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Current Opinion in Structural Biology

Working in concert: the metalloaminopeptidases from *Plasmodium falciparum*☆

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Malaria remains the world's most prevalent human parasitic disease. Because of the rapid spread of drug resistance in parasites, there is an urgent need to identify diverse new drug targets. One group of proteases that are emerging as targets for novel antimalarials are the metalloaminopeptidases. These enzymes catalyze the removal of the N-terminal amino acids from proteins and peptides. Given the restricted specificities of each of these enzymes for different N-terminal amino acids, it is thought that they act in concert to facilitate protein turnover. Here we review recent structure and functional data relating to the development of the *Plasmodium falciparum* metalloaminopeptidases as drug targets

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Current Opinion in Structural Biology 2013, **23**:828–835

This review comes from a themed issue on **Catalysis and regulation**

Edited by **Ben M Dunn** and **Alexander Wlodawer**

For a complete overview see the Issue and the Editorial

Available online 13th August 2013

0959-440X/$ – see front matter,

http://dx.doi.org/10.1016/j.sbi.2013.07.015

Introduction

The metalloaminopeptidases (MAPs) constitute a diverse set of protease enzymes with essential roles in cell maintenance, growth and development, and defense. Their active sites coordinate essential metal ion(s) that activate a water molecule to form an hydroxide nucleophile which attacks the scissile peptide bond, releasing the P1 position amino acid[1] [1]. The aminopeptidases are classified into families based on structural patterns and substrate specificity in particular by the preference shown for specific amino acids at the P1 position [2].

Parasites of the genus *Plasmodium* are the causative agents of malaria. While four *Plasmodium* species commonly infect humans, *Plasmodium falciparum* (*Pf*) causes the most deaths [3–5]. To date, nine MAPs have been identified in *Pf* (3D7) [6]. Four of these are methionine aminopeptidases. The other five enzymes comprise a prolyl iminopeptidase (or post-prolyl aminopeptidase),[2] a prolyl aminopeptidase (see footnote 2), a leucine aminopeptidase, an alanine aminopeptidase and an aspartyl aminopeptidase. Given the restricted specificities of each of these enzymes for particular N-terminal amino acids, it is thought that they act in concert to facilitate hemoglobin (Hb) catabolism [7,8], a process essential to the survival of *Pf*. However, recent studies show that the *Pf*MAPs may have additional roles to that of Hb digestion, including important housekeeping functions and in conjunction with the parasite proteosome [9••,10]. The essential nature of the *Pf*MAP activity has sparked interest in using them as targets for the development of novel antimalarials [11,12].

Drug campaigns have successfully targeted proteases; for example, the angiotensin-converting enzymes (hypotension drugs including the original *Captopril*, Bristol Myers Squibb) and HIV protease (HIV/AIDs anti-retrovirals including the original *Saquinavir*, Hoffman-La Roche). However, developing drugs for new protease targets has proven to be particularly challenging, in part due to the importance of achieving selectivity [13]. Since the parasite and host MAPs show limited sequence identity (<35%) and have differing substrate specificities, rational design and delivery of selective parasite-specific inhibitors is an exciting possibility. A general approach for therapeutically targeting proteases is to identify specific inhibitors — generally small molecules — that can block the active site. The search for inhibitors can be accelerated with knowledge of both the active site structure and the intrinsic subsite occupancy. If rational drug design platforms are going to succeed, it will be absolutely essential that precise structure–activity-relationship and mechanistic data are available that can be used to exploit selective intricacies of the parasite enzyme over its mammalian homologs.

The methionine aminopeptidases

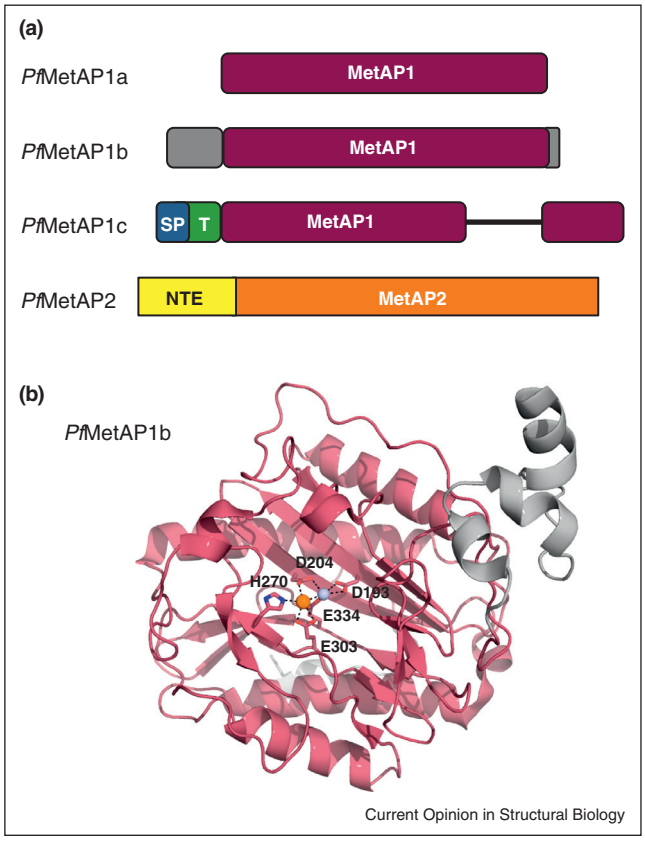
The methionine aminopeptidases (MetAP) perform the essential housekeeping role of catalyzing the removal of

[1] The nomenclature of Schechter and Berger [1] is used here. P1 and P1′ refer to substrate residues while the S1, S1′ refer to the corresponding enzyme subsites.

