Proteases of Parasitic Helminths: Their Metabolic Role in Establishment of Infection in the Host

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Abstract

Proteases catalyze hydrolysis of peptide bonds in proteins and play an important role in the survival of living organisms, encoded by about 2% of the whole genome in all kind of organisms. Mostly they are nonspecific, while some are highly specific toward a peptide bond. Generally, proteases are grouped into different clan, family, and type, depending on kinds of reaction they catalyze, mechanism of catalysis, and their molecular structure and homology. Proteases control many biological processes in living organisms including helminths. There are about 1828 sequences that pertain to 25 genera of helminth parasites. In this chapter, we have discussed various types of proteases found in helminth parasites, like aspartic-, cysteine-, metallo-, and serine proteases, and their possible role in these parasites and their hosts.

Keywords

Protease · Parasite · Helminths

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S. Chakraborti et al. (eds.), *Proteases in Human Diseases*,
DOI 10.1007/978-981-10-3162-5_12

1 Introduction

Proteases are the biological scissors, which hydrolyze the peptide bonds in proteins, and may have appeared in biological systems during early periods of protein evolution. Protein degradation plays an important role in the survival of all living organisms as it is involved in food digestion and defense mechanism against the pathogen. Activation and inactivation of proteins by degrading some specific portion of them regulate some physiological and cellular processes, thus preventing the accumulation of unwanted or abnormal proteins within the cells [1]. Proteases are found in almost all living organisms from viruses to human, and they are encoded by about 2% of the whole genome in all kind of organisms [2]. Most of these proteases are relatively nonspecific for their substrates, like proteinase K, while some are highly specific toward a particular peptide bond, like angiotensin-converting enzyme; the later group, thus, launched a new and exciting filed of protease research [3]. The proteases also vary in their molecular sizes from simple catalytic units ($\sim$20 kDa) to sophisticated molecular machines, like the proteasome and meprin metalloproteinase isoforms ($\sim$6000 kDa) [4]. Till today, even after 100 years of their discovery, these enzymes remain at the cutting edge of research in most laboratories around the globe.

Generally proteases are classified into endopeptidases, which target internal peptide bonds, and exopeptidases, which catalyze the terminal NH_2 (aminopeptidases) or COOH (carboxypeptidases) bonds. However, the availability of structural and mechanistic information on these enzymes facilitates new classification schemes [5]. According to recent information, proteolytic enzymes may be grouped depending on the kinds of reaction they catalyze (like endopeptidases, omega peptidases, exopeptidases, aminopeptidases, carboxypeptidases, dipeptidyl peptidases, tripeptidyl peptidases, peptidyl dipeptidases, and dipeptidases), or on the mechanism of catalysis (such as aspartic-, glutamic-, cysteine-, serine-, threonine-, and metalloproteases) [6], or on their molecular structure and homology (such as clan, family, and type) [7].

Proteases control many biological processes in living organisms, like regulation of proteins, creation of new bioactive molecules, processing of cellular information, and molecular signaling [8–16]. Hence, alterations in proteolytic systems in human cause many diseases such as cancer, neurodegenerative disorders, and inflammatory and cardiovascular diseases. Accordingly, many proteases are of special attention for the pharmaceutical industry as potential drug targets or as biomarkers [17]. Unswerving with other higher animals, in parasitic helminths too, proteases play crucial roles, like tissue penetration, digestion of host's tissue for nutrition and evasion of host's immune response, and thus determine the establishment and survival of the pathogen in its host [18].

In "protease.lib" of the latest MEROPS-10.0 database (http://merops.sanger.ac.uk), a huge number (362,652) of protease sequences (including protease inhibitors) are found. Of these, only 1828 sequences pertain to 25 genera of helminth parasites (Table 1). In class/clan-wise categorization, the protease sequences in helminths fall

