ABSTRACT

The metabolism of xenobiotics and endogenous substances is mediated by a mixed function oxidase system which includes cytochrome b_5 participating in catalytic activities of CYP. The mechanism of action of the cytochrome b_5 has not been fully elucidated yet. But it is assumed that cytochrome b_5 is involved either in direct electron transfer within the mixed function oxidase system or in induction of conformational changes in CYPs. So it is important to gain the pure form of apo-cytochrome b_5, devoid of heme, which is not capable of electron transfer and further study the effect of this form on CYP-catalyzed reactions. The obtained results can contribute to understanding the mechanism of cytochrome b_5 effects.

The transformation of bacterial cells of *Escherichia coli* BL-21 (DE3) Gold was performed by expression vector pET22b which contained genes for microsomal and erythrocyte cytochrome b_5.

In order to produce a high level of apoprotein form, the heterologous expression of cytochrome b_5 was induced by addition of higher amount of IPTG. Expression was performed at 37°C.

This bachelor thesis is primarily engaged in purification of both microsomal and erythrocyte form of cytotochrom b_5, especially in its apo-form. However, the productions of holo-cytochrome b_5 form always occur in a greater or lesser extent during the expression, so this was also isolated.

During isolation, holo- and apoprotein form of microsomal and erythrocyte cytochrom b_5 were divided and processed separately. Microsomal apo-cytochrome b_5 was purified by chromatography on DEAE-Sepharose and erythrocyte on DEAE-Sepharose and Sephadex G-75.

The native structure of apo-cytochrome b_5 was verified by its ability to accept the heme group in its molecule by titration with hemin.

Keywords:

cytochrome b_5, cytochromes P450, protein expression, bacterium *E. coli*, solubilization, isolation

(In Czech)