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Plasmodium spp. Enolase: A Housekeeping Protein with Complex Moonlighting Functions

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Abstract

This article examines the evidence and the structural basis for a host of moonlighting functions of enolase in malarial parasite *Plasmodium*. Blood stages of the malarial parasite have excessive expression of enolase that is distributed in multiple organelles viz. cytosol, nucleus, food vacuole and cell membrane. Stage-dependent variation, diverse sub-cellular distribution and a host of electrophoretic species support the view that post-translational modifications may dictate spatio-temporal distribution. Growth neutralizing activity of anti-enolase antibodies has further attracted attention to investigate its role in host cell invasion. Its surface expression at multiple invasive stages, complete lack of polymorphism and being a target for growth neutralizing antibodies have made it a potential anti-malarial vaccine candidate. Current status of these efforts is discussed.

Keywords: *Plasmodium* enolase; Moonlighting; Vaccine; Malaria

Abbreviations: rPfeno: Recombinant *Plasmodium falciparum* enolase; PTMs: Post-Translational Modifications; Δ^5-rPfeno: Pentapeptide ^{104}EWGWS^{108} Deleted rPfeno; S108G-rPfeno: S108G Variant of rPfeno

Introduction

Enolases are highly conserved proteins that catalyze the inter-conversion of 2-phosphoglyceric acid and phosphoenolpyruvate [1]. This is an essential step during glycolysis as well as gluconeogenesis. Interestingly, evolution has incorporated multiple novel non-glycolytic functions in several glycolytic enzymes (Reviewed in [2]). Such non-glycolytic or 'moonlighting functions' of enolase range from its role in transcription regulation [3-6], to invasion of host cells [7-10], to structural roles [11]. Enolases from apicomplexan parasites are more similar to plant orthologs as compared to host enolases. A distinct feature of malarial parasite *Plasmodium* enolase is its diverse distribution inside the cell. Such multi-compartmental localization is indicative of this protein possessing organelle-specific physiological functions. Recent observations have detected the presence of this enzyme in food vacuole [12]; nucleus; cytoskeletal elements ; cell surface of invasive stages - sporozoites, merozoites [13] and ookinetes [14] etc. Although the protein doesn't have any organelle-specific signal sequence [15], yet it finds its diverse destinations in the cell. Structural diversity as evident from the multiple electrophoretic variants [12,16-20] that largely arises due to post-translational modifications (PTMs) is likely to form the molecular basis for its stage-dependent diverse sub-cellular functions. Although the primary sequence of this protein is highly conserved, minor structural alterations have accumulated over the course of evolutionary time scales [21]. A distinct structural feature of *Plasmodial* enolase is the presence of a five amino acid insert ^{104}EWGWS^{108} in a surface loop [22]. It is instructive to investigate whether such unique insert elements indeed form the structural basis for parasite specific moonlighting functions. Multiple strategies (NMR, ITC, site-directed mutagenesis, immunological etc.) have been employed to understand its functional significance [23-28].

Biochemical role of ^{104}EWGWS^{108}

The *Plasmodium* proteome is unique in having numerous low complexity insert sequences [21]. The functional significance of these elements is yet to be understood. *Plasmodium* spp. enolase has one distinct five amino acid insert with a sequence ^{104}EWGWS^{108} [22]. This insert is present in plant and apicomplexan enolases and absent in other eukaryotic organisms. Sequence

and location of this insert is highly conserved among different species of the malarial parasite. In an earlier study, it was shown that the presence of this insert sequence stabilized the 'Apo form' of the parasite enolase in an active 'closed' conformational state [25]. Deletion of this pentapeptide (Δ^5-rPfeno) led to reduction in binding affinity of the cofactor Mg (II), substantial decrease in enzyme activity and dissociation of dimeric form in to monomers [27]. For a detailed insight of structure-activity relationship, a single residue replacement approach was taken. The insert sequence was perturbed using site-directed mutagenesis. Kinetic (Km and *kcat*) and structural properties (oligomeric state) of these variants were evaluated and compared with the wild type enzyme. Comparison with the wild type enzyme allowed us to infer the functional significance of the residues concerned. Replacement of S108 by glycine resulted in the conversion of apo-enolase from a closed active conformation to an open inactive state. S108G variant of rPfeno behaved much like other enolases that require Mg (II) induced conformational changes to acquire an active conformation.