Table 1 Genus-wise distribution of 1828 proteases found in helminths

S. No.	Helminth parasite (Genus)	CsP	AsP	MtP	SeP	MxP	UsC	Total
Trematodes								
1.	*Clonorchis*	60	34	50	20	16	0	180
2.	*Fasciola*	41	2	2	0	0	0	45
3.	*Metagonimus*	7	0	0	0	0	0	7
4.	*Opisthorchis*	6	1	0	1	1	0	9
5.	*Paragonimus*	12	0	1	0	0	0	13
6.	*Schistosoma*	130	40	173	71	58	0	472
7.	*Trichobilharzia*	6	0	0	0	0	0	6
Cestodes								
8.	*Echinococcus*	56	6	81	43	33	9	228
9.	*Spirometra*	2	0	0	0	0	0	2
10.	*Taenia*	4	0	1	0	0	0	5
Nematodes								
11.	*Ancylostoma*	7	3	14	0	0	0	24
12.	*Angiostrongylus*	4	1	1	0	0	0	6
13.	*Anisakis*	1	1	0	0	1	0	3
14.	*Ascaris*	40	15	94	40	16	0	205
15.	*Brugia*	58	13	77	30	22	0	200
16.	*Dirofilaria*	1	0	1	1	0	0	3
17.	*Gnathostoma*	1	0	1	0	1	0	3
18.	*Loa*	60	12	117	19	10	0	218
19.	*Necator*	5	2	1	1	0	0	9
20.	*Onchocerca*	2	1	1	2	0	0	6
21.	*Strongyloides*	1	10	1	1	0	0	13
22.	*Toxocara*	3	0	0	0	0	0	3
23.	*Trichinella*	38	10	67	17	37	0	169
24.	*Trichuris*	1	0	1	0	0	0	2
25.	*Wuchereria*	0	1	0	0	0	0	1
Total		546	152	681	245	195	9	1828

under six protease groups; cysteine protease (CsP-546 sequences), aspartic protease (AsP-152 sequences), metalloprotease (MtP-681 sequences), serine protease (SeP-245 sequences), mixed protease (MxP-including cysteine, aspartic, and serine clans; 195 sequences) and unassigned protease (UsP-9 sequences) (Fig. 1). Using Database of Essential Genes (eDEG; http://tubic.tju.edu.cn/deg/) and applying a two-layered filtering approach via protein–protein BLAST against (mammalian) host proteases, highly diverse and essential proteases could be sieved out of the total sequences identified to exist among helminths (Fig. 2). Out of 1828 sequences, only 108 are predicted as highly diverse and essential proteases, of which 36, 12, 26, 1, 24, and 9 sequences belong to AsP, CsP, MtP, SeP, MxP, and UsP,

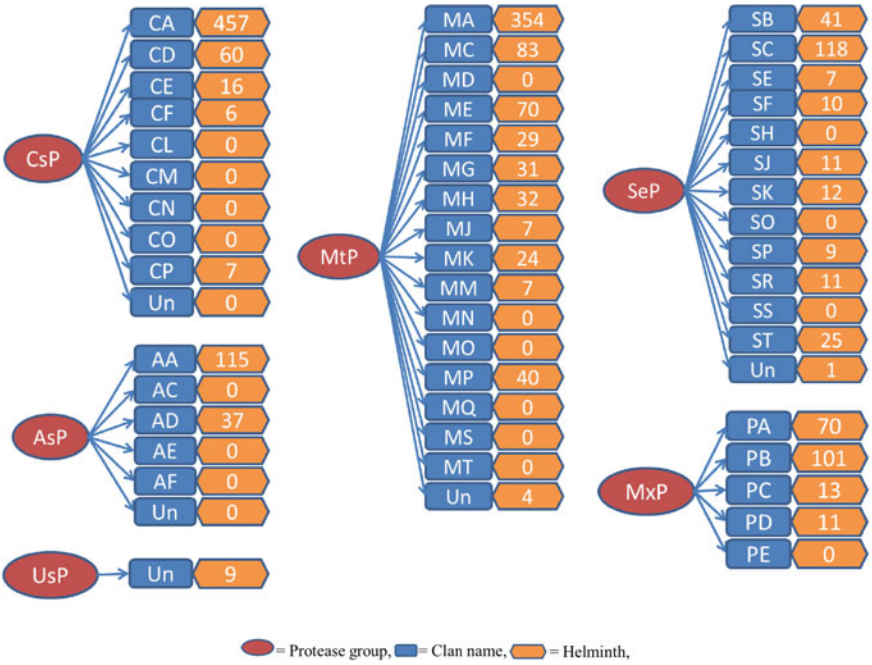

Fig. 1 Total protease sequences available in "protease.lib" of MEROPS-10.0. Available type of proteases and their clan-wise distribution in helminth parasites

