ABSTRACT

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Title of dissertation thesis:

DEVELOPMENT AND APPLICATION OF AFFINITY CARRIER FOR ISOLATION OF HUMAN CARBONYL-REDUCING ENZYMES

For several millennia the human medicine is based on application of small bioactive molecules that are administered in the form of plant extracts or synthetic compounds. However, their use in modern medicine is not possible without a detailed understanding of their biochemical effects and identification of their molecular targets. Chemical proteomics based on the specific recognition between the bioactive molecule and the target molecule is currently the most widely used techniques for identification of molecular targets of small molecules. Compared to conventional biochemical methods (e.g. 2D electrophoresis), chemical proteomics represents particularly sensitive and very selective technique that enable successful identification of biomolecules from complex biological samples that are naturally presented in very small concentrations. Carbonyl-reducing enzymes, which play an important role in physiology due to their involvement in metabolism of various endogenous (e.g. prostaglandins, steroid hormones) and xenobiotic (e.g. anthracyclines, oracin) substrates, also represent such low abundance biomolecules. Although the majority of today known carbonyl-reducing enzymes represent soluble proteins, there are many membrane-bound members in short chain dehydrogenases/reductases (SDR) superfamily. However, the knowledge on their role in metabolism of xenobiotics is quite poor. Based on the research on the reduction stereospecificity of anticancer drug oracin, there were predicted microsomal carbonyl-reducing enzymes involved in the metabolism and inactivation. However, previous attempts to purify these enzyme failed. Although the obtained protein fractions exhibited metabolic activity towards oracin with desired stereospecificity, using non-selective

purification techniques (ion-exchange chromatography, hydrophobic-interaction chromatography) was not possible to obtain desired enzymes in sufficient quantity and purity for their subsequent identification by MS.

The aim of this project was to develop suitable affinity carrier capable to selectively purify carbonyl-reducing enzymes from complex biological samples. For this purpose several types of magnetic and non-magnetic beads were modified with anticancer drug oracin to form an affinity carrier. The carrier showing the best binding capacity was subsequently tested with a series of pure carbonyl-reducing enzymes and complex biological samples. The in-house developed affinity carrier was able to capture carbonyl-reducing enzymes having affinity towards oracin in all cases. Thus, affinity carrier was implemented into purification protocol of human microsomal carbonyl-reducing enzymes. Obtained fractions exhibited metabolic activity towards oracin with desired stereospecificity of its reduction. Using mass spectrometry proteins DHRS1, RDH16 and 17β-HSD6, with unknown affinity and metabolic activity towards oracin were successfully isolated and identified. Furthermore, enzyme 11β-HSD1 with already described affinity towards oracin was identified too. The affinity of enzymes DHRS1 and RDH16 towards oracin was subsequently demonstrated by using recombinant proteins. Although these recombinant proteins have not confirmed significant metabolic activity of this drug yet, their isolation and identification as its potential molecular target may indicate their role in biotransformation of other xenobiotics.