Abstract

Cytochromes P450 are the major xenobiotics converting enzymes. They are classified as mixed function monooxygenases (MFO). Isoform 1A1 is a extrahepatic form found mainly in the lung and other tissues. It is strongly induced by polycyclic aromatic hydrocarbons and their derivatives via the Ah receptor. As a marker reaction for this enzyme can be used hydroxylation of Sudan I, which has previously been widely used as a azo dye in industry, but since 1980s it is banned for coloring food and cosmetics for its negative influence on the organism. NADPH:cytochrome P450 reductase is the major electron donor for cytochrome P450 catalyzed monooxygenation reactions. Another electron carrier for cytochrome P450 catalyzed reactions is cytochrome b_5. It was shown that cytochrome b_5 can stimulate, inhibit or have no effect on P450 catalyzed reactions.

This thesis aims to evaluate the influence of the ration between NADPH:cytochrome P450 reductase and cytochrome b_5 on cytochrome P450 1A1 catalyzed Sudan I hydroxylation. The main goal is to characterize the influence of electron donor and electron transfer ratios on hydroxylation of Sudan I, and to determine the kinetic parameters K_M and V_{MAX} for selected protein ratios. Partial aims of the thesis were to characterize the recombinant proteins used in this study and possibly to purify them, to optimize the methods of sample preparation and to refine the qualitative and quantitative determination of Sudan I metabolites.

(In Czech)

Key words: cytochrome P450, NADPH:cytochrome P450 oxidoreductase, cytochrome b_5, protein-protein interaction, Sudan I, HPLC, enzyme kinetics