Thesis Title	Isolation and Characterization of DNA Polymerases and DNA Topoisomerase II from Plasmodium falciparum
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ABSTRACT

Plasmodium falciparum is the sporozoan parasite that causes the most serious form of human malaria. Widespread multi-drug resistance raises an urgent need for new antimalarial drugs and investigation of potential target enzymes at the biochemical and molecular level.

Two enzyms involving DNA replication, DNA polymerases and DNA topoisomerase II, were chosen as possible target enzymes in this study. Fractionation of Plasmodium falciparum cellular extracts by FPLC identified at least two different DNA polymerases. One DNA polymerase fraction co - purified with a primase activity and therefore contained DNA polymerase α. Its sensitivity to a range of inhibitors was consistent with this conclusion.

The only exception was its relative resistance to Butyl-phenyl dGTP indicating possible structural differences between host and parasite DNA polymerase α. The other DNA polymerase matched eukaryotic DNA polymerase ϑ in all properties tested in this study. DNA polymerase α-like enzyme was inhibited by diphosphorylated (S)-9-(3-hydroxy-2-phosphonyl-methoxypropyl)adenine with an IC_{50} at 40 μM and a ϑ-like DNA polymerase was 40-fold more sensitive.

DNA topoisomerase II was partially purified by FPLC. The enzyme showed ATP-and Mg^{2+}-dependent activities in a decatenation assay and the highest activity was detected in the presence of 100 mM KCl. Enzyme activity was not inhibited by the DNA topoisomerase I inhibitor, camptothecin, but was sensitive to both prokaryotic and eukaryotic DNA topoisomerase II inhibitors.

An in vitro investigation of the structure - activity profiles for a range of 9-anilinoacridines on drug-resistant Plasmodium falciparum showed that C-3, 6-diamino substitution, low lipophilicity, and high pKa values substantially increased inhibitory activity and there appeared to be no correlation with DNA binding. 3, 6-Diamino-1'-amino-9-anilinoacridine was the most active and least toxic compound. It and pyronaridine, a 9-anilino-aza-acridine inhibited decatenation activity of parasite DNA topoisomerase II.