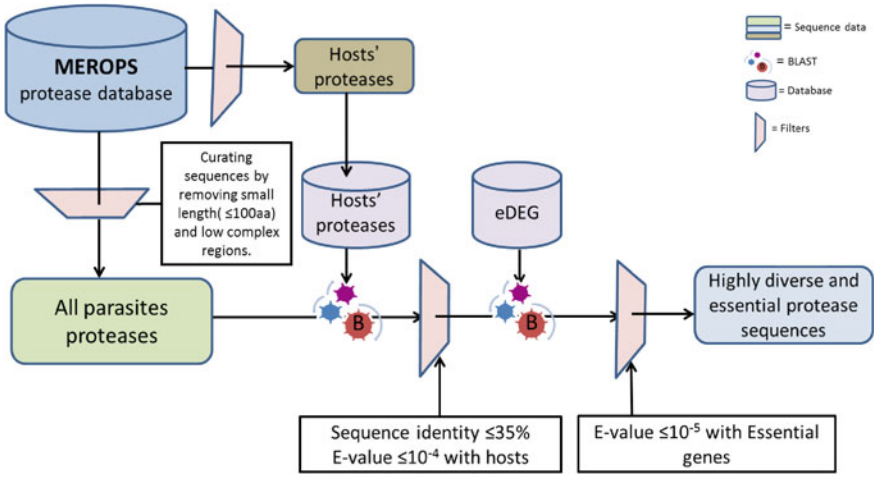

Fig. 2 Two-level filtering approach for finding highly diverse and essential proteases. Two-level-filtering approach means two times protein–protein BLAST program was used with mammalian (human and cattle) proteases and database of essential genes

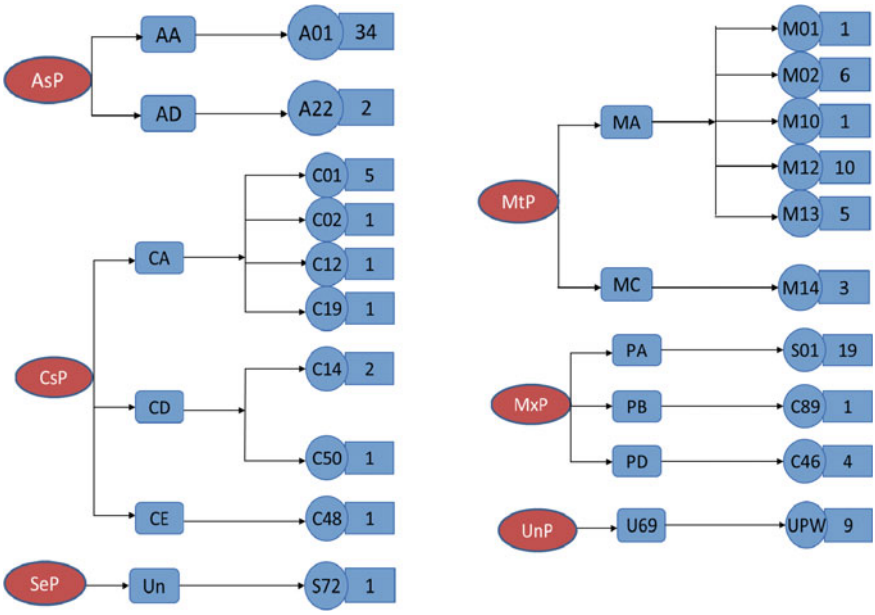

Fig. 3 Types and clan-wise distribution of 108 protease sequences after two-level filtration by BLAST with mammalian (human and cattle) proteases and database of essential genes (DEG)

respectively (Fig. 3). It is noteworthy that protease sequences of glutamine (GuP) and asparagine (AnP) groups are conspicuously missing in helminths group (http://merops.sanger.ac.uk).

