1994) isolated and characterized another novel serpin from *S. mansoni*. The serpin was partially purified from an adult worm extract by gel filtration on an HPLC superose-12 column as a complex with a 28 kDa protease, and the protease-inhibitor complex immunoprecipitated with rabbit

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anti-28 kDa protease antibodies. Analysis of the immunoprecipitated proteins by SDS-PAGE and autoradiography demonstrated a major band at 74 kDa which represented a protease-inhibitor complex. Incubation of [^{35}S] methionine-labelled adult worm extracts with biotinylated elastase and subsequent precipitation with streptavidin-agarose isolated the 74 kDa band and 2 other smaller bands of 64 kDa and 56 kDa. Antibodies raised in rabbits against the inhibitor-biotinylated elastase-streptavidin-agarose complex immunoprecipitated a protein of 56 kDa from metabolically labelled and extracted AW proteins; hence this novel AW protease inhibitor was named *S. mansoni* protease inhibitor56 (Smpi56) (Ghendler *et al*. 1994). Protease inhibition assays showed that Smpi56 strongly bound and inhibited human neutrophil elastase suggesting that Smpi56 might protect the parasite from elastase released from neutrophils.

A schistosome homologue of mouse contrapsin – a serpin present in serum that reacts specifically with trypsin-like proteases (Nathoo *et al*. 1982; Takahara and Sinohara, 1983*a*,*b*) – has been identified in *S. mansoni* adult worm homogenates (Modha and Doenhoff, 1994). Modha and Doenhoff (1994) demonstrated that contrapsin from mouse serum and from *S. mansoni* homogenates were immunologically identical, despite the significant difference in their molecular weights. These authors showed that contrapsin is a tegumental protein which bound to and inhibited host trypsin with high specificity and the binding caused the serpin to lose its immunogenicity so that an antibody response was not mounted (Modha and Doenhoff, 1994). Additional studies are required to determine the precise biological function(s) of this *S. mansoni* serpin and to investigate further its possible role in host immune evasion.

Microtus fortis is an Asian vole that is naturally resistant to *S. japonicum* infection (He *et al*. 1999). With the aim of identifying *S. japonicum* molecules associated with this resistance, Yan *et al*. (2005) screened an adult worm cDNA expression library with sera from *M. fortis* and identified a cDNA clone that encoded a sequence homologous to the serpin superfamily. Full-length sequence analysis of the Sj serpin clone revealed a 1200-bp open reading frame encoding a protein of 400 amino acids. Multiple sequence alignment of the *S. japonicum* reactive centre loop (RCL) showed high sequence similarity with serpins from *S. mansoni* (Smserpin Accession number AAA29938) and *S. haematobium* (SH serpin) (Huang *et al*. 1999). Sj serpin is a tegumental protein that is only expressed in the adult and cercarial stages of the parasite. C57BL/6 mice immunized with the Sj serpin induced the production of high levels of specific IgE and IgG1 antibodies as well as a marked IL-4 response. Lymphocyte surface marker analysis revealed proliferation of CD19-expressing B cells, indicating a predominant Th2-type response to the serpin. Immunized mice developed some protection against *S. japonicum* suggesting a potential role for Sj serpin as a vaccine candidate or as a novel target for anti-schistosome drugs although additional study is required to characterize the precise biological function(s) of this protein as well as its possible role in host immune modulation.

Using phylogenetic analysis, published sequences and information from the completed and annotated genomes of *S. mansoni* (Berriman *et al*. 2009) and *S. japonicum* (*Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium, 2009), Quezada and McKerrow (2011) identified 2 major serpin clades with homology to the gene encoding human α1-antitrypsin; there were 8 serpin gene sequences in the *S. mansoni* database compared with 4 serpin genes in the *S. japonicum* gene database. Most of the variation in serpin genes occurred in the reactive centre loop (RCL) and these authors suggested the greater multiplicity of serpin genes in *S. mansoni* perhaps reflects adaptation to infection of the human host.

Serpins from Clonorchis sinensis

Clonorchis sinensis is endemic to Southeast Asia, resides in the liver of humans and many other mammals, and causes clonorchiasis (Lun *et al*. 2005). A cDNA of 1149 bp encoding a novel serpin of 42·2 kDa (CsproSERPIN) has been isolated and characterized from *C. sinensis* (Yang *et al*. 2009). Semi-quantitative RT-PCR analysis of the infective metacercaria and adults showed a higher level of CsproSERPIN expression in the former suggesting an important biological role, possibly in metacercarial excystment (Yang *et al*. 2009). Kang *et al*. (2010) biochemically characterized another serpin (CsSERPIN) with a molecular weight of 44 kDa from *C. sinensis*. While transcriptional analysis of CsSERPIN showed expression in all developmental stages of the parasite, the highest levels were seen in adults and eggs. Amino acid sequence analysis demonstrated that CsSERPIN lacked a N-terminal signal peptide, a C-terminal extension and a transmembrane domain, suggesting a cytosolic location, a feature supported by phylogenetic and immunoblotting analyses (Kang *et al*. 2010). Immunofluorescence studies showed that CsSERPIN was localized in the eggs within the uterus and in the vitelline glands of adult worms (Kang *et al*. 2010). Protease inhibition assays carried out with a panel of mammalian serine proteases revealed that CsSERPIN inhibited the enzymatic activity of chymotrypsin in a dose-dependent manner but showed little or no inhibitory activity against trypsin, thrombin, elastases or cathepsin G. Due to its localization in the uterine eggs, CsSERPIN may be involved in the development and/or maturation of the miracidia within the egg by modulating the activities

