

## Structure-function studies on *Plasmodium falciparum* GMP synthetase

Javaid Yousuf Bhat, V. Roopa & Hemalatha Balaram

Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research,  
Jakkur, Bangalore 560 064. INDIA

*Plasmodium falciparum* the causative agent of the fatal form of malaria synthesizes GMP primarily from IMP and hence, needs active GMP synthetase (GMPS) for its survival. GMPS, a G-type amidotransferase catalyzes the amination of XMP to GMP with the reaction occurring in two domains, the glutamine amidotransferase (GAT) and ATP pyrophosphatase (ATPPase). The GAT domain hydrolyzes glutamine to glutamate and ammonia while the ATPase domain catalyzes the formation of the intermediate AMP-XMP from ATP and XMP. *P. falciparum* GMPS (PfGMPS) has an insertion of 20 residues in the GAT domain that is absent in all other known GMPS. Variations seen in the inhibition by nucleosides and nucleotide analogs between human and *P. falciparum* GMPS highlighted differences in ligand specificity that could serve as a basis for the design of specific inhibitors.

The kinetic mechanism of *Plasmodium falciparum* GMPS (PfGMPS) is steady state ordered with ATP binding first, followed by XMP to the ATPase domain and glutamine binding in a random manner to the GAT domain. We attribute the irreversible, ping-pong step seen in initial velocity kinetics to the release of glutamate prior to the attack of adenylyl-XMP intermediate by ammonia<sup>1</sup>.

The parasite GMPS exhibited ammonia dependent activity whose efficiency ( $k_{cat}/K_m$ ) was 10 fold lower than that of the glutamine dependent activity. pH optimum of the ammonia dependent reaction at 9.2 was higher than that for the glutamine dependent activity (7.4) indicating the direct channeling of ammonia to the ATPase domain as seen in CTP synthetase, carbamoyl phosphate synthetase and *E. coli* GMP synthetase. Augmentation of activity seen upon increasing the concentration of glutamine or ammonia at fixed saturating concentration of the other suggests the presence of two separate entry routes for ammonia.

Though unlike the human enzyme, PfGMPS exhibited significant 'leaky' glutaminase activity, formation of the reaction intermediate, AMP-XMP, in the ATPase domain enhanced glutamine hydrolysis in the GAT domain, indicating the presence of inter-domain cross talk. Enhanced inactivation of the GAT domain by the inhibitor, acivicin, in the presence of ATP or ATP+XMP further supported inter-domain modulation of activity in the two catalytic pockets. Mass spectrometric analysis of tryptic digests of PfGMPS both in the presence and absence of ATP or ATP+XMP showed an alteration in susceptibility to proteolysis upon ligand binding to the ATPase domain. Most evident was the observed inaccessibility of Lys 38 in the GAT domain brought about by conformational changes induced in this domain upon ATP or ATP+XMP binding to the ATPase domain. These results will be presented.

<sup>1</sup> Bhat JY, Shastri BG, Balaram H. (2008) Kinetic and biochemical characterization of *Plasmodium falciparum* GMP synthetase. *Biochem J.* 409, 263-73.