

Abstract

The last step of molybdenum cofactor (Moco) biosynthesis is catalyzed by two proteins in prokaryotes, whereas in eukaryotes those activities are fused in multi-domain proteins such as Cnx1 in plants and gephyrin in mammals. In addition, mammalian gephyrin also functions as a scaffold at inhibitory synapses in the central nervous system, where it undergoes extensive alternative splicing. The first part of this work focuses on the Moco function of gephyrin with the overall aim to elucidate the evolutionary significance of the fusion of both catalytic centers (G and E domain) in eukaryotes. By establishing the *in vitro* synthesis of Moco, a product-substrate channeling between both active sites within gephyrin was found to be 60-fold faster as compared to the isolated G and E domain, and operates at physiological molybdate concentrations in an ATP-dependent manner. Thus, this study provides experimental evidence, that product-substrate channeling in basic metabolism can be a driving force in the fusion of proteins that are separately expressed in bacteria, while being multi-domain proteins in eukaryotes. Alternative splicing mainly affects the unique central C domain, which has been shown to be crucial only for gephyrin's synaptic function. However, the impact of alternative splicing within the C domain on the catalytic efficacy of gephyrin reported in this study, suggests an important structural role of the C domain in controlling the conformational arrangement of gephyrin's catalytic domains.

The second part of this work focuses on Moco deficiency (MoCD) following two strategies. First, a new HPLC method for fast quantification of S-sulfocysteine (SSC) has been developed, as this is the main stable accumulating metabolite in MoCD patients. Validation of the SSC quantification method revealed high sensitivity and accuracy. Thus, it was possible to use this method for diagnosis and treatment monitoring of MoCD patients. Second, as it was firmly established that MoCD symptoms are mainly caused by the inactivation of sulfite oxidase (SO), which oxidizes toxic sulfite into sulfate, two potential therapies were investigated for their ability to reduce sulfite levels in the organism. As a first approach, an enzyme replacement therapy was studied by treating MoCD-mice with plant SO (PSO), as this enzyme, in contrast to the vertebrate SO enzymes, uses oxygen as terminal electron acceptor. Using PEGylation as a masking method for therapeutic proteins, an extension of life span from 7.5 to 16 days was achieved following treatment with PSO and no MoCD symptoms were observed. However, premature death of treated-mice was due to the probably toxic effect of hydrogen peroxide, as this is a byproduct of the PSO reaction, whose identity was demonstrated by using purified human catalase enzyme. Enzyme replacement therapy was extended to the use of murine and human SO proteins. Two SO variants lacking the heme domain were generated, purified, kinetically characterized, and their ability to use oxygen as electron acceptor was demonstrated for the first time in this study. As hydrogen peroxide will most likely cause toxicity when MoCD-mice are treated with SO, an enzyme replacement therapy using the here generated SO and catalase enzymes may be a starting point for the establishment of potential therapies towards MoCD as well as other sulfite toxicity disorders.

The second approach towards treatment of MoCD, started with the characterization of human cysteine dioxygenase (CDO), as this enzyme is responsible for 80 % of cysteine catabolism in mammals, leading to sulfite and taurine formation. Thus, a possible inhibition of this enzyme may lead to an overall reduction of sulfite levels in MoCD patients. CDO was cloned, purified and kinetically characterized. Furthermore, a fast inhibitor screening assay was established and its use in future experiments may provide evidence for the feasibility of this approach towards MoCD-treatment. It may also answer the question whether or not cysteine accumulation in the organism is toxic, as this has been previously reported for many neurodegenerative diseases.