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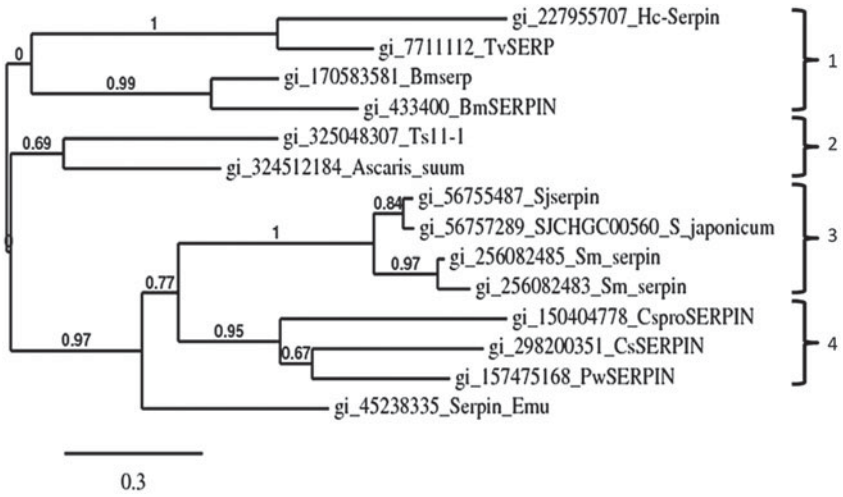

Fig. 3. Multifurcating phylogenetic tree showing relationships between a number of the helminth parasite serpins described in this review. The 14 serpins were aligned using MUSCLE and a bootstrapped maximum likelihood tree was generated using PhyML 3.0. The branch bootstrap support values are shown on branch splits. The analysis was carried out as described by Dereeper *et al*. (2008).

of the parasite's endogenous serine proteases (Kang *et al*. 2010).

A serpin from Paragonimus westermani

Paragonimus westermani is of both public health and veterinary importance, causing pulmonary and/or extrapulmonary granulomatous disease in humans and other mammalian hosts. Hwang *et al*. (2009) obtained a complete cDNA sequence encoding a novel serine protease inhibitor (PwSERPIN) from *P. westermani* during analysis of EST sequences randomly selected from an adult worm cDNA library. Subsequent analysis of the PwSERPIN sequence indicated that it was probably a cytosolic protein (Hwang *et al*. 2009). Although PwSERPIN was shown to be expressed in all stages of the life cycle there was a clear gradual increase in transcription levels as the parasite developed from metacercaria to adult. It effectively inhibited porcine trypsin, bovine chymotrypsin and human thrombin but had little inhibitory activity against human neutrophil cathepsin G or human and porcine elastases, suggesting a role in the regulation of endogenous cytosolic serine proteases (Hwang *et al*. 2009).

CESTODE SERPINS

The cestodes are a highly diversified group and cause a range of diseases including echinococcocosis, and taeniasis/cysticercosis (Spakulová *et al*. 2011).

Serpins from Echinococcus spp.

Antigen B (EgAgB) is a highly immunogenic protein produced in great abundance by the larval hydatid cyst of *Echinococcus granulosus* (Li *et al*. 2003; Zhang *et al*. 2003, 2010). The protein, encoded by 5 subclasses of at least 10 genes (Zhang *et al*. 2010), is synthesized and secreted by the cyst germinal layer and protoscoleces (Sanchez *et al*. 1991) but its precise function remains unclear.

With the aim of identifying *E. granulosus* antigens that might interfere with the host immune response, Shepherd *et al*. (1991) isolated and characterized the smallest 12 kDa subunit of EgAgB. Multiple sequence alignment of the deduced amino acid sequence of the 12 kDa-subunit with baboon and human $\alpha-1$ antitrypsin amino acid sequences showed some shared sequence homology but not with the reactive site. Subsequent protease inhibition assays demonstrated that the electrophoretically purified 12-kDa antigen inhibited the activity of porcine elastase at similar concentrations as commercially produced $\alpha-1$ antitrypsin; furthermore, the 12 kDa antigen inhibited human neutrophil chemotaxis indicating that the native protein might play an important role in the survival of the parasite in an immunocompetent host (Shepherd *et al*. 1991). Although these data suggested that *E. granulosus* antigen B was a serpin due to its sequence similarity with other well characterized serpins as well as its capacity to inhibit serine proteases, further studies are required to determine the precise role of this protein family in the biology of *E. granulosus*.

