Abstract

NADPH:cytochrome P450 reductase (CPR) is a 78 kDa flavoprotein, which is together with cytochrome P450 component of monooxygenase system bound in the membrane of the endoplasmic reticulum. Monooxygenase system is involved in the metabolism of a wide range of organic substances, including drugs or various pollutants present in the environment (polycyclic aromatic hydrocarbons, aromatic amines, etc.). CPR works as a transporter of reducing equivalents from NADPH to the cytochromes P450. For proper interaction with cytochromes P450, intact N-terminal hydrophobic domain anchoring protein in the membrane is needed. Removing this domain, e.g. during trypsin proteolysis, gives rise a soluble CPR (72 kDa) and cause loss of catalytic activity towards cytochrome P450. During heterologous expression in *E. coli* proteolytically sensitive site of CPR (Lys56 - Ile57) is cleaved by intracellular trypsin-like proteases, that may negatively affect the yields of native 78 kDa protein.

This thesis describes the heterologous expression, purification and characterization of two forms of rat CPR. WtCPR is a protein naturally occurring in rats (Wistar strain), while mCPR contains one amino acid substitution (K56Q) in the site of proteolytic degradation. The result of that substitution is proteolytically stable CPR, resistant to proteases with trypsin activity. Both forms of CPR were expressed in *E. coli* BL21 (DE3) RIL cells using the expression vector pET22b. Recombinant proteins were purified to homogeneity using ion exchange (DEAE Sepharose) and affinity (2'5' ADP Sepharose) chromatography. Enzyme activity of wtCPR and mCPR was verified. Rat CYP1A1 reconstituted with wtCPR and mCPR was capable to oxidize Sudan I, a marker substrate of this CYP. Furthermore, both forms of CPR were capable of reducing human cytochrome b_5.