Functional Characterization of Inhibitor-Resistant Mutants of cGMP-Dependent Protein Kinase (*Pf*PKG) from Plasmodium falciparum

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malaria, *Pf*PKG, kinase, mutations, resistance

Malaria is a severe infectious disease caused by protozoa of the genus Plasmodium and remains one of the greatest challenges to global public health. Despite advances in disease control, the increasing resistance to antimalarial drugs, especially to artemisinin-based combination therapies (ACTs), threatens to undermine recent progress. In this context, the cGMP-dependent protein kinase of P. falciparum (*Pf*PKG) has emerged as a promising molecular target for developing new antimalarial drugs. *Pf*PKG plays an essential role in the parasite's life cycle and exhibits structural differences from its human homologs, making it possible to design selective inhibitors. Mutations in this protein induced by inhibitors have already been described, suggesting possible mechanisms by which the parasite may develop resistance to compounds with this mode of action. In this study, we investigated the biochemical effects of mutations in the catalytic site of *Pf*PKG (I602M, T618I, and T618Q). We assessed how these alterations impact protein expression, enzymatic activity, and their potential influence on interactions with active-site inhibitors. The genes encoding the mutant proteins were generated by site-directed mutagenesis and cloned into expi-Sf9 insect cells for heterologous expression. The introduction of the T618I and T618Q mutations was confirmed by sequencing, and the T618Q variant was successfully expressed and purified. Enzyme production was verified by Western blot, and its molecular mass was determined by mass spectrometry, revealing a value consistent with that reported in the literature. The purified protein displayed kinase activity in the Kinase-Glo assay comparable to the wild-type protein, indicating that catalytic function was preserved. These results suggest that the T618Q mutation does not compromise the enzymatic activity of *Pf*PKG, allowing further studies to assess how this and other mutations (I602M and T618I) affect the affinity and susceptibility to inhibitors. Therefore, these findings contribute to the characterization of mutations associated with drug resistance and aid in the development of selective *Pf*PKG inhibitors with a reduced likelihood of inducing resistant parasites.