Identification of Mouse Selenomethionine $\alpha,\gamma$-Elimination Enzyme

Cystathionine $\gamma$-Lyase Catalyzes Its Reaction to Generate Methylselenol

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ABSTRACT

The purpose of this study was to identify the seleno-L-methionine (L-SeMet) $\alpha,\gamma$-elimination enzyme that catalyzes L-SeMet to generate methylselenol (CH$_3$SeH), a notable intermediate for the metabolism of selenium compounds, in mammalian tissues. The enzyme purified from ICR mouse liver was separated by one-dimensional gel electrophoresis, and the specific band was subjected to in-gel trypsin digestion followed by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometric analysis. In the peptide mass fingerprinting search, the mass numbers of 14 peptides produced by tryptic digestion of the enzyme were consistent with the theoretical mass numbers calculated from the amino acid sequence of murine cystathionine $\gamma$-lyase (E.C. 4.4.1.1). The peptide sequence tags search was also performed to obtain the amino acid sequence data of five tryptic peptides. These peptides were significantly identical to the partial amino acid sequences of cystathionine $\gamma$-lyase. This enzyme was clearly shown to catalyze the $\alpha,\gamma$-elimination reaction of L-cystathionine by the enzymological research. The $K_m$ value for the catalysis of L-cystathionine was 0.81 mM and $V_{\text{max}}$ was 0.0013 unit/mg protein. These results suggested that cystathionine $\gamma$-lyase catalyzes L-SeMet to generate CH$_3$SeH by its $\alpha,\gamma$-elimination reaction.

Index Entries: L-Selenomethionine; $\alpha,\gamma$-elimination enzyme; identification; matrix-assisted laser desorption/ionization–time-of-flight mass

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INTRODUCTION

Selenium (Se) is an essential trace element for mammals. However, it is also known to be highly toxic, and the range of Se concentrations between physiological requirement and toxic appearance is narrow. In humans, Se is supplied by various kinds of food. Seleno-L-methionine (L-SeMet) is a major chemical form of Se in plant foods (1) and Se-containing supplements such as Se-enriched yeast (2). An experimental study has shown that L-SeMet is an appropriate supplemental form of essential Se for humans (3).

Biological functions of Se are mainly based on a catalytic role of a variety of enzymes that contain seleno-L-cysteine (L-SeCyH) residues as a part of their active site (4,5). The metabolism by which L-SeMet is used for the synthesis of selenoproteins probably proceeds as follows: It is converted to L-SeCyH by the enzymes responsible for L-methionine metabolism (6,7) and then to H2Se by selenocysteine β-lyase (6,8,9). H2Se is a principal metabolite in the utilization of Se and is transformed into selenophosphate, which is required for selenoprotein biosynthesis. Alternatively, it can be metabolized to methylated metabolites by S-adenosylmethionine-dependent methyltransferases, which lead to excretion (10–12). A monomethylated metabolite, CH3SeH, is being watched with keen interest because it might be involved in the tumor chemopreventive effect (13).

We previously found the enzyme that catalyzes L-SeMet to produce CH3SeH in mouse liver (14). The enzyme was purified to homogeneity from mouse liver, and the physicochemical and enzymological properties were investigated. This purified enzyme differed from the bacterial L-methionine γ-lyase (E.C. 4.4.1.11), which catalyzed both L-SeMet and L-methionine, because it hardly catalyzes L-methionine (15). Furthermore, the primary structure of murine enzyme is yet unresolved.

The present study was performed to identify the L-SeMet α,γ-elimination enzyme purified from mouse liver. The purified enzyme separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was digested in the gel, and then the digested peptide was subsequently extracted and analyzed by a matrix-assisted laser desorption/ionization tandem quadrupole/orthogonal time-of-flight mass spectrometer (oMALDI-Qq-TOF MS/MS). We identified the purified enzyme as cystathionine γ-lyase by both the peptide mass fingerprinting (PMF) search (16–18) and the peptide sequence tags (PST) search (19,20). This is the first report showing that mammalian cystathionine γ-lyase catalyzes the α,γ-elimination reaction of L-SeMet.