Abstract

Mixed-function oxygenase systém (MFO systém) plays a vital role in the metabolism of a variety of both endogenous substrates and xenobiotics. This membrane systém consists of cytochrome P450s, NADPH:cytochrome P450 oxidoreductase (POR), cytochrome b_5 and NADH:cytochrome b_5 oxidoreductase (b_5R). Cytochrome P450 catalyzes a monooxygenation of a substrate, while POR and cytochrome b_5 represent its redox partners. Cytochrome b_5, itself having a redox partner in b_5R, effects the reactions catalyzed by the MFO system in various ways, through mechanisms that are not fully understood.

This paper focuses on the purification of b_5R and POR from rabbit liver. The microsomal fraction obtained by differential centrifugation contained 42 mg of protein per ml.

From a portion of the microsomal fraction, b_5R was obtained using chromatography on DEAE-Sepharose, CM-Sepharose and 5‘-ADP agarose columns. The yield was 0,3 % of ferricynide-reductase activity and the product contained several contaminants in the molecular weight range of 50-70 kDa. A second purification of b_5R from the microsomal fraction was carried out using a column of DEAE-Sepharose directly connected to a 5‘-ADP agarose column. The b_5R product was purified with a yield of 10,9 % and it once again contained several contaminants in the molecular weight range of 50-70 kDa. The prudct was able to reduce cytchrome b_5 in the presence of NADH.

During both of the purification attempts, POR was also purified using chromatography on DEAE-Sepharose and 2‘, 5‘-ADP-Sepharose columns. In the first case, POR was purified in electrophoretically pure form with the molecular weight of 78 kDa, with a yield of cytochrome c-reductase activity of 3,4 %. On second attempt, the yield was 4,7 %. In addition to POR with the molecular weight of 78 kDa, the product contained the truncated form of POR, lacking its membrane anchoring domain (72 kDa).

Keywords: cytochrome b5 reductase, cytochrome P450 reductase, purification, rabbit

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