ABSTRACT

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

Candidate: Tereza Lundová
Supervisor: Prof. Ing. Vladimír Wsól, Ph.D.
Title of diploma thesis: Cloning, expression and purification of human AKR1B1

Aldose reductase (EC 1.1.1.21) AKR1B1 is one of the 13 human enzymes of the AKR superfamily. All human AKRs are cytosolic and NADP(H) dependent. AKR1B1 plays an important role in metabolism of endogenous and exogenous substances. The main endogenous substrate is glucose. Its reduction to sorbitol is consistently linked to secondary diabetic complications. From xenobiotics metabolized by AKR1B1, daunorubicin, an anticancer drug from the group of anthracyclines, is reduced to daunorubicinol. This metabolite is less active than parent drug and is the cause of anthracycline related cardiotoxicity. At present, many projects are focused on AKR1B1 as a target enzyme and specific inhibitors of AKR1B1 are looking for.

The recombinant protein of AKR1B1 was prepared in *E. coli* together with the pET expression system. First, cDNA for AKR1B1 in pOTB7 was isolated from *E. coli*. The coding sequence of AKR1B1 was amplified by a PCR. PCR was performed with Phusion Hot Start II polymerase and pair of forward and reverse primers, which contained *Nde*I and *Xho*I restriction sites. As the vector, pET-28b(+) plasmid was used. Both the purified PCR fragments and the plasmid were double digested with the above mentioned restriction endonucleases and purified, followed by the ligation into the multiple cloning site. After ligation, the transformation using the heat shock method was performed. *E. coli* BL21 (DH3) strain was used to overexpress the protein. The overexpression was induced by the addition of 1 mM IPTG. The protein was purified by Ni-NTA Fast Start Kit, where the purification is based on the affinity of the protein to nickel ions. Concentration of protein was determined by Bradford assay.