The fact that many infectious microorganisms require proteases for replication or use them as virulence factors has facilitated the development of protease-targeted therapies for diseases of great relevance to human life [3]. Besides, proteases are also important tools of the biotechnological industry because of their usefulness as biochemical reagents [19]. Like microbes, macropathogens such as helminths also possess proteases, which have diverse functions in parasite biology. These enzymes are secreted by helminth parasites and used for their entry into the host as well as feeding and migration. In this chapter, we focus on various types of proteases in parasitic helminths and their metabolic roles in establishment of infection in the host.

2 Role of Proteases in Parasitic Helminths

Besides therapeutic importance of proteases in various types of cancers, respiratory and cardiovascular disorders, they have also been considered as potent therapeutic targets for various infections. When a parasite infects a host, it survives by escaping from the host immune system; parasites must have sufficient mechanisms by which

they can cross various barriers, such as epithelial cell wall, connecting tissues and extracellular matrix to reach their final destination [20]. The roles of various proteases in helminth parasites are discussed under.

2.1 Aspartic proteases are associated with digestion of host hemoglobin in blood-feeding nematodes such as the trichostrongylids, *Haemonchus contortus*, hookworms (*Ancylostoma caninum* and *Necator americanus*), and *Angiostrongylus costaricensis* [21, 22]. Consequently, many aspartic proteases are being used to develop vaccines against trichostrongylids and hookworm infections [23, 24]. It has been observed that aspartic proteases of hookworms are also capable to degrade skin macromolecules and aid skin penetration, suggesting that their role in nematode parasitism is not limited to digestion of hemoglobin but they also function in intestinal digestion and tissue degradation of the host [25, 26]. Substrate specificity of hemoglobin-degrading proteases employed by blood-feeding helminth parasites influences the parasite–host species range. The differences in amino acid sequences in the catalytic sites of these proteases interact less or more efficiently with hemoglobin of permissive or nonpermissive hosts [27]. In the human liver fluke, *Opisthorchis viverrini,* cathepsin D-like aspartic protease, Ov-APR-1, is expressed in the gut and reproductive tissues of the mature hermaphroditic parasite. This is also present in the developing larval miracidium stage within the eggshell, and in the excretory/secretory products (ESPs) of the cultured adult flukes. The presence of Ov-APR-1 in all developmental stages and ESPs of *O. viverrini* shows its indispensable role in the host–parasite relationship [26].

2.2 Cysteine proteases are the most widely reported class of proteases from parasitic nematodes and are secreted by larval and adult parasites for tissue invasion, feeding and defense against effector mechanisms of the host immune response. Major cysteine proteases belong to the papain superfamily (Family C1) and are common in nematodes. Cathepsins B of *H. contortus* are expressed in the intestine of adult worms that are capable of digesting hemoglobin (Hb), fibrinogen, collagen, and immunoglobulin-G. Similarly, cysteine proteases in ES products of *N. americanus* have lytic activity against Hb, fibrinogen and antibodies [28, 29]. Comparative analysis of the transcriptome of various life-cycle stages of *Brugia malayi* shows that cathepsin-like cysteine proteases (including Bm-cpl-1, 4 and 5) are expressed at every stage, though more prominently in MF, L3, and L4 stages [30]. The expression of cathepsin B, AcCP-2, was more abundant in eggs and larval developmental stages of *A. caninum*, showing that cathepsin B might play a role in the early development of the dog hookworm [31]. Similarly, intestinal expression of four distinct Cathepsin B (Na-CP-2, -3, -4, -5) from the human hookworm, *N. americanus*, shows that these cysteine proteases and likely to be involved in nutrient acquisition [32].

