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VANIDA NOPPONPUNTH : MOLECULAR CLONING AND EXPRESSION OF DIHYDROPTEROATE SYNTHASE FROM *M. TUBERCULOSIS* AND *M. LEPRAE*. THESIS ADVISORS : WORACHART SIRAWARAPORN, Ph.D., YONGYUTH YUTHAVONG, D.Phil., PRAPON WILAIRAT, Ph.D., MATHUROSE PONGLIKITMONGKOL, Ph.D. 240 p. ISBN 974-663-535-2

Dihydropteroate synthase (EC.2.5.1.15, DHPS) is an obligatory enzyme in the *de novo* folate biosynthesis of prokaryotes and protozoan, whereas human cells can salvage and utilize exogeneous folates. Such a difference in folate metabolism has made DHPS an attractive target for chemotherapy. This research concerns the cloning and expression of DHPS from *M. tuberculosis* and *M. leprae*. Sufficient amounts of recombinant enzymes will provide opportunities for further studies that might lead to rational drug design of new effective anti-mycobacterial agents. The gene coding for DHPSs of *M. tuberculosis* and *M. leprae* were cloned from gDNA libraries by hybridization using probes derived from the polymerase chain reaction. DNA sequencing revealed an open-reading-frame of 840 bp, encoding a protein of 280 amino acids for *M. tuberculosis* DHPS, and of 852 bp encoding a protein of 284 amino acids for *M. leprae* DHPS. Both DHPSs were monofunctional enzymes. The enzymes were successfully expressed under the control of T5 promoter in a DHPS deficient strain of *E. coli*C600 Δ *folP*::Km^r. Using three chromatographic steps, i.e. DEAE-Sepharose, DyeMatrex Gel Green A and Hydroxyapatite, the DHPSs of *M. tuberculosis* and *M. leprae* were purified to ~98% homogeneity. SDS-PAGE revealed molecular mass of 29 kDa and 30 kDa for *M. tuberculosis* and *M. leprae* DHPS, respectively. Gel filtration of both enzymes showed a molecular mass of ~ 60 kDa indicating the existence of a dimer of identical subunits in the native enzyme. Steady-state kinetic analysis showed that the K_m for *p*-aminobenzoic acid and 6-hydroxymethyl-7,8-dihydropterin pyrophosphate of *M. tuberculosis* DHPS were $0.37 \pm 0.08 \mu\text{M}$ and $1.03 \pm 0.07 \mu\text{M}$, respectively, whereas those for *M. leprae* DHPS were $0.6 \pm 0.1 \mu\text{M}$ and $1.2 \pm 0.3 \mu\text{M}$, respectively. The inhibitory effects of sulfa drugs, dapsone and *p*-aminosalicylic acid were studied. Dapsone was found to be the most potent inhibitor against the recombinant mycobacterial DHPSs, whereas *p*-aminosalicylic acid, a putative DHPS inhibitor, was a poor inhibitor of the enzyme.