DISTINCT DIMER INTERFACE OF *PLASMODIUM FALCIPARUM* THYMIDYLATE SYNTHASE: IMPLICATION FOR SPECIES-SPECIFIC ANTIMALARIAL DRUG DESIGN

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Abstract. Thymidylate synthase (TS), a homodimer with two active sites near the dimer interface, plays a central role in DNA biosynthesis. Each active site is located on each subunit but shares a number of amino acid residues for catalytic activity. As TS is highly invariant across species, this has raised problems in designing inhibitors selective against *Plasmodium falciparum* (*Pf*) TS, but not against the human (h) counterpart. However, there exists differences in amino acids at the TS interface of *P. falciparum* and human enzyme, which are critical for dimerization in each species. Here, we employed *in vivo* genetic complementation and 6-[^{3}H]-FdUMP binding assays of transformants from TS-deficient *Escherichia coli* with a variety of pairs of inactive *Pf*TS(R470) and inactive hTS(C195) mutants, and vice versa, to demonstrate none of the combinations formed active cross-species TS heterodimers. Visualization by structural superposition of TS from the two species revealed incompatible interface amino acids stemming from residues of different porality. Key residues at hTS dimer interface (Q62, Q211 and T251) and their equivalence in *Pf*TS (I357, I506 and V546) could not be interchanged to generate active TS cross-species heterodimers using the co-transformation complementation assay. These results demonstrate that the TS interface of *P. falciparum* is unique and completely different from that of the human enzyme, suggesting that this domain provides a target for development of novel antimalarials.

Keywords: *Plasmodium falciparum*, antimalarial, malaria, subunit complementation, thymidylate synthase

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