Thesis Tittle *Plasmodium falciparum* Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS) and their Mutants from Synthetic Gene

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ABSTRACT

We have exploited a synthetic gene for *P. falciparum* dihydrofolate reductase (DHFR) to identify putative amino acids involved in anti-folate resistance in Plasmodium. Based on the known mutations thus far observed from Pyr- and Cyc-resistant field isolates, DHFR mutants with single and multiple mutations at residues 16, 51, 59, 108 and 164 of the DHFR domain were constructed by cassette mutagenesis. The mutants, some of which are never found in nature, were subcloned into expression vector and expressed under the control of T7 promoter. The enzymes were expressed in *E. coli* as insoluble inclusion bodies with molecular mass ~27 kDa upon SDS-PAGE. Soluble enzyme activity could be recovered by unfolding and refolding. All refolded mutant enzymes were purified by MTX-Sepharose CL-6B affinity chromatography to give the homogeneous enzyme except for that from quadruple mutant (N51I+C59R+S108N +I164L) which bound to the affinity resin only 20%. Alternative purification scheme

employing hydroxyapatite resin had to be used for the quadruple mutant.

The DHFRs from mutation at residue 16 from Alanine to Valine (A16V) associated with that at residue 108 from Serine to Threonine (S108T) were resistant to Cyc but sensitive to Pyr. The DHFRs from single mutant (S108N) were slightly resistant to Pyr and Cyc. The DHFRs from double mutants (N51I+S108N and C59R+S108N) were moderately resistant to Pyr and Cyc. The DHFRs from triple mutants (N51I+C59R+S108N and C59R+S108N+I164L) were 5-10 times more resistant to Pyr and Cyc than those observed from double mutants. The quadruple mutant N51I+C59R+S108N +I164L showed the most Pyr- and Cyc- resistant enzyme. These results provide proof of the linkages of these residues to anti-folate resistance. A sequential development of anti-folate resistance in malaria parasites was proposed by involving primary mutation at residue 108 and subsequent mutations at residues 51, 59 and 164.