Abstract

Enzymes involved in sulfur metabolism have gained growing interest as potential targets for the diagnosis and therapy of inflammatory and neurodegenerative diseases. In particular, the study of inborn errors of metabolism has unequivocally contributed to the current understanding of human sulfur metabolism. The current study focuses on two different aspects of oxidative cysteine catabolism in humans. First, two enzymes involved the oxidative degradation of cysteine leading to sulfite were characterized.

The first reaction catalyzed by the cytosolic cysteine dioxygenase (CDO) results in the irreversible formation of cysteine sulfinic acid (CSA) by addition of molecular oxygen to cysteine. Steady state kinetics of human CDO revealed an important role of the characteristic protein-derived Cys-Tyr crosslink in enhancing catalytic activity. Moreover, iron titration experiments revealed a decreased K_d of iron upon crosslink formation in CDO. These findings suggest a structural role of the crosslink in coordinating the ferrous iron in the active site of CDO, which is in line with the previously postulated reaction mechanism.

In the second enzymatic reaction the deamination of CSA by aspartate aminotransferases (AAT) leading to pyruvate and terminally sulfite release was proposed. The existence of two AAT isoforms, a cytosolic (cAAT) and mitochondrial (mAAT), raised the question of the subcellular localization of sulfite formation. Steady state kinetics of CSA deamination and cell culture experiments in human embryonic kidney cells revealed a higher activity for mAAT in comparison to cAAT, suggesting a favored mitochondrial localization of sulfite formation. Several studies have further pointed out a potential role of CSA in the brain. Excitotoxicity and cellular uptake in cultured primary neurons identified a novel role of CSA as a potential neurotransmitter. CSA deamination by AAT was proposed to result in the formation of the putative intermediate ß-sulfinylpyruvate, which non-enzymatically decomposes into sulfite and pyruvate. The development of a coupled enzyme assay, which combines CSA deamination and sulfite oxidation by using AAT and sulfite oxidase (SO) allowed direct measurement of sulfite oxidation following CSA deamination and suggested that ß-sulfinylpyruvate does not accumulate. Furthermore, an increased catalytic efficacy for sulfite formation from CSA of the mitochondrial over the cytosolic AAT isoform was determined, which is in accordance with the mitochondrial localization of SO as the terminal enzyme in the oxidative cysteine catabolism.

A further aspect of this study dealt with the possibility of inhibiting sulfite formation from cysteine as a potential therapy for two inborn errors of metabolism, molybdenum cofactor deficiency (MoCD) and isolated SO deficiency (SOD), which are characterized by an impairment of sulfite oxidation resulting in severe neurodegeneration and death in early childhood. For this purpose, a simplified well plate-based CDO activity assay was used for inhibitor identification. Inhibition of CDO through α-ketoglutarate and glutarate was identified and further characterized using HPLC-based steady state kinetics. The utilized assay can be used for high-throughput identification of additional inhibitors, which coupled with *in vivo* experiments, may evaluate the potential of CDO inhibition as a therapeutic approach for the treatment of sulfite toxicity disorders and may contribute to the understanding of the functional importance of CDO and its relationship to neurodegenerative disorders.