Abstract

Reduction is the reverse of oxidation and therefore it can involve loss of oxygen atom or the addition of two hydrogen atoms. The reduction of carbonyl groups in xenobiotics was the main topic of this thesis.

We tried to identify and characterize human carbonyl reductases responsible for anticancer drugs deactivation. When cancer is among the most common death causes in the developed world, it is necessary to look for new and efficient ways of its treatment. Inhibition of enzymes, which may contribute to disease development or relapses and/or treatment efficacy decrease by drug inactivation, could be a possible way of treatment improvement and might also lead to decrease of drug doses and side effects of cytostatics.

In the first part of our project, we focused on a soluble cytosolic reductase AKR1C3. This enzyme is involved in sex hormone metabolism and might play an important role in breast and prostate cancer development. We tested its ability to metabolize anticancer drugs by its incubation with oracin and doxorubicin with subsequent metabolite determination with use of HPLC. Our experiment proved that it can deactivate these two drugs with K_m 355 μM for doxorubicin and 110 μM for oracin, respectively. AKR1C3 can therefore influence the anticancer therapy, expecially when overexpressed.

The second part of our project was targeted on purification of a novel human liver microsomal reductase with help of ÄKTA design chromatography system. We used several chromatographic techniques (ion-exchange, affinity, and size exclusion chromatography, electrofocusing) and separation modes. Oracin was used as a model substrate to evaluate reductive activity. SDS-PAGE and Native PAGE were necessary to determine the purity of fractions obtained after protein separation, active bands were analyzed by means of MALDI-TOF. To determine the protein content in the active fractions, Bradford protein assay and BCA assay were used.

Various separation methods and conditions were tested and introduced, some of them were ruled out, and some were used in further experiments. In this long process we followed several leads and thanks to the hard work of the whole team, we managed to partially purify a new human membrane bound reductase which is able to metabolize anticancer agent oracin.