The whole-genome analysis of various trematode parasites, for example *Schistosoma japonicum* and other *Schistosoma* species reveals that cysteine proteases are the largest and most important protease family. A total of 102 cysteine protease sequences have been identified from the whole genome of *S. japonicum* and all are assigned to 17 subtypes. Among them, the cathepsins B, C, F, L, K, and S have a pivotal role in schistosome feeding and nutrition, as well as in migration through

human tissues [33]. Analysis of the expressed sequence tags (ESTs) of *Clonorchis sinensis* revealed that proteases are the largest proportion of the protein population of this fluke, which are essential for stage transition, nutrient uptake, and immune evasion [34, 35]. Furthermore, the partially purified cysteine proteases from ESPs of *C. sinensis* adult worms show cytotoxic effects on cultured cells, and the endogenous cysteine proteases of the metacercaria appear to be involved in excystation from the cyst [36]. Several cysteine proteases were identified in *C. sinensis* and are phylogenetically more close to the mammalian cathepsin F enzymes [37]. Recently, the published draft genome of *C. sinensis* shows that the largest part of protease members belong to cysteine protease superfamily. Some cysteine proteases are also reported for the first time and probably contribute to the catabolism of bilirubin and other host proteins [38].

Various developmental stages of the liver fluke *Fasciola hepatica* also express different types of cysteine proteases. The transcriptome analysis of the invasive juvenile stage of *F. hepatica* shows that cathepsins L3, L4, and L6 are specifically identified in the juvenile ESTs, while these are not present in the adult stage. Some isoforms of various cathepsins are expressed in different developmental stages. For example, the secretome analysis of the adult *F. hepatica* shows that FhCL1 and FhCL2 peptidases are the most abundant proteins, comprising 67 and 27%, respectively of the total cathepsin Ls. A total 31% cysteine peptidases are made up of the total proteins secreted by the newly excysted juvenile (NEJ), in which various isoforms of cathepsin Ls; L3, L4, and L6 (37%), Cathepsin B (45%), and Asparaginyl endopeptidases (18%) have been identified [39, 40]. The total RNA analysis of the adult form of another liver fluke, *O. viverrini*, the causative agent of cholangiocarcinoma, shows two novel cysteine proteases—cathepsin F (Ov-CF-1) and cathepsin B1 (Ov-CB-1). Ov-CF-1 is secreted as an inactive zymogen that autocatalytically processes and activates itself to mature enzyme at pH 4.5 via an intermolecular cleavage at the prosegment–mature domain junction. Also, Ov-CB-1 is secreted as a zymogen but, in contrast to Ov-CF-1, is fully active against peptide and macromolecular substrates despite retaining the N-terminal prosegment [41].

The cysteine proteases of the lung fluke *Pargonimus westermani* newly excysted metacercariae (PwNEM) play a role in host tissue invasion. Two isoforms (PwMc27 and PwMc28) of a particular type of cysteine protease enzyme having 27 and 28 kDa molecular weight and purified from PwNEM ESPs, preferentially degrade fibrillar proteins, but not globular proteins [42].

In the case of *Echinococcus multilocularis* metacestodes, two cDNA clones, encoding cathepsin L-like (EmCLP1 and EmCLP2) and cathepsin B—like (EmCBP1 and EmCBP2) cysteine proteases, are isolated from ESPs and the extract of the metacestodes. These are suggested to play a key role during protein digestion for the parasite's nutrition and in parasite–host interactions [43, 44]. *Taenia solium* and other species of *Taenia* are the cause of neurocysticercosis in human and other animals, respectively. Immunoglobulin degradation by cysteine proteinases of the pathogenic *Taenia* species is suggested to play a key role in escaping from the host immune system and thus could be employed as a target for chemotherapy [45]. Another cysteine protease, cathepsin L-like peptidase, is secreted by *Taenia*

species, which can be utilized as immunodiagnostic antigen for cysticercosis treatment [46]. In another tapeworm, *Spirometra erinaceieuropaei*, a cysteine protease (SeCP), recognized in the sparganum ES proteins by early infection sera and identified by MALDI-TOF/TOF-MS, is a 336-amino acid long chain. In SeCP, 15 potential antigenic epitopes and 19 HLA-I restricted epitopes are computationally predicted, giving insights on the diagnostic antigens and target molecular sites of antisparganum drugs [47].

