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RESEARCH ARTICLE

METHIONINE- γ-LYASE (MGL) – ROLE IN COMBATING CANCER

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Abstract

L-Methionine γ-lyase (EC 4.4.1.11; MGL), also known as methionase, is a pyridoxal phosphate (PLP)-dependent enzyme that catalyzes the direct conversion of L-methionine to a ketobutyrate, methanethiol, and ammonia by an α,γ-elimination reaction. The enzyme also catalyzes the β-replacement reactions of sulfur amino acids. MGL is widely distributed in bacteria, especially in pseudomonads. Many cancer cells have an absolute requirement for plasma methionine, whereas normal cells are relatively resistant to the restriction of exogenous methionine. Methionine depletion has a broad spectrum of antitumor activities. Under methionine depletion, cancer cells were arrested in the late S-G2 phase due to the pleiotropic effects and underwent apoptosis. Thus, therapeutic exploitation of MGL to deplete plasma methionine has been extensively investigated. Growth of various tumors such as Lewis lung carcinoma, human colon cancer lines, glioblastoma, neuro-blastoma and other types of cancer was arrested by MGL.
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Keywords: Methionine γ-lyase, Cancer, Anti-cancerous

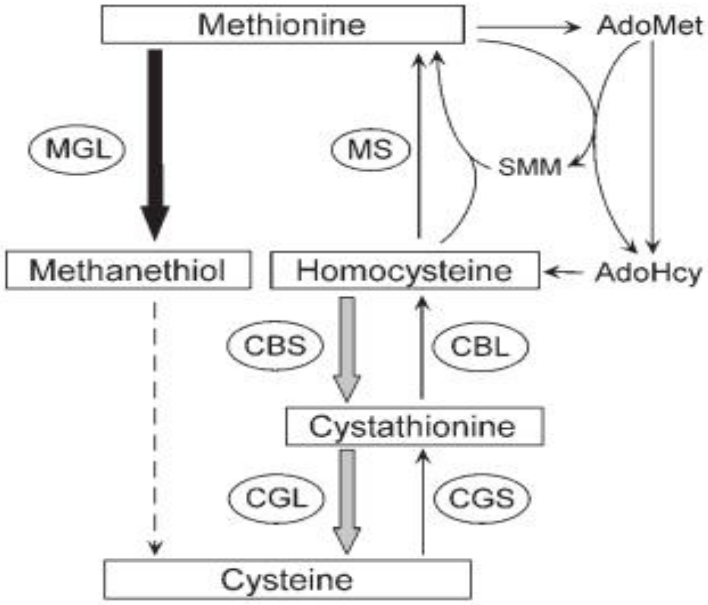
Introduction

Cysteine is formed from sulfide and O-acetylserine via the enzyme O-acetyl serine (thiol) lyase[1]. Cysteine is incorporated into proteins and glutathione, and serves as a sulfur donor for the synthesis of sulfur containing compound such as methionine. Like bacteria and fungi, plants have a trans-sulfuration pathway, mediated by cystathionine γ-synthase and cystathionine β-lyase that converts cysteine to homo cysteine via cystathionine, the homocysteine then being used to make methionine (Fig.1). Methionine is a protein constituent and the precursor of S-adenosyl methionine (SAM), the universal methyl donor, and of S-methyl methionine (SMM), a major form of sulfur transport in some plants[2].

Sulfur is an essential element in all living organisms. Bacteria and plants incorporate sulfur as inorganic compounds such as sulfate, sulfite, and sulfide. In contrast, most of heterotrophs take in sulfur as sulfur-containing amino acids (SAAs) synthesized by other organisms. SAAs play critical roles in a variety of biological processes including protein synthesis, methylation, biosynthesis of vitamins, polyamines and antioxidants. SAAs are ubiquitously distributed, but their metabolic pathways diverged among organisms, and are modulated in the life cycle and upon stresses and changes in environmental conditions[3].

Both biosynthesis and degradation of SAAs (Fig. 2) must be tightly regulated. The maintenance of low homocysteine concentrations is essential not only for proper flow of sulfur in the transsulfuration pathway and the methionine cycle, but also for

Fig.1: Scheme showing methionine/ cysteine interconversion pathways



evading toxic effects of the molecule, which has been implicated in pathological conditions associated with various genetic disorders causing homocystinuria and homocysteinemia[4]. Homocysteine has also been shown to be the pro-oxidant causing damage to the vascular endothelia[5], and associated with an increased cardiovascular risk[6] and Alzheimer's disease[7].

