Use of Chiral Separations for the Determination of Enzyme Enantioselectivity

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Enantioselectivity is the ability of chiral environment to distinguish between two enantiomers. Enantioselective enzyme prefers one enantiomer as a substrate or preferentially forms one enantiomer over the other in an enzymatic reaction.

Enantioselective enzymes are very useful biocatalyzators allowing cheap preparation of optically pure chemicals. In this work, we determined enantioselectivity of novel microbial epoxide hydrolases. Enantiopure epoxides prepared by an enzyme kinetic resolution of racemates can serve as valuable building blocks in an organic synthesis. In order to measure the enantioselectivity, we developed methods for analyses of various chiral epoxides using a chiral GC on cyclodextrine-based stationary phases. We showed, that epoxide hydrolase from Aspergillus niger M200 reacts with tert-butyl glycidyl ether in enantioselective manner.¹

In the second part of this thesis, we elucidated the basis of enantioselective oxidation of trans-4-hydroxy-2-nonenal (HNE) by brain mitochondria.² HNE is a cytotoxic product of lipid peroxidation involved in numerous diseases, including Alzheimer’s disease. Here, we described enzyme kinetics of HNE enantiomers detoxification to trans-4-hydroxy-2-nonenoic acid (HNEA) by aldehyde dehydrogenases (ALDHs).³ Furthermore, we developed direct and indirect HPLC methods for HNEA enantioseparation⁴ and LC-MS method for determination of HNE enantiomers⁵. Our results showed, that rat ALDH5A enantioselectively oxidized (R)-HNE with retention of stereoconfiguration, whereas rat ALDH2 was not enantioselective.

REFERENCES