2.3 Metalloproteases comprise a heterogeneous group of proteolytic enzymes whose main characteristic is the utilization of a metal ion to polarize a water molecule for performing hydrolytic reactions. A major group of metalloproteases includes zinc-dependent endopeptidases, which have the ability to cleave one or more extracellular matrix components as well as non-matrix proteins [48]. According to their substrate specificity, MMPs can be categorized as collagenases, gelatinases, elastases, stromelysins, and membrane-type. In nematodes, metalloproteases including collagenases, gelatinases, and elastases play an important and essential role in larval and adult migration and invasion through host's connective tissues [48]. A novel astacin-like metalloprotease (Ac-MTP-1) is characterized in ESP of *A. caninum* L3 larvae. Ac-MTP-1 has significant sequence similarity with Zinc-metalloprotease and is exclusively expressed in L3 stage, indicating its role in host tissue invasion [49]. More recently, an ortholog of Ac-MTP-1, known as Ay-MTP-1, has also been identified in *Ancylostoma ceylanicum* and is believed to be a plausible protein target for vaccine development to prevent larval migration through tissues [50]. Recently, two metalloproteases, a 175 kDa collagenase and another leucine aminopeptidase (LAP), have been purified and characterized from adult female *Setaria cervi* (a filarial parasite of Indian buffalo). In vivo study for these enzymes reveals that collagenase plays an important role in host immune evasion and immunoprotection by specifically cleaving human IgG in vitro [51].

2.4 Serine proteases, secreted by the intestinal nematode parasites, have the ability to change the properties of the mucus barrier, making it more porous by degrading the mucin component of the mucus gel [52]. The parasitoid nematode, *Steinernema carpocapsae*, is capable of killing its insect host within 48 h. The ESP of the parasitic stage of this worm shows high proteolytic activity; a chymotripsin-like serine protease, Sc-SP-3, participates in degradation of extracellular proteins and is thus involved in nematode pathogenesis [53, 54]. Serine proteases derived from ESPs of in vitro cultures of *Trichinella spiralis* L1 muscle larval have been shown to participate in hydrolysis of collagens and elastin proteins [55].

3 Proteases as Drug Targets in Helminths

From the foregoing account, it clearly emerges that proteases have multifarious functions in the biology and pathogenesis of parasitic organisms. They are unusually immunogenic and have been exploited as serodiagnostic markers and vaccine targets. Although host homologs exist, parasite proteases have distinct structural and

biochemical properties including optimum pH and stability, alteration in peptide loops or domain extensions, diverse substrate specificity, and cellular location. The disparate nature of parasite proteases compared to the host orthologous proteins has opened opportunities for chemotherapy [56]. For comprehensive understanding of the role proteases in molecular and biochemical mechanisms for survivability, nutrition, metabolism, host-dependent development and maturation, immune evasion and evolution, numerous parasite whole genomes have been sequenced in recent years and some are still in the pipeline [57, 58]. Depending on the chemical groups in their active sites, proteases found in helminths parasites are grouped as four major classes (aspartic-, cysteine-, metallo-, and serine proteases), which are discussed below.

3.1 Aspartic proteases, like pepsin proteases (Clan AA and Family A01), digest the ingested food. In blood-feeding nematodes, aspartic proteases, along with cysteine proteases, are involved in degrading the host's blood in a multienzyme cascade manner [59]. Aspartic proteases in adult *O. viverrini* and *Heligmosomoides polygyrus* secretome are found to play a key role in hemoglobin degradation [60, 61]. Comparative genomic analysis of aspartic proteases in eight parasitic platyhelminths reveals that aspartic protease members of family A01 are prevalent in schistosomes than in cestodes. Proteases of family A22 are evolutionarily highly conserved among all the parasites, and one retroviral-like AP in family A28 shares a highly similar predicted 3D structure with the HIV protease, thus implying its potential to be inhibited by HIV inhibitor-like molecules [62]. Analysis of secretory cDNA of *C. sinensis* reveals a 425 amino acids-long Cathepsin D-like Aspartic protease that may be potential to diagnose the antigen and the drug target of clonorchiasis [63].