Merckelbach and Ruppel (2007) cloned and bacterially expressed a serpin gene ($\text{serpin}^{\text{Emu}}$) from *E. multilocularis* and tested the inhibitory potential of the purified recombinant protein against a number of mammalian proteases involved in cellular immune defense, blood clotting and digestion. Multiple sequence alignment of its deduced amino acid sequence with mammalian serpins suggested $\text{serpin}^{\text{Emu}}$ is an intracellular protein due to the lack of a signal sequence and no N- or C-terminal extensions. Protease inhibition assays showed that $\text{serpin}^{\text{Emu}}$ inhibited

https://doi.org/10.1017/S0031182011002435 Published online by Cambridge University Press

mammalian trypsin and pancreatic elastase (PE) with high specificity but no inhibition was evident with cathepsin G or chymotrypsin. Serpin^{Emu} was highly expressed in *E. multilocularis* oncospheres, likely playing a similar role to that of the human intracellular serpin B9 in cytotoxic lymphocytes, which is thought to protect immune effector cells against endogenous proteases (Hirst *et al*. 2003; Zhang *et al*. 2006).

Trypsin and PE, which were most readily inhibited by serpin^{Emu} are mammalian digestive enzymes, suggesting a probable extracellular role for serpin^{Emu}, a hypothesis supported by the fact that plasminogen-activator inhibitor 2, an intracellular serpin, has been shown to be secreted by monocytes through a pathway independent of the endoplasmic reticulum and Golgi apparatus (Ritchie and Booth, 1998). A serpin lacking a signal sequence has also been shown to be excreted into the saliva of the ectoparasitic tick, *Ixodes ricinus* (Prevot *et al*., 2006). Merckelbach and Ruppel (2007), therefore, suggested that, if serpin^{Emu} were to be excreted by *E. multilocularis* oncospheres, it might be able to block attack by host digestive enzymes thereby making this serpin an important target of the intestinal immune system and a possible candidate for vaccine development.

A PHYLOGENY OF HELMINTH PARASITE SERPINS

Comprehensive phylogenetic analysis of a number of the helminth serpins discussed in this review was undertaken in order to shed some light on their evolutionary relationships. The phylogenetic analysis assigned the serpins to 4 major branches (Fig. 3). Branch 1 consists of Hc-Serpin and Tv-SERP clustering closely together with Bmserp and BmSERPIN more distantly related but still falling within this grouping. The second major branch comprises Ts11-1 and *A. suum* serpin. The third major branch includes sm_serpin, (gi256082483), sm_serpin (gi256082483), Sj serpin and SJCHGC00560 which cluster closely together suggesting a possible common ancestry. Branch 4 consists of CsproSERPIN, CsSERPIN and PwSERPIN. Suprisingly, the phylogenetic tree reveals that CsSERPIN is more closely related to PwSERPIN than CsproSERPIN (Fig. 3). The clustering pattern of serpins from branches 1 and 2 as well as those of branches 3 and 4 is not surprising given the representatives belong to the same nematode or trematode classes, respectively. The *E. multilocularis* serpin (serpin_Emu) is distantly related to those of the other helminths, suggesting early evolutionary divergence.

FINAL COMMENTS

Although recognized for their involvement in many important endogenous regulatory processes, it has been suggested that serpins from pathogens, including those of helminth parasite origin, may have evolved specifically to limit or hinder the activation of the host immune response by inhibiting enzymes involved in generating immuno-stimulatory signals (Chopin *et al*.1997; Chopin, 1998*a*,*b*). Many of the studies presented here strongly support the idea that serpins not only perform endogenous physiological and regulatory functions in parasitic helminths but may also be actively involved in host-parasite interplay as well as possible host immune modulation and/or evasion processes. These findings highlight the potential of serpins and smapins as possible drug targets as well as potential anti-helminthic vaccine candidates. Additional studies, building on the findings presented in this review are, however, needed to functionally characterize the biological importance of the native molecules from each of the parasitic helminth species. With the recent publication of the draft genomes of *B. malayi* (Ghedin *et al*. 2007) *T. spiralis* (Mitreva *et al*. 2011) and *A. suum* (Jex *et al*. 2011), more serpin genes are likely to be identified by data mining and, with their subsequent biochemical characterization, more light will be shed on their roles in the biology of the parasitic helminths. In turn, this may lead to the identification of further intervention targets against this important group of pathogens.

FINANCIAL SUPPORT

The authors' research has received financial support from various sources including: the UNICEF/UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases; the National Health and Medical Research Council (NHMRC) of Australia; the Wellcome Trust (UK); the Sandler Foundation (USA); the Dana Foundation (USA); and the National Institute of Allergy and Infectious Diseases. Donald P. McManus is a NHMRC senior principal research fellow. A. J. Molehin is supported by a University of Queensland International Research Tuition Award (UQIRTA) and University of Queensland Research Scholarship (UQRS).

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