Abstract

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Candidate: Petra Kořínková
Supervisor: Prof. Ing. Vladimír Wsól, Ph.D.
Title of diploma thesis: Cloning and expression of human AKR1A1

The aldo–keto reductase 1A1 (AKR1A1) is monomeric cytosolic enzyme from aldo-keto reductase superfamily (AKR) with carbonyl reducing activity. The members of AKR superfamily have an important role in reductive reactions in metabolism of some endogenous substrates and in the first phase of biotransformation of xenobiotics. Fifteen members of this large family have been found in the human body. These proteins are expressed in different tissues within all our organism. The highest concentrations of proteins have been detected in the hepatocytes and in the renal cells.

The function of AKR1A1 is to catalyse reductive reactions of aromatic and aliphatic aldehydes to the respective alcohols. Among its important substrates belong mevalonate (endogenous substance) and some drugs like anthracykline antibiotics – doxorubicin and daunorubicin. AKR1A1 also participates in biotransformations of sorbitol and in the development of diabetes complications.

Preparation of recombinant protein was performed in *E. coli* with using of pET28b(+) as an expression vector. Isolated cDNA was reproduced by polymerase chain reaction (PCR) catalysed by Phusion Hot Start II polymerase with specific forward and reverse primers, with restriction sites for endonuclease *NdeI* and *XhoI.* Those ones were used for induction of the purified products of PCR into the treated vector. The products were ligated by DNAligase and transformed into competent cells by the heat shock method. In order to express the protein a strain of *E. coli* BL21 was used and reaction was induced by isopropyl-β-D-1-thiogalaktopyranoside. Concentration of protein was determined by Bradford method and protein was purified on Äcta purifier. Verification of the protein activity was made by incubation with 4–nitrobenzaldehyde.

key words: AKR1A1, carbonyl-reducing enzymes, recombinant enzymes