Abstract

Cytochrome P450 1A1 (CYP1A1) is one of the major isoforms of the cytochrome P450 superfamily. It is primarily an extrahepatic enzyme which is responsible for oxidation of many polycyclic aromatic hydrocarbons and other xenobiotics. Besides of the role in detoxification metabolism CYP1A1 is the one most important isoform involved in activation of procarcinogens.

The main aim of this project was preparation of two modifications of the rat CYP1A1 gene with codon optimization for expression in *E. coli* by gene synthesis. One was wild type (wt1A1) and the other was with modified N-terminal anchor (mod1A1) - for both modifications with or without His Tag at the C-end of CYP1A1. Furthermore, an aim was to compare their level of expression in different strains of *E. coli* and try to purify and assess enzymatic activity of the gene's products.

From pre-prepared oligonucleotides 2 „syntons" (parts of gene) were synthetized and separately inserted into pUC19. After verified sequence of the „syntons" they were cleaved from pUC19 and inserted together into pET-22b. These vectors were prepared for transformation of 3 strains of *E. coli* (BL-21 (DE3) GOLD, RIL a RIPL). For production of proteins many conditions were tested: temperature (18, 22, 24, 27 a 37 °C), time of production (untill 48 hours), concentration of IPTG (0,1; 0,2; 0,5 a 1mM), induction in different OD_{600} (0,4; 0,6; 0,8; 0,9 a 1) and addition of ALA (0, 30, 60, 120 or 150 minutes before induction). Within 3 strains of *E. coli* expression of all *CYP1A1* modifications were on the same level with much higher expression for *wt1A1* than for *modCYP1A1*. The highest yield in production and activity of CYP1A1 was observed within production of wt1A1 in the BL-21 (DE3) GOLD cells (22 °C, 12 hours, induction in 0,9 OD_{600} with 0,1mM IPTG). In the next steps conditions for solubilization of wt1A1 were optimized. The most effective solubilization of wt1A1 was observed with 0,6% (v/v) Triton X-100 together with 0,6% (v/v) sodium cholate.

Within the project 1) expression vectors for preparation of recombinant rat wt1A1 and his 3 other modifications were prepared, 2) conditions for production of 4 modifications of CYP1A1 in *E. coli* were optimized and 3) solubilization step of wt1A1 from membranes of *E. coli* BL-21 (DE3) GOLD was optimized.

Key words: recombinant rat CYP1A1, gene synthesis, EROD assay, solubilization, heterologous expression, *E. coli* (In Czech)