[2] To date, there is no structural information for either prolyl aminopeptidase and as such, these two proteases will not be discussed further in this review.

the N-terminal initiator methionine during protein synthesis [14]. Two distinct types of MetAP enzymes exist, MetAP1 and MetAP2, having mutually redundant yet essential functions within all organisms [15]. In comparison to MetAP1, MetAP2 has a 65 amino acid insertion in its catalytic domain and an additional N-terminal domain (in eukaryotes) [14]. The *Pf*MetAP2 enzyme contains a 274 residue N-terminal domain and a conserved catalytic C-terminal domain [16] (Figure 1a). Recombinant *Pf*MetAP2 binds fumagillin derivatives with high selectivity and these compounds are now being considered as antimalarial agents targeting *Pf*MetAP2 [16].

Figure 1



The structure of the *Pf*MetAPs. **(a)** The domain structure of the *Pf*MetAPs. The three isoforms of *Pf*MetAP1 all contain a canonical MetAP1 catalytic domain (shown in ruby). *Pf*MetAP1c contains an N-terminal signal peptide (in blue, SP) followed by a transit peptide domain (in green, T) to target the enzyme to the apicoplast [16]. *Pf*MetAP1c also contains a ~210 residue insertion in the MetAP1 catalytic domain (shown as black line). The *Pf*MetAP2 enzyme has a 274 residue N-terminal extension (in green, NTE) before a canonical MetAP2 domain. **(b)** The 1.95 Å X-ray crystal structure of *Pf*MetAP1b shows a canonical MetAP1 fold (in ruby) (3S6B, MolProbity Quality Score 1.42 = 97th percentile). The *Pf*MetAP1b enzyme has an N-terminal extension of 56 amino acids when compared to the human enzyme representatives (in gray in a and b), extra 10 unstructured amino acids at its C-terminus (in gray). A single FeIII ion (orange sphere) is coordinated by residues H270, E303 and E334 (shown in sticks). A water molecule (light blue sphere) is located in the second metal site in other MetAP1 structures and is coordinated by conserved active site residues D193 and D204 (shown in sticks). The water molecule contributes to an apparent pentavalent coordination of the FeIII ion (metal bonds shown as black dashes).

There are three isoforms of MetAP1 in *Pf*; *Pf*MetAP1a, *Pf*MetAP1b and *Pf*MetAP1c [6] (Figure 1a). The three MetAP1 isoforms are active and have comparable activities to other members of the mammalian MetAP1 family [17]. *Pf*MetAP1a contains the minimal catalytic domain and *Pf*MetAP1c has a signal peptide in its longer N-terminal for targeting the enzyme to the apicoplast [16] (Figure 1a). *Pf*MetAP1b is the most characterized of the three MetAP1s. It is expressed in the early intra-erythrocytic stage of the *Pf* lifecycle [18]. Inhibition of *Pf*MetAP1b with the highly selective compound XC11 that contains a 2-(2-pyridinyl)-pyrimidine core resulted in inhibition of *Pf* proliferation *in vitro* and was active in mouse malaria models that included drug-resistant *Pf* strains [17]. It has been suggested that *Pf*MetAP1b is a promising target for the development of novel antimalarial drugs [17]. There are currently no published structures of the *Pf*MetAPs; however, the Structural Genomics Consortium solved the structure of *Pf*MetAP1b, the target of the antimalarial XC11 compound (3S6B, deposited RCSB 2011). The structure shows a typical MetAP1 core fold of pseudo twofold-related N-terminal and C-terminal domains, containing two α-helices and two antiparallel β-strands (Figure 1b). Unlike characterized mammalian homologs, the *Pf*MetAP1b active site contains a single FeIII ion rather than a di-metal core. The biological relevance of this difference remains unknown. Structural alignment of 3S6B with other known MetAP1 folds identifies the human MetAP1b as the closest structural homolog (and 4FLI the closest structural homolog) [19]. Structural alignment of the human enzyme with *Pf*MetAP1b identifies that the FeIII ion in *Pf*MetAP1b is equivalent to one metal ion site present in the human MetAP1 structures and that a water molecule occupies the second metal site (Figure 1b).

The M1 alanyl aminopeptidase, *Pf*A-M1

The *Pf*A-M1 alanyl aminopeptidase enzyme is responsible for the final stages of hemoglobin (Hb) digestion [9••], a process that provides a source of amino acids for incorporation into parasite proteins [20–22]. The *Pf*A-M1 enzyme has a broad specificity for the P1 amino acid and includes most basic and hydrophobic amino acids [23,24,25••] but preferentially cleaves leucine and methionine [25••]. *Pf*A-M1 also has subsite specificity requirements for activity [26••]. Engagement of the S1, S1′ and S2′ subsites can contribute to the catalytic efficiency [26••]. Proteolytic activity of *Pf*A-M1 has been identified in both the parasitic cytosol and the digestive vacuole, the membrane vesicle where Hb digestion begins [7,9••,23,24,27]. Whether *Pf*A-M1 digests Hb peptides in the digestive vacuole or the cell cytosol has been the topic of debate [7,9••,23,24,27–29,30••]. The *Pf*A-M1 enzyme shows a 100-fold decrease (k_{cat}/K_m) in overall efficiency in an acidic