DIHYDROFOLATE REDUCTASE OF *PLASMODIUM FALCIPARUM*: EFFECTS OF JUNCTIONAL PEPTIDE ON ENZYME KINETICS

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Erythrocyte Dihydrofolate Reductase (DHFR) and Thymidylate Synthase (TS) are enzymes that are crucial in the process of creating thymidylate which is an essential component of DNA synthesis in Plasmodium falciparum. They are known to be on the same polypeptide chain and are connected by a short polypeptide (Junctional Region, JR) which connects between the enzymes. DHFR-TS is an enzyme that is crucial for the development of new drugs for the treatment of malaria, as such knowledge will be useful in understanding the structure and function of the enzymes. Researchers want to study the role of the polypeptide connection in the function of DHFR enzyme. They made clones and expressed them in bacteria and studied the structure and inhibition of the enzymes. Results showed that the enzymes with different lengths of JR at the C-terminus have similar characteristics with pfDHFR 74.
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ABSTRACT

Dihydrofolate reductase and thymidylate synthase are two essential enzymes in the *de novo* synthesis of thymidylate required for DNA synthesis. In a malaria parasite, *Plasmodium falciparum*, these two enzymes exist as a bifunctional protein and are linked by a junction region. The junction of these two enzymes in *P. falciparum* is an important drug target of antifolate antimalarials such as pyrimethamine and cycloguanil in malarial chemotherapy. A thorough understanding of the enzyme structure and its interaction with antifolates would aid in the development of new antifolates effective against resistant malaria. Comparative analysis of the kinetic properties of dihydrofolate reductase and the kinetics of the enzyme with junction peptides of varying lengths would be a possible means to investigate their effects on the enzyme’s properties.

The goal of this study was to investigate the roles of the junction region on the catalytic activity and kinetic properties of dihydrofolate reductase in *P. falciparum*. I have constructed four recombinant clones of genes encoding for this enzyme containing different lengths of junction peptides extending toward the carboxyl-terminus of this enzyme. Expression and purification of the recombinant proteins in *E. coli* was performed. Kinetic studies and inhibition by antifolates were carried out on the recombinant proteins and the results obtained were compared to those of the dihydrofolate reductase in *P. falciparum*.

My results showed that the presence of junction peptide extending toward the carboxyl-terminal of the dihydrofolate reductase in *P. falciparum* did not improve the level of expression of the enzyme nor affect its stability. The kinetic properties of enzymes with different lengths of junction peptides were comparable to those of the dihydrofolate reductase in *P. falciparum*.

KEY WORDS: DIHYDROFOLATE REDUCTASE / JUNCTION REGION / *PLASMODIUM FALCIPARUM*

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