# Abstract

The system of mixed-function oxidases (MFO system) has a significant role in metabolism of many endogenous compounds, as well as xenobiotics (for ex. karcinogens, drugs). Membrane-bound haemoproteins called cytochromes P450 are a vital part of that system. Reactions catalyzed by cytochromes P450 are influenced by another protein of the MFO system, cytochrome $b_5$. The mechanism of this cyt $b_5$ agency has not yet been fully described. One of methods used for study of this protein-protein interaction is covalent cross-linking. By replacing one of three methionines in the cyt $b_5$ structure by a photo-reactive analogue (photo-methionine), an analogue of cyt $b_5$ (photo-cyt $b_5$) can be obtained. When activated by UV radiation, the protein covalently bonds cytochrome P450 in a membrane environment.

This paper focuses on expression and isolation of a recombinant cyt $b_5$ analogue with only one methionine position (96) in the protein structure and substitution by photo-methionine. Protein was purified in a yiedl of 6 mg from 1 liter of baterial suspension. Analysis by mass spectrometry (MALDI-TOF/TOF) showed methionine to have been substituted by the photo-reactive analogue in approx. 30 %. Photo-cyt $b_5$ was used to fixate transient protein-protein interactions with cytochrome P450 2B4 (CYP2B4). Photo-cyt $b_5$ was reconstituted in a lipid system with CYP2B4 and photolyzed. With SDS electrophoresis, 3 heterooligomers were found. Peptide mapping showed presence of both the peptides originating from cyt $b_5$ and CYP2B4 in all of the three complexes.



**Keywords:**

NADPH:cytochrome P450 oxidoreductasa, cytochrome b5, protein expression, fotocrosslinking