ABSTRACT

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Title of diploma thesis: Cloning, expression and purification of mycobacterial dihydrofolate reductase

Dihydrofolate reductase is an enzyme essential for the metabolism of folic acid – it catalyzes the reduction of dihydrofolate to tetrahydrofolate. Tetrahydrofolate is an important cofactor involved in one-cabron transfer reactions. Dihydrofolate reductase plays a key role in the synthesis of DNA, RNA and proteins.

Dihydrofolate reductase was also found in *M. tuberculosis*. This bacterium is the most common causative agent of tuberculosis in humans. Thus dihydrofolate reductase could be a potential target for the design of new antituberculotics.

The recombinant protein dihydrofolate reductase was prepared in several steps. The coding sequence of the protein was first amplified by polymerase chain reaction. A recombinant plasmid, obtained by the ligation of an amplified segment of DNA with plasmid pET-28b(+), was transformed into competent cells *E. coli* strain BH101 by the heat shock method. Cells *E. coli* strain BL21(DE3) were used for the protein expression. The expression was induced by the addition of isopropyl-β-D-thiogalactopyranosid (IPTG). The prepared recombinant protein was isolated and purified by the affinity chromatography method. The concentration of the enzym was determined by the Bradford method. The activity of the enzym was verified by the reaction of the enzyme with dihydrofolate in the presence of NADPH. This enzyme can be used for further research, particularly to search compounds inhibiting its activity.

Key words:

mycobacterium, dihydrofolate reductase, cloning, expression, purification, activity