Further, this replacement did not affect the oligomeric state of the protein [25]. The deletion (Δ^5-rPfeno) induced dissociation of the dimer into monomers and lack of dissociation of S108G variant suggests the likely role of Trp residues in stabilizing the subunit-subunit interface interactions. Examining the modeled structure of Pfeno (based on TgENO1 [29]) revealed that none of the insert residues were a part of the active site or inter-subunit interface. Thus, the effect of perturbation of insert residues was transmitted to the active site and dimer interface via the intervening structural elements. Further, the observed enhancement in the activity of Δ^5-rPfeno upon stabilization of dimeric interface [27] indicated that allosteric type of communication prevailed among the three regions viz. EWGWS insert, subunit interface and the active site of the molecule. If EWGWS insert sequence constitutes the site for protein-protein interaction for performing moonlighting functions, it is likely that such interactions may result in subunit-subunit dissociation and consequent loss of enzyme activity. This may provide a free subunit surface available for other molecules to interact for non-catalytic functions.

Role of enolase in host cell invasion

Unusual cell surface presence of this glycolytic protein has been observed in multiple prokaryotic and eukaryotic cells. Realization that enolase acts as a cell surface receptor for plasminogen has implicated it in assisting pathogens to invade and establish infection [7,10,14,30,31]. Malignant cells and certain immune cells [32] use the same system to move through the extracellular matrix. To explore the function of this protein on the merozoite surface, mice were immunized with recombinant *Plasmodium falciparum* enolase (rPfeno). Such mice on subsequent challenge with malaria parasite exhibited considerable survival advantage [23]. These protective antibodies when tested for cross reactivity with wild type rPfeno (WT-rPfeno) and a Δ^5 variant lacking the pentapeptide insert ^{104}EWGWS^{108} showed exclusive reactivity against WT-rPfeno [27]. Polyclonal anti-enolase antibodies were also found to inhibit both the parasite growth in *in vitro* RBC cultures [23] as well as the invasion of mosquito mid-gut epithelium by ookinetes [14]. One of the barriers in invasion of mosquito mid-gut epithelium is the peritrophic matrix containing multiple extracellular proteins some of which have a -PWWP- protein-binding domain. Insert motif ^{104}EWGWS^{108} of Pfeno has been shown to recognize this sequence [26]. The dual ability of Pfeno on the surface of ookinete to serve as a plasminogen receptor and possibly anchor the parasite on the peritrophic matrix (by interacting with -PWWP- domain proteins) could facilitate efficient traversal of ookinete across the matrix barrier, thus highlighting the potential of Pfeno as an anti-malarial candidate. The idea that EWGWS could be a protective antigenic epitope was further tested by studying the protective potential of a peptide embedded with the insert sequence [24]. Although active immunization yielded rather low antibody response in mice, yet significant growth retardation in parasitemia and prolongation of survival suggested EWGWS to be a promising protective epitope against malaria.

Conclusion

Plasmodium spp. enolase has emerged as a potential protective antigen present on the surface of all three invasive stages. Efforts should be made to identify the cognate enolase receptor(s) on the surface of host cells and identify the structural elements in *Plasmodium* enolase where the invasion related functions map. Identification of such elements will provide epitopes that could yield subunit vaccine against malaria. Further, complete lack of polymorphism and not having any strain-dependent variation in sequence (strain and species transcending) would make it a promising multi-stage broad-spectrum vaccine candidate.

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