Abstract

Cytochrome b_5 is a small amphipathic protein. The human form is anchored to the outer membrane of the endoplasmic reticulum and mitochondria, a free form is located in red blood cells. It consists of two domains: a large hydrophilic domain binds heme, a small hydrophobic domain anchors cytochrome b_5 to the microsomal membrane. Both domains are connected by linker chain of about 15 amino acids, which gives a flexibility to the protein. Its length plays an important role in transferring electrons to cytochrome P450. If the linker domain is too short, cytochrome b_5 is not able to tranfer electrons to cytochrome P450 and not participates in the reactions of MFO system. Other functions are preserved.

The aim of this study was to design and build 4 deletion mutants of cytochrome b_5 using gene synthesis. The linker domain contains long and short deletions, which are expected to have distortion interaction with cytochrome P450.

Part of this thesis was the expression of heterologous proteins by cells of *Escherichia coli* strain XL10-Gold and DH5α. As expression vectors for the transformation were used plasmids pET-30a(+) and pET-22b. DNA from cells was isolated and the accuracy of the genetic code was verified using the sequencing.

Keywords: cytochrome b_5, heterologous expression, gene synthesis

(In Czech)