Abstract

Cytochromes P450 represent a large group of proteins metabolizing variety of substrates. Many of them are responsible for metabolism of xenobiotics including drugs and chemical carcinogens.

Heme-protein cytochrome b_5 is a single-electron donor cooperating with a NADPH:cytochrome P450 reductase and NADH:cytochrome b_5 reductase 3 enzyme. Cytochrome b_5 can affect the xenobiotic metabolism via modulation of the cytochromes P450 activity.

One of the goals of the Ph.D. thesis was to utilize site directed mutagenesis of cytochromes P450 family 1 to elucidate the mechanism of their nitroreductase activity. Another aim was to study the interaction between cytochrome b_5 and cytochromes P450 of the 1A subfamily using site directed mutagenesis on presumed protein-protein contact interface. Another goal was to utilize the combination of theoretical and experimental approaches to explain variance in the reduction state of several human cytochromes P450 heterologously expressed in intact bacterial cells.

The results found in the thesis show that nitroreductase activity of CYP1A1, CYP1A2 and CYP1B1 is mediated by the presence of a particular hydroxyl group in their active centre. Single mutation introducing a hydroxyl group to the specific part of CYP1B1 active site to the active site turned on its artificial nitroreductase activity. Other results identified several amino acids residues located on the contact of cytochrome b_5 with CYP1A1 or CYP1A2. Finally the results of combined theoretical and experimental approaches suggest that the differences in reduction state of human cytochromes P450 heterologously expressed in intact bacterial cells, which can be related to the differences in their interaction with bacterial electron donating protein - flavodoxin.