3.2 Cysteine proteases are the most studied proteases in helminth parasites and involved many aspects of hosts–parasite relationship. These proteases are mainly intracellular in higher organisms, but are often extracellular proteases in helminths parasites [64]. Such diverse utility of this group of proteases reveals their therapeutic importance against helminth infections. Recently, K11777, a potent cysteine protease inhibitor has been developed and its clinical trial is being tested [64]. A recent study about Cathepsin F (member of Cysteine protease group) of *Trichinella* spp has shown that it is a major virulence factor for parasitic helminths, and it may be a potential anthelmintic drug target and vaccine candidate of trichinellosis, a reemerging infectious disease [65]. Whole genome sequencing of the whipworm *Trichuris* and its comparative analysis to find new predicted drug targets based on transcript-level expression, essentiality of protein homologs. It has been shown that Cathepsin B and gut-specific cysteine protease-1, and -2 are novel drugable targets in the parasite [66]. Phylogenetic analysis using Bayes approach of *T. solium* genome shows their functional divergence among regulatory cysteine- and serine proteases from their hosts, hence, these proteases can be used for drug targets [67]. Analysis of the ESPs of the encysted progenetic metacercariae of the digenetic trematode parasite, *Euclinostomum heterostomum,* has shown that cysteine proteases, being a major component, can be exploited as serodiagnostic markers and therapeutic and vaccine targets [68]. Similar study of *C. sinensis* ESPs shows the presence of four *C. sinensis* cathepsin B cysteine proteases (CsCB1, CsCB2, CsCB3, and CsCB4), and immunological and biochemical studies reveal

their potential to be vaccine candidates and drug targets in *C. sinensis* prevention [69]. *S. mansoni* cathepsin B1 (SmCB1) is the most abundant papain-like cysteine peptidase in the parasite gastrodermis, gut lumen, and probably in caeca and protonephridia [70]. Immunological studies of schistosomiasis patients suggest that SmCB1 is an immunodominant target of the immune response during pre-patent schistosome infection. It has been demonstrated that SmCB1 is targeted by IgG- and IgE-specific antibodies. In the first situation, SmCB1 significantly reduces the worm burden (by 66–73%), eggs in the liver (51%), and in the small intestine (25%). However, when SmCB1 is incubated with the proteinase-inhibitor prior to immunization, levels of protection decrease significantly. This study points out the importance of the peptidase activity in protective potential. Therefore, SmCB1 has been considered a strong candidate for drug development and vaccine designing [71]. Some natural and synthetic compounds are being developed as cysteine protease inhibitors for pathogenic helminths [72–75].

3.3 Metalloproteases (like aspartic- and cysteine proteases) also play an important role in survival of the parasite in the host system. From bioinformatics analysis, 26 metalloproteases are mined from MEROPS database which are considered highly diverse and essential, and thus, potent therapeutic drug targets. Two novel leucine aminopeptidases (CsLAP1 and CsLAP2), identified from *C. sinensis*, are suggestedly involved in the digestion of intestinal peptides of the host [76]. Another study of the ESPs of *C. sinensis* suggested that methionine aminopeptidase 2 (MAP-2), belonging to metallopeptidase family M24, is highly expressed in eggs, metacercaria, and adult stages of *C. sinensis*. These proteases have been considered as potential drug targets in immunotherapy of clonorchiasis and biliary diseases [77]. The differential expression profile of proteases in *S. japonicum* degradome reveals that 14 putative M8 family members are surface proteases. Their annotation reveals similarity with leishmanolysin protease of protozoan parasites, which plays a crucial role in host body invasion. It has been speculated that leishmanolysin (invadolysin) may also contribute to tissue invasion by schistosome cercaria; thus leishmanolysin inhibition could serve as a novel intervention strategy for schistosomiasis [78]. In protease analysis of *T. solium* genome, it has been revealed that 27% of total metalloprotease are membrane bound. It has been speculated that their inhibition will be effective in eradication of *T. solium* infection [67]. Neurocysticercosis (NCC) is the central nervous system (CNS) infection caused by the larva of *T. solium* tapeworm. Its matrix metalloprotease (MMP) expression plays a crucial role in the differential breakdown of the blood–brain barrier (BBB), and inhibition of this enzyme has been considered its therapeutic importance [79]. Inhibition of metalloprotease by selected chemical compounds shows prolongation of the moulting process in nematode larvae [80]. In *A. cantonensis*, the L3 larva releases heavily secreted proteases, in which metalloproteases are dominantly found. The study shows the capability of *A. cantonensis* secretory metalloproteases to degrade human metalloproteinase (MMP-9). This analysis has revealed the therapeutic importance of the metalloprotease of *A. cantonensis* [81]. The third-stage larvae of *Strongyloides stercoralis* have an ability to migrate through

tissues at a speed of 5–15 cm per hour. This process of migration is facilitated by a metalloproteinase with elastase activity, which is determined by inhibition study [82]. The parasitic virulence factor may be guided by metalloproteinase and could be a molecule for therapeutic purpose [23].

