Amplification and cloning of the enzyme dihydrofolate reductase from *Schistosoma mansoni*

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The enzyme dihydrofolate reductase (DHFR) (E.C 1.5.1.3) catalyzes the reduction of 5,6-dihydrofolate + NADP+ to 5,6,7,8-tetrahydrofolate and NADPH in the Schistosoma pyrimydine "de novo" pathway. This enzyme is an attractive drug target, indeed is one enzyme in the TDR Targets. The importance of DHFR in chemotherapy of parasites is due to their function in DNA biosynthesis and in cell replication. The DHFR inhibition results dTMP deficiency and causes cell grown inhibition. The Schistosoma DHFR gene has 561pb and codifies a protein with 187 amino acids that has 37% identity when compared to their human homologue. In order to obtain the recombinant enzyme to functional and crystallographic studies we amplified and cloned the DHFR gene in pGEM and pET28a expression vector. The sequence of Schistosoma DHRF was obtained in the Schistosoma mansoni GeneDB (Smp_175230). The amplification of the DHFR gene was done by PCR using enriched cDNA with DHFR gene, this library was prepared using reverse primer and Schistosoma total mRNA by RT-PCR. The amplification product was inserted in the pGEM, the recombinant plasmid, purified and submitted to DNA sequencing, confirming the gene sequence. The pGEM-DHFR vector was digested with *Nde*I and *Xho*I, and the purified DHFR insert and was ligated in the pET28a. The expression testes were initiated. Using Modeller homology modeling program a molecular model was built using chicken DHFR that possess 41% sequence identity. This project belongs to the Pyri-purinome Schistosoma Project that intends solve the crystallographic structure of several enzymes involved in the purine salvage and pyrimidine "de novo" and salvage pathways.

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