

Molecular and Structural Basis of the Functional Differences Among *Plasmodium falciparum* Enolase Mutants

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Abstract

Enolase mutants enzymatic and structural features were evaluated and provided the structural basis for protein function.

Introduction

Malaria is a global threat that requires new safe therapeutics.¹ Enolase (*Pfen*) is a glycolytic dimeric metalloenzyme (100 KDa) from *Plasmodium falciparum*, which catalyzes the ninth reaction converting 2-phosphoglycerate (2-PGA) in phosphoenolpyruvate (PEP). The enzyme is essential for parasite development.² Additionally, *Pfen* has been related to moonlight functions becoming an even more interesting molecular target for antimalarial drug design.³ Recently, Dutta et al⁴ showed that Ser108 and Leu49 that could be involved in stabilizing the enzyme in an active conformation. Here, we evaluate the impact of replacement of Ser108 with Gly/Ala/Thr on enzyme activity as well as the effect of the deletion of a five amino acid insert -104EWGWS108- that is found in apicomplexan enolases, only.

Results and discussion

In total, four *Pfen* mutants were obtained (S108A, S108G, S108T, and Δ^{104} EWGWS¹⁰⁸). The kinetic studies were performed on all mutants and compared with the wild-type enzyme. The assays were conducted by spectrometric measurements on 240 nm. The titration of 2-PGA (substrate) was conducted using concentrations in the range of 10–900 μ M in buffer solution of MOPS 100 mM, pH 7.3, 1.5 mM MgCl₂, and 1–2 μ g of protein. As can be seen in Table 1, all the mutants showed similar K_m and V_{max} values, hence, the results obtained support the view that Ser108 and the absence of the exclusive apicomplexan pentapeptide are unlikely to be crucial for enzyme activity.

To shed some light on the structural basis of these mutations, crystallographic studies on the mutated enzyme were conducted. We solved the crystal structure S108A-*Pfen* mutant at 2.0 Å resolution.

Table 1. Kinetics studies on *Pfen* wild-type and mutants. Data are expressed as the mean of three independent assays \pm SD. K_m and V_{max} values were calculated using the Michaelis-Menten equation.

Enzyme	K_m (μ M)	V_{max} (μ mol PEP.min ⁻¹ .mg ⁻¹)
wt- <i>Pfen</i>	50 \pm 5	2.4 \pm 0.5
S108A	48 \pm 6	2.1 \pm 0.6
S108T	49 \pm 6	2.3 \pm 0.3
S108G	51 \pm 8	2.5 \pm 0.3
Δ^{104} EWGWS ¹⁰⁸	48 \pm 3	2.3 \pm 0.2

The structural analysis indicated no significant differences between the mutant and the wild-type enzyme (rmsd = 0.7 Å) (Figure 1).

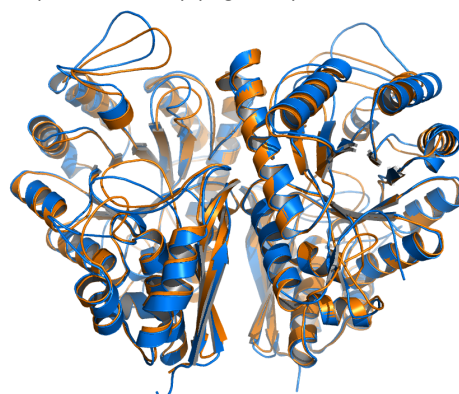


Figure 1. Structural superposition between wt-*Pfen* (blue) and S108A-*Pfen* mutant (orange).

Conclusions

The kinetic experiments have shown that either the change of Ser108 residues and the deletion of amino acids (104–108) have no impact on the enzyme activity. The X-ray crystallographic structure of the mutant supported this observation and provided a better understanding of kinetic behavior.

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¹ http://www.who.int/malaria/media/world_malaria_report_2013/en/
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