In mammals, SAAs are mainly degraded via oxidative cysteine catabolism, where cysteine dioxygenase (EC. 1. 13. 11. 20) catalyzes the oxygenation of cysteine to 3-sulfinoalanine, a key intermediate of cysteine metabolism leading to hypotaurine, taurine, pyruvate, and sulfate[8]. The other cysteine degradative pathway in mammals is initiated by cysteine aminotransferase (EC. 2. 6. 1. 3), which deaminates cysteine to form 3-mercapto-pyruvate. In the organisms that possess a methionine biosynthetic pathway, such as bacteria and plants, cystine (a pair of cysteines joined by a disulfide bond) is also degraded at least *in vitro* by cystathionine β-lyase (EC. 4. 4. 1. 8) to thiocysteine, pyruvate, and ammonia[9, 10]. On the other hand, a limited lineage of organisms possesses the unique pathway, in which SAAs are converted to α-keto acids, ammonia, and volatile thiols by methionine gamma-lyase (MGL, EC. 4. 4. 1. 11).

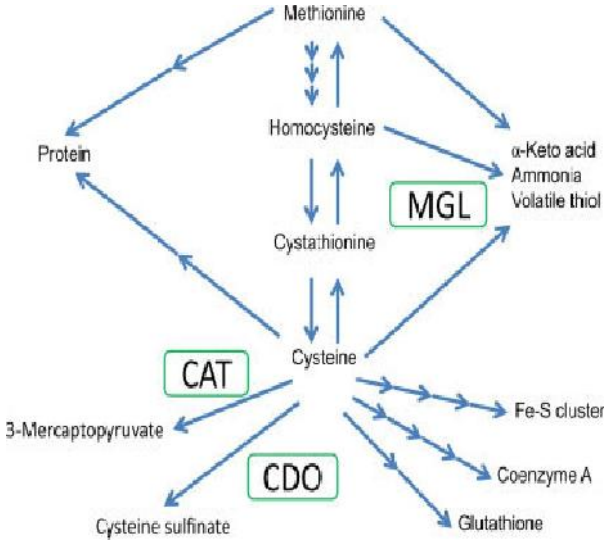
It was reported 70 years ago that some bacteria produced methanethiol[11], MGL has been characterized from bacteria, including *Clostridium porogenes*[12], *Pseudomonas ovalis*[13], *Pseudomonas putida*[14], *Aeromonas* sp.[15], *Citrobacter intermedius*[16], *Brevibacterium linens*[17], *Citrobacter freundii*[18], *Porphyromonas gingivalis*[19] and *Treponema denticola*[20], parasitic protozoa such as *Trichomonas vaginalis*[21], *Entamoeba histolytica*[22] and a model plant *Arabidopsis thaliana*[23]. MGL activity was also detected from archaeon *Ferroplasma acidarmanus*[24], cheese surface bacteria such as *Micrococcus luteus*, *Arthrobacter* sp., *Corynebacterium glutamicum* and *Staphylococcus equorum*[25]. Crystal structures have been reported from *Pseudomonas putida*[26], *Citrobacter freundii*[27, 28], *Trichomonas vaginalis* and *Entamoeba histolytica*[29, 30].

Enzymological properties

Basic Reactions, Size, and Cofactor

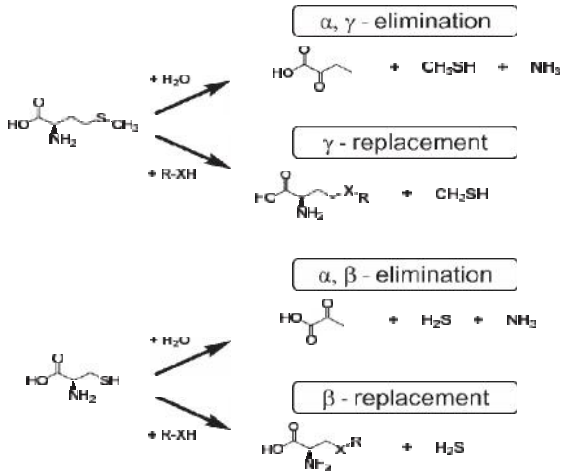
MGL catalyzes the α,γ-elimination of L-methionine and its derivatives such as L-homocysteine, L-ethionine, and L-seleno- methionine (Fig. 3). It also catalyzes the α,βelimination of L-cysteine and its analogs such as S-methyl-L-cysteine[31]. These reactions yield α-keto acid (2-oxobutyrate and pyruvate), ammonia, and thiols (methanethiol and hydrogen sulfide). It also degrades O-substituted serine or homoserine such as O-acetyl-L-serine, O-acetyl-L-homoserine, and O-succinyl-L-homoserine, and release organic acids instead of thiols. This

