Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2016, Article ID 6143753, 11 pages
http://dx.doi.org/10.1155/2016/6143753

Research Article

Alternatively Spliced Methionine Synthase in SH-SY5Y Neuroblastoma Cells: Cobalamin and GSH Dependence and Inhibitory Effects of Neurotoxic Metals and Thimerosal

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Received 6 September 2015; Revised 28 December 2015; Accepted 10 January 2016

Academic Editor: Antonio Ayala

The folate and cobalamin (Cbl-) dependent enzyme methionine synthase (MS) is highly sensitive to oxidation and its activity affects all methylation reactions. Recent studies have revealed alternative splicing of MS mRNA in human brain and patient-derived fibroblasts. Here we show that MS mRNA in SH-SY5Y human neuroblastoma cells is alternatively spliced, resulting in three primary protein species, thus providing a useful model to examine cofactor dependence of these variant enzymes. MS activity was dependent upon methylcobalamin (MeCbl) or the combination of hydroxocobalamin (OHCbl) and S-adenosylmethionine (SAM). OHCbl-based activity was eliminated by depletion of the antioxidant glutathione (GSH) but could be rescued by provision of either glutathionylcobalamin (GSCbl) or MeCbl. Pretreatment of cells with lead, arsenic, aluminum, mercury, or the ethylmercury-containing preservative thimerosal lowered GSH levels and inhibited MS activity in association with decreased uptake of cysteine, which is rate-limiting for GSH synthesis. Thimerosal treatment decreased cellular levels of GSCbl and MeCbl. These findings indicate that the alternatively spliced form of MS expressed in SH-SY5Y human neuronal cells is sensitive to inhibition by thimerosal and neurotoxic metals, and lower GSH levels contribute to their inhibitory action.

1. Introduction

MS is a multidomain enzyme which transfers a folate-derived methyl group to homocysteine (HCY), thereby creating methionine. The cobalamin (Cbl) cofactor of MS, its Cbl[I] form, directly participates in the transfer reaction by abstracting a folate-derived methyl group, temporarily creating methylcobalamin (MeCbl), and then transferring the methyl group to HCY [1]. However, if Cbl[I] oxidizes prior to MeCbl formation, enzyme activity is temporarily halted, increasing HCY diversion to the transsulfuration pathway and augmenting formation of cysteine, the rate-limiting metabolite for synthesis of the antioxidant GSH [2, 3]. In this manner Cbl serves as a redox sensor whose oxidation leads to increased antioxidant synthesis in proportion to cellular demand. MS inactivation is accompanied by decreased methylation activity, caused by lower levels of the methyl donor SAM and higher levels of the methylation inhibitor S-adenosylhomocysteine (SAH) [4]. Thus MS and Cbl link redox status to methylation status, including methylation of DNA and