

ABSTRACT

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Title of rigorous thesis: Implementation of experimental techniques for preparing of recombinant proteins on the model of CBR3.

This study was focused on implementation of experimental techniques for preparing of recombinant proteins. As a model protein was used the human carbonyl reductase 3 (CBR3). The recombinant CBR3 is used for numerous metabolic studies and its structure is already well described on both amino acids sequence and structure levels. Several of its substrates have been described, but its physiological functions and its role in metabolism of endogenous substances and xenobiotics are still not sufficiently described.

The preparation of the recombinant protein started from isolation of the plasmid carrying its code sequence (vector pOTB7), followed by the polymerase chain reaction (PCR) with appropriate primers containing restriction sites for the choosen endonucleases. The PCR product was ligated into the amplification vector (pCR[®]2.1-TOPO[®]) and the vector was multiplied in transformed competent cells. The code sequence was removed from the vector by using restriction endonucleases and subsequently was subcloned into the open express vector (pET-15b). The verification of the stages was performed by using agarose gel electrophoresis (0.8 and 2% agarose gel).