ABSTRACT

Using novel high-performance liquid chromatographic (HPLC) techniques to study pterin and folate metabolism of Plasmodium falciparum in in vitro, and to develop enzyme assays, it was shown that P. falciparum can synthesize pteroylpolyglutamate de novo from its metabolic radiolabeled precursors, which are guanosine 5'-triphosphate (GTP), 4-aminobenzoate (PABA), and L-glutamate (L-Glu). The parasite also synthesized a pteroylpolyglutamate cofactor as a major product from both intact and degraded forms (4-aminobenzoylglutamate, PABG; and 6-aldehydepterin) of exogenous folate in its growth medium. The major product was identified as 5-methyl-tetrahydropteroylpentaglutamate (5-methyl-$H_4PteGlu_5$) through comparison with authentic $PteGlu_n$, UV spectrum, exposure to pteroylpolyglutamate hydrolase (conjugase), and oxidative cleavage of C9-N10 bond with subsequent identification of products by a reversed-phase HPLC. The de novo synthesis of pterins by P. falciparum-infected red cells was demonstrated,inhibitable by N^7-methylguanosine (N^7MeGR), an innibitor of GTP cyclohydrolase from the parasite which had moderate antimalarial activity against P. falciparum growth. The GTP cyclohydrolase activity was shown in 3 species of Plasmodium : P. berghei, P. knowlesi, and P. falciparum. The 190-fold purified enzyme by a high-performance size-exclusion chromatography (HPSEC) on a TSK-G-3000 SW column had different characteristics from the host lymphocyte enzyme e.g. K_m for GTP, K_i for N^7MeGR, molecular weights.

The de novo 5-methyl-$H_4PteGlu_5$ synthesis was found to be inhibited by N^7 MeGR, sulfadoxine and pyrimethamine, whereas the folate salvage for 5-methyl-$H_4PteGlu_5$ synthesis was inhibited by sulfadoxine and pyrimethamine. Pyrimethamine-resistant parasites showed less sensitivity in the cofactor synthesis to the drugs. It is suggested that P. falciparum has both the de novo and folate salvage pathways for 5-methyl-$H_4PteGlu_5$ synthesis which are analogous to bacterial and mammalian systems, respectively. Furthermore, the salvage from the folate degradative products (PABG and 6-aldehydepterin) which be strongly inhibited by sulfadoxine is a unique pathway for the parasite.