3.4 Serine proteases also play an important role in a broad range of biological processes, such as intra- and extracellular protein metabolism, digestion, blood coagulation, regulation of development, and fertilization. Therefore, this group of enzymes is also therapeutically important like aspartic-, cysteine-, and metalloproteinases. Expression of different types of serine proteases is observed in some nematodes parasites. In *T. spiralis* a novel serine protease (i.e., TsSerP) is found during all life-cycle stages, whereas two TsSP-1 and TsSP-2 are expressed only during the invasive larvae stage in the host muscle [83]. Recently, a newborn larval stage-specific serine protease gene (NBL1) has been identified via a subtractive cDNA library of *T. spiralis* newborn larvae. Based on the high immunogenicity of its C-terminal domain, it is speculated that it may be important therapeutic target [84]. Novel serine protease was isolated from the infective larvae of *Anisakis simplex,* which is similar to the extracellular serine protease of the pathogenic bacterium *Dichelobacter nodosus*, which can degrade elastin, keratin, and collagen [85]. Blisterase, a subtilisin-like serine protease, is expressed in *Onchocerca volvulus,* and plays an important role in the nematode biology including the cuticle production and maintenance, neural signaling, and development. Thus, it is a potential drug target for controlling the parasite infection [86]. Three neutral serine proteases, two trypsin-like proteases (198 and 104 kDa) and one chymotrypsin-like protease (36 kDa), have been extracted from the plerocercoid larvae (spargana) of *S. mansoni*. These purified proteins elicit strong antibody responses in infected patients, suggesting that they could be potential antigens in serologic diagnosis of human sparganosis [87].

A novel antigen 5 (Ag5) proteins that harbors two subunits has been characterized by RT-PCR from *Echinococcus granulosus*. One of them, the 22-kDa subunit, contains a highly conserved glycosaminoglycan-binding motif, and the other is a 38 kDa subunit that shows high similarity to serine proteases of the trypsin family. But, biochemical tests reveal that in native purified Ag5 neither the proteolytic activity nor the binding to protease inhibitors could be found. This intriguing feature of Ag5 suggests that it could be a new drug target [88]. In a recent study, a trypsin-like serine protease TsAg5 protein has been identified from *T. solium*, which has high homology with the *E. granulosus* antigen Ag5. Detected from the cyst fluid and ES of the cysticercus, TsAg5 seems to be a potential candidate for immunodiagnostic and drug designing [89].

A secreted dipeptidylpeptidase (DPP) (a type of serine protease) has been isolated from *F. hepatica* and characterized [90]. Recently, serine proteases like proteins (Pic and PII) have been partially purified from *Fasciola gigantica* [91]. Genomes and transcriptomes analysis of blood flukes, *S. mansoni*, *S. japonicum S. haematobium,* and *S. douthitti,* reveal that the skin invasion is facilitated by secretions from the acetabular and head glands, which contains cercarial elastase, a chymotrypsin-like protease. Disruption of the enzyme activity by specific drugs/vaccines may provide therapeutic benefits in schistosomiasis [33, 92–95].

4 Conclusions

Finally, detailed analyses of complex protease-mediated processes in helminths and their role in parasitic metabolic activities will help in better understanding the establishment of infection in their host. However, proteolytic regulation of the transcription factor activity, protein ectodomain shedding, and regulated intra-membrane proteolysis are challenges to be addressed in the near future. Hopefully, this chapter will provide a current view various types of proteases found in helminth parasites.

Acknowledgements Authors thank all the workers in the field of protease research in helminths. We also apology to the authors if some of their works were not accommodated in this compilation.

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