

Thesis Title	Mutagenesis of <i>Plasmodium falciparum</i> dihydrofolate reductase: linkage between anti-folate resistance and mutation at residue 108
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## ABSTRACT

The roles of residue 108 of *Plasmodium falciparum* DHFR in conferring anti-folate resistance were investigated. Mutants containing mutations at residue 108 of synthetic plasmodial DHFR were constructed by combinatorial cassette mutagenesis, a process by which the target codon is mutated to encode for all possible amino acids. With the exception of S108K, all other DHFR mutants highly expressed DHFR as inactive inclusion bodies of molecular mass about 27 kDa in *E. coli* system. Active DHFR activity could be recovered upon refolding of the inclusion bodies. Substitution of Ser108 by other amino acids affected the kinetic parameters of the enzymes and altered the inhibitory effects against pyrimethamine and cycloguanil. Mutants with Thr or Asn at residue 108 caused approximately 50% reduction in DHFR activity as compared to the wild-type enzyme. Poor

DHFR activities were observed from mutants with Gly, Ala, Gln at residue 108, and mutants with Cys, Val, Leu and Met showed very poor DHFR activities. Mutations at residue 108 from Ser to Ile, Arg, Pro, Asp, His, Tyr, Phe, Trp, Glu yielded inactive enzyme. The mutant DHFRs from S108G, S108C, S108A and S108Q were purified to homogeneity by methotrexate-sepharose affinity chromatography. The kinetic parameters and inhibition by antifolates of the mutant enzymes were investigated. Mutant DHFR with Ser108 to Gln mutation was highly resistant to both pyrimethamine and cycloguanil with elevated  $K_m$  values for the substrates. However, other mutant DHFRs were susceptible to the drugs. Inhibition studies also revealed that the wild-type DHFR and DHFRs from S108N and S108Q mutants were competitively inhibited by pyrimethamine and cycloguanil. The data suggested that mutation at residue 108 from Ser to Asn was more preferably selected over other amino acids in nature, presumably due to its stability and ability to confer resistance to anti-folates, while substitutions with other amino acids might not be favorable or parasite survival.