ABSTRACT

Betaine homocysteine *S*-methyltransferase (BHMT) and betaine homocysteine *S*-methyltransferase 2 (BHMT-2) are mammalian cytosolic metalloenzymes. They both participate in the metabolism of homocysteine (Hcy), specifically Hcy remethylation, mainly in liver and kidney cells. BHMT catalyzes the transfer of a methyl group from betaine to L-Hcy, yielding L-methionine and dimethylglycine (DMG). BHMT-2 catalyzes the transfer of a methyl group from *S*-methylmethionine (SMM) to L-Hcy as well, yielding two molecules of L-methionine. Disorders in Hcy metabolism could lead to the so called hyper-homocysteinemia and homocystinuria, which can be connected with several pathological conditions. BHMT is already relatively well characterized enzyme. Its crystal structure and reaction mechanism have been described and a series of BHMT inhibitors have been prepared. The specific inhibitors enabled further *in vivo* studies and, recently, *Bhmt-/-* mice model has been successfully developed. In contrast, the research of BHMT-2 is still at the beginning and physiological functions of the enzyme are unknown so far. The reason is that BHMT-2 is a highly unstable enzyme and also there is a lack of selective BHMT-2 inhibitors. BHMT and BHMT-2 are very similar enzymes which have 73% amino acid identity.

This thesis provides new information about properties and structural requirements of the active site of BHMT. This information was obtained after testing the BHMT inhibition by 30 newly prepared low molecular weight compounds. We further optimized the purification of BHMT-2 and stabilized it by a co-purification with its related enzyme, BHMT. These results indicate specific interaction of these enzymes which was proved by native electrophoresis. We developed the first potent and selective inhibitor for BHMT-2. The compound can be an important tool for further *in vivo* studies on BHMT-2. Studies on an impact of BHMT inhibition and simulated hyperhomocysteinemia, which were tested on HepG2, ^{BHMT}HepG2 cell lines and human hepatocytes using 2-DE, MS and LC-MS/MS, shed light on a response and an ability of liver cells to cope with disturbed Hcy metabolism. An important result of this thesis is also the discovery of a catalytic activation of BHMT together with changes in its substrate specificity caused by potassium ions. Moreover, we described binding and important structural role of potassium ions close to the BHMT active site using theoretical calculations, newly prepared BHMT mutants and protein crystallography.

In conclusion, this work provides innovative and valuable findings about physico-chemical and biochemical properties of BHMT and BMHT-2. It provides new BHMT and BHMT-2 inhibitors and brings new knowledge about BHMT function in liver cells.