Sulfite Metabolism in Health and Disease: Biogenesis, Biochemical Changes and Mitochondrial Morphology

Abstract

Isolated sulfite oxidase deficiency (ISOD) and molybdenum cofactor deficiency (MoCD) are autosomal recessive inborn errors of metabolism characterized by progressively severe, neurodegenerative symptoms such as pharmacoresistant seizures, which often lead to early childhood death. The underlying cause for both deficiencies is the loss of the mitochondrial enzyme sulfite oxidase (SO), which is catalyzing the terminal reaction of cysteine catabolism, the oxidation of sulfite to sulfate. Sulfite is a strong nucleophile and has long been regarded as a cellular toxin. Sulfite is known to react with cystine to form the neurotoxin S-sulfocysteine (SSC), which is a major contributor to the neurodegeneration observed in SO deficiencies.

Despite recent progress, there are still several unknown aspects of cellular sulfite metabolism: Firstly, which enzyme of cysteine catabolism is primarily responsible for the biogenesis of sulfite. Secondly, although it is generally known that sulfite reacts with disulfides to form S-sulfonates, the impact of those reactions on cellular redox balance is not well understood. Lastly, while studies have suggested that sulfite may impair cellular respiration and mitochondrial energy metabolism, the exact effect of sulfite on mitochondria is still ill defined. In this work, these three important aspects of sulfite metabolism were investigated.

At the beginning of this work, a comprehensive review of cellular cysteine catabolism in health and disease was conducted (chapter II), thus gaining important insights into the cross regulation of different pathways of cysteine catabolism. For instance, it was highlighted that sulfite metabolism is intricately connected with H_2S metabolism, a second branch of cysteine catabolism, thus providing important implications for future projects presented in this work.

The two glutamate oxaloacetate transaminase isoenzymes GOT1 (cytosolic) and GOT2 (mitochondrial) have both been proposed to contribute to cellular sulfite production. Using an enhanced sulfite detection method and newly generated cell lines, the cytosolic protein GOT1 could be identified as the main producer of sulfite in cells (chapter III). Furthermore, to investigate possible impacts of sulfite accumulation on the regulation of cysteine catabolism, expression studies of key enzymes of cysteine catabolism were performed. These studies showed a broad rewiring of cellular cysteine catabolism in SO-deficient cells and revealed that mitochondrial GOT2 contributes to elevated H_2S levels upon impairment of SO.

Regarding the impact of sulfite on cellular redox balance, two new mechanisms of how sulfite influences cellular glutathione homeostasis in a cell type specific manner are described in chapter IV. On the one hand, a sulfite-induced increase of cellular glutathione levels was detected, that depended on the uptake of SSC via the system x_c^- antiporter. On the other hand, sulfite treatment protected WT and SO deficient cells from glutathione oxidation upon acute H_2O_2 stress, most probably by reacting with oxidized glutathione to S-sulfonated glutathione, thereby leading to faster recovery of reduced glutathione.

Finally, the effect of sulfite on mitochondria was analyzed in chapter V. It was discovered that mitochondria show altered morphological features and impaired ATP production in cell culture models of SO deficiency. It could furthermore be shown that sulfite directly alters mitochondrial morphology in a dose-dependent manner by inducing an interconnected, hypertubular mitochondrial network in concentrations lower than 50 µM, and mitochondrial fragmentation

and ultimately cell death in higher concentrations. These findings therefore collectively characterize MoCD and ISOD as novel mitochondrial disorders.