Thesis Title	Purification and characterization of dihydrofolate reductase from P. falciparum K_1
Name	Siriluk Atamaprasangsa
Degree	Master of Science (Biochemistry)
Thesis Supervisory Committee	Yongyuth Yuthavong Pintip Ruenwongsa M.R. Jisnuson Svasti
Date of Graduation	April, 16, 1988

ABSTRACT

Dihydrofolate reductase and thymidylate synthetase are key enzymes for DNA synthesis in the malarial parasites where dTMP is synthesized de novo . Attempts have been made to purify dihydrofolate reductase from *P. falciparum* K_1. The enzyme was first partially purified by folate-Sepharose CL-4B column chromatography. About 30 fold purification with 69% yield was obtained from this column . However , the enzyme from folate-Sepharose CL-4B was shown to contain several protein bands on SDS-polyacrylamide gel electrophoresis. Methotrexate (MTX)-Sepharose CL-4B column was used to obtain better purification . The enzyme was purified about 150 fold at 63% yield by MTX-Sepharose column. The molecular weight of the enzyme estimated by gel filtration on Sephacryl S-300 column was about 160,000 . The molecular weight of the enzyme was estimated to be 67,000 on SDS-polyacrylamide gel electrophoresis . The optimal pH and the optimal temperature

for the enzyme were about 6.0 and 40 ^{o}C respectively. The enzyme from *P. falciparum* K_1 was slight activated by 0.1-0.5 M KCl . However , a slight decline in its activity was observed as KCl concentrations increased from 0.5 M to 1.0 M . The K_m values for dihydrofolate and NADPH of the enzyme was 7.6 μM and 15.9 μM respectively. Inhibition of dihydrofolate reductase from *P. falciparum* K_1 by pyrimethamine was found to be inhibition of mixed type with K_{is} of 0.7 nM and K_{ii} of 2.35 nM.