Thesis Title Studies on Dihydrofolate Reductase from *Mycobacterium smegmatis*

Name Atid Chanpongsri

Degree Master of Science (Biochemistry)

Thesis Supervisory Committee

Worachart Sirawaraporn, Ph. D.

Yongyuth Yuthavong, D. Phil.

Prapon Wilairat, Ph. D.

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ABSTRACT

Dihydrofolate reductase (5,6,7,8-tetrahydrofolate:$NADP^+$ oxidoreductase E.C. 1.5.1.3) is an enzyme of chemotherapeutic interest. It is a target for a number of antitumor, antibacterial and antiprotozoal agents. However, there has been very little study of the enzyme from mycobacterium despite the fact that the organisms are causative agents of the diseases such as leprosy and tuberculosis. The present study involves purification and characterization of dihydrofolate reductase from *M. smegmatis* , a saprophytic organism closely related to *M. leprae* and *M. tuberculosis* . Dihydrofolate reductase from wide-type *M. smegmatis* was purified by affinity chromatography on Methotrexate-Sepharose followed by Mono Q^{TM} FPLC. These resulted in homogeneous enzyme of 26% yield with 25,000 fold purification. The enzymes from both wild-type and trimethoprim-resistant *M. smegmatis* showed similar apparent molecular weight of approximately 23 kDa upon gel filtration on Sephadex G-100 and SDS-PAGE. The optimum temperature for both enzyme was 55°C. However, the enzyme from wild type was more sensitive to thermal denaturation than the trimethoprim-resistant enzyme. The enzyme from trimethoprim-resistant organism was not significantly inhibited by NaCl and KCl, whereas 1 M NaCl and KCl inhibited about 50% of the activity of the trimethoprim-sensitive enzyme. The enzymes from both sources were slightly affected by urea (0.1-3.5 M). The K_m values for dihydrofolate and NADPH of the highly purified enzyme from sensitive organism were 0.50 ± 0.1 μM and 11.4 ± 0.4 μM, respectively. The K_i value for trimethoprim was 4.0 ± 0.1 nM.

The k_{cat} of about 400 min^{-1} was determined for the highly purified enzyme from this organism. The amino terminal sequence of highly purified DHFR from trimethoprim-sensitive *M. smegmatis* showed homology with DHFRs from other gram-positive organisms. The data suggested that mutation of DHFR gene and probably in combination with other mechanisms may contribute to trimethoprim resistance in *M. smegmatis*.

DHFR from *E. coli* harboring cosmid ec^2 493 was purified by combination of affinity chromatography, FPLC gel filtration and FPLC anion exchange chromatography. The final step yielded two homogeneous proteins which had the same apparent molecular weight of 23 kDa from FPLC gel filtration and SDS-PAGE. Both enzymes had the same amino terminal sequence which showed high homology to the sequence of DHFR from trimethoprim-resistant *M. smegmatis* . The results of hybridization have also proved that both enzymes should have resulted from the expression of the inserted DHFR gene in the cosmid.