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Review

Parasite Cathepsin D-Like Peptidases and Their Relevance as Therapeutic Targets

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Inhibition of aspartic cathepsin D-like peptidases (APDs) has been often discussed as an antiparasite intervention strategy. APDs have been considered as virulence factors of *Trypanosoma cruzi* and *Leishmania* spp., and have been demonstrated to have important roles in protein trafficking mechanisms of apicomplexan parasites. APDs also initiate blood digestion as components of multienzyme proteolytic complexes in malaria, platyhelminths, nematodes, and ticks. Increasing DNA and RNA sequencing data indicate that parasites express multiple APD isoenzymes of various functions that can now be specifically evaluated using new functional-genomic and biochemical tools, from which we can further assess the potential of APDs as targets for novel effective intervention strategies against parasitic diseases that still pose an alarming threat to mankind.

Relevance of Parasite Cathepsin D-Like Peptidases

Parasitic diseases represent major global health and economic problems. Novel therapeutic drugs and vaccines are urgently needed so as to limit the socioeconomic burden and overcome increasing parasite resistance to current therapeutics [1,2].

Parasite peptidases (proteases, proteolytic enzymes) are involved in various adaptive functions including tissue penetration, larval migration, molting, immune evasion, coagulation, digestion of host blood proteins, and degradation of the cellular matrix [3]. Aspartic peptidases (AP) of the **cathepsin** D type (**peptidase clan** AA, A1 family; APDs; see Glossary), in contrast to other peptidase families (e.g. cysteine, serine, metallopeptidases) [4], represent a relatively small group of enzymes with enormous therapeutic potential. Their unique mechanism of activation and substrate binding [5] predetermines them for specific and non-redundant endopeptidolysis in key physiological processes. APDs are important virulence factors for HIV and malaria, and numerous other parasite APDs, notably members of the A1b subfamily plasmepsin V [6] and TgASP5 [7,8], have been demonstrated to play crucial roles in host–parasite interactions. Parasite APDs operating within multienzyme hemoglobinolytic complexes in malaria, platyhelminths, nematodes, and ticks [9] have primary roles in cleaving intact hemoglobin before it can be processed by other digestive peptidases, and their inhibition thus presents a promising strategy to block amino acid supply to the parasite.

This review provides a global insight into the relevance of parasite APDs for parasite survival and replication, and evaluates their potential as therapeutic targets. This work focuses on parasite A1

Trends

Increasing DNA and RNA sequencing data indicate that parasites express multiple aspartic peptidases of cathepsin D type (APDs) with several functions.

APDs are involved in numerous parasitic mechanisms, from protein trafficking to tissue penetration, immune evasion, and digestion of host blood proteins.

APDs are a relatively small group of enzymes with a unique mechanism of activation and substrate binding, which makes them attractive drug targets.

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family enzymes, and excludes other parasite aspartic peptidases such as the plasmodial signal peptide peptidase (clan AD, family A22) [10]. We explicitly highlight the need to functionally characterize candidate parasite APDs before novel antiparasitic compounds and vaccines can be designed and tested. Providing a *trans*-parasite review of this topic allows us to highlight potential analogies between research findings from distinct parasitic organisms.

Evolution of Parasite APDs

Parasitic life strategies have evolved convergently and have appeared multiple times during evolution, leading to the adaptation of cathepsin D-like enzymes to diverse biological functions connected with a parasitic lifestyle (Figure 1). Alveolate APDs have undergone rapid evolution that divides them into six distinct clades represented by apicomplexan groups A–E and the newly described group F comprising *Toxoplasma gondii* TgASP1, *Neospora caninum* NsASP1, and the free-living *Vitrella brassicaformis* VbASP1. Convergent adaptation of metazoan APDs to parasitism is demonstrated by isoenzyme clustering within different phylogenetic clades, and provides a good reason not to compare the individual parasite APDs by their functional analogies but instead by their relation to organismal groups. Of interest are some molecular details, such as the presence/absence of the polyproline loop, that warrant further functional analysis.

