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AMORNRAT BUNWATTANAKUL : *PLASMODIUM FALCIPARUM* DIHYDROFOLATE REDUCTASE: INTERACTION AMONG AMINO ACID RESIDUES RESPONSIBLE FOR ANTIFOLATE BINDING, THESIS ADVISOR: WORACHART SIRAWARAPORN, Ph.D., PRAPON WILAIRAT, Ph.D. 88 p. ISBN 974-663-161-6

Linkages between point mutations in the dihydrofolate reductase (pfDHFR)domain of *Plasmodium falciparum* bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS) and antifolate resistance have been widely observed and well documented. Previous laboratory studies on the kinetic properties and inhibition by antifolate agents, e.g. pyrimethamine (Pyr) and cycloguanil (Cyc) of naturally occurring pfDHFR mutants revealed that the mutated amino acids synergistically interact for binding to antifolates Pyr and Cyc. The results raise questions as to whether such phenomenon are unique only for the naturally occurring mutants or can be observed in the laboratory constructed of naturally unfound mutant DHFRs.

In this study, a series of fifteen pfDHFR mutants were constructed by cassette mutagenesis in positions 16, 51, 59, 108, and 164. Expression in *E. coli* of each mutant were monitored by DHFR activity assay and SDS-polyacrylamide gel electrophoresis. Except for N51I+I164L mutant which did not express DHFR, all other mutants yielded protein band of molecular mass ~27 kDa in the insoluble fraction. Only six out of fifteen mutants had detectable DHFR activity in the soluble extract. Among these are five double mutants (A16V+N51I, A16V+C59R, N51I+C59R, C59R+I164L, S108N+I164L) and one triple mutant (N51I+C59R+I164L). The DHFR from these six mutants were purified and characterized with respect to their kinetics and inhibition by Pyr and Cyc. The mutants with N51I+C59R, C59R+I164L, S108N+I164L, and N51I+C59R+I164L mutations conferred cross resistance to both Pyr and Cyc and showed good correlation with the naturally occurring mutant DHFRs ($R = 0.934$), while the mutants with A16V+N51I and A16V+C59R mutations conferred resistance to Cyc but remained susceptible to Pyr. The free energy ($\Delta G°$) and the interaction energy ($\Delta\Delta G°$) were calculated from the inhibition data to explain the molecular interactions among the combinations of mutagenized residues. Data for the six unnaturally occurring mutants reveal cooperative interactions among mutated residues for Pyr binding but not for Cyc. The results support the hypothesis that combinations of multiple mutations at specific residues contribute toward antifolate resistance, and that mutants which are either poorly resistance or have insufficient catalytic DHFR activity to support DNA synthesis do not survive under antifolates pressure.

Studies were also extended to two new DHFR mutants, C50R and Bolivia repeat, recently found in Bolivia. The results revealed no drastic changes in their kinetic parameters as compared to the wild-type enzyme. The C50R mutant and Bolivia repeat, however, did not markedly affect the drug resistivity to antifolate agents.