APDs of Endoparasites

Trypanosomatidae family (order Kinetoplastida) APDs have been only poorly or indirectly characterized, primarily by activity assays of crude parasite extracts with APD-specific substrates and inhibitors, or by studying the effects of these inhibitors on parasite cultures (Table 1). These studies have highlighted the need to identify and functionally characterize APD target enzyme(s) before they are tested as chemotherapeutic targets [11]. The finding that HIV protease inhibitors (HIV PIs) have significant effects on parasitic kinetoplastida is paradoxical because APD coding sequences are absent from the genomes of *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania braziliensis* [11], and most likely reflects off-target effects. In support, there is experimental evidence that HIV PIs can interact with non-A1 family aspartic peptidases [12], and some clan AD, family A22 (presenilin-like) and clan AA, family A28 (Ddi-1-like) protease coding genes are found in the above-listed genomes [13]. Importantly, off-target inhibition of cytochrome P450s by HIV PIs is known to occur in mammalian systems [14], and should not be ruled out as a potential explanation [11].

For a long time APDs have been considered as potential antimalarial drug targets given the inhibitory effects of HIV PIs on plasmodia [15]. *Plasmodium falciparum* expresses 10 different APDs during its life cycle: plasmepsins PfPMI, II, IV–X, and histoaspartic peptidase HAP (Table 2, Figures 1 and 2). It was first suggested that the inhibitory effects of HIV PIs on malaria operated via inhibition of plasmepsins involved in blood digestion: PfPMI, PfPMII, PfPMIV, and HAP [16]. Despite functional redundancy among these isoenzymes, other plasmodial species express a single digestive vacuole APD, and it was believed that at least one functional digestive vacuole plasmepsin is necessary for hemoglobin digestion [17]. However, this was later ruled out by the generation of quadruple digestive plasmepsin knockout strains of *P. falciparum*, confirming their non-essential roles [18]. However, this probably reflects to functional redundancy of digestive plasmepsins and vacuolar cysteine hemoglobinases (falcipains), as indicated by the synergistic effects of cysteine and aspartic peptidase inhibitors on hemoglobin degradation [17]. PfPMVI, PfPMVII, and PfPMVIII are three malarial APDs that could be excluded as therapeutic targets for malaria because they are not expressed during the blood stages. *P. berghei* PMVI was shown to play a crucial role in malaria–mosquito interactions [19], and it has been speculated to be substrate for rhomboid-like protease ROM3 in a pathway that regulates sporogony in oocysts [20]. *P. berghei* PMVII knockdown displayed no apparent loss of fitness with respect to progression through the life cycle [20]. Significant interest has recently focused on PfPMV, an endoplasmic reticulum (ER)-resident A1b family (Figure 4) peptidase that cleaves the

Glossary

Albuminolysis: hydrolysis of albumin to peptides and amino acids by peptidases.
Bradyzoite: the slowly-dividing stage of *Toxoplasma gondii* that makes up tissue cysts.
Cathepsins: typical peptidases of endosomes/lysosomes involved in intracellular protein degradation. They have also have specific functions in other cellular compartments and outside cells. There are about 15 types of cathepsins and cathepsin-like peptidases in vertebrates and invertebrates that belong to different **peptidase classes**. From *kathepsein* (Greek), to digest.
Endopeptidases: peptidases that catalyze the cleavage of internal peptide bonds in a (poly)peptide or protein.
Epimastigote: morphological form of trypanosomatids with the flagellum of the unicellular parasite positioned at anterior face of the cell, and where the flagellum is connected to the cell body by an undulating membrane.
Fat body: an invertebrate analog to vertebrate adipose tissue and liver that functions as a major biosynthetic and storage organ in the body.
Gastrodermis: cellular or syncytial (schistosomes) layer of endodermal cells in trematode gut (caeca).
Hemoglobinolysis: hydrolysis of hemoglobin to peptides and amino acids by peptidases.
Merozoite: a motile, pre-, and extra-erythrocytic form of Apicomplexa (e.g., plasmodia), resulting from the asexual division of a schizont during schizogony, which in *Plasmodium* spp. occurs in the liver or red blood cells.
Nepenthesin loop: first described from the digestive aspartic protease of the carnivorous plant *Nepenthes gracilis*, the loop comprises an N-terminal cysteine-rich three-leaf-clover configuration that is present in the A1b subfamily of peptidases, and which interacts with the active-site flap and probably influences the substrate specificity of the enzymes.
Nymph: the second and last sexually undifferentiated developmental stage of ticks.
Oviposition: the process of laying eggs.
P and P' positions: in the nomenclature of peptidase substrates, the number of amino acid residues that are N- and C-terminal