Fig.2: A general scheme of transsulfuration, methionine cycle, and sulfur-containing amino acid degradation



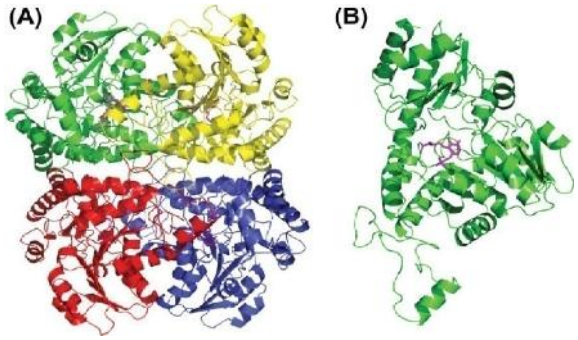
The enzymes involved in sulfur-containing amino acid degradation are MGL, methionine gamma-lyase; CDO, cysteine dioxygenase; CAT, cysteine aminotransferase

Fig.3: Catalytic reactions of MGL



α, γ-elimination and γ-replacement of L-methionine (upper) and α, β-elimination and β-replacement of L-cysteine (lower) are indicated. X ¼S or Se

Figure 4.Crystal structure of MGL



A. The overall structure of E. histolytica MGL2
B. Single subunit of E. histolytica MGL2

enzyme alternatively catalyzes β- or γ-replacement reactions, where the sulfur or oxygen atom at the β- or γ-position of the substrate is replaced with the thiol. For example, the methylthiol moiety of L-methionine is replaced by ethanethiol to yield ethionine and mechanethiol[13]. MGL also catalyzes deamination and γ-addition reaction of L-vinylglycine[31]. MGL consists of 389–441 amino acids, and forms homo tetramer. The active MGL tetramer consists of two sets of the catalytic dimers (Fig. 3) that are tightly associated[26, 32, 27]. The active site is formed at the interface of the two neighboring subunits. Each subunit contains one pyridoxal 5^0-phosphate (PLP) as a cofactor (Fig. 4). MGL is categorized into the γ-family of PLP-dependent enzymes[33].

Reaction Mechanisms

Based on the reaction mechanism of PLP γ-family enzymes and the enzymological analysis of *P. putida* MGL wild-type and mutants, it has been proposed that MGL catalyzes elimination reaction in the following order:

1. a Schiff-base linkage between PLP and the lysine residue displaces the binding of the primary amino group of the substrate and PLP, to form an external aldimine,
2. α- and β-hydrogens of the substrate are shifted to PLP,
3. the phenolic group of the adjacent tyrosine residue attacks the γ-position of the substrate as an acid catalyst,
4. the thiol group is eliminated from the substrate, and
5. α-keto acid and ammonia are released from PLP (Fig. 5) [31, 34].
6. The mutational studies of *E. histolytica* MGL supported the assumption of an acid catalyst of the tyrosine residue[35].

Amino Acid Residues Implicated in Catalysis

Structural analysis of *P. putida* MGL revealed that the six amino acid residues, Tyr59, Arg61, Tyr114, Cys116, Lys240, and Asp241 are located in the vicinity of the substrate binding pocket, close to PLP[32]. Aside from the amino acid residues conserved among PLP γ-family enzymes[22, 36, 37], a line of evidence indicates that Cys116 of *P. putida* MGL takes part in the unique enzymatic reactions of MGL. This cysteine is not conserved in other PLP γ-family enzymes and substituted by glycine or proline in cystathionine γ-lyase, cystathionine β-lyase, and cystathionine β-synthase[32], and thus was previously suggested to be involved in the recognition and γ-elimination of methionine[38]. Unlike other MGLs, B. linens MGL degrade neither cysteine nor cystathionine, whereas A. thaliana MGL degrades cysteine, but hardly cystathionine[39]. In both *B. linens* and *A. thaliana* MGL, the corresponding cysteine residue was substituted by glycine[17, 39].

The mutational studies of *E. histolytica* and *T. vaginalis* MGL isozymes also demonstrated that the corresponding cysteine residue directly contributes to the substrate specificity. When this cysteine was replaced with glycine or serine, the Km values of one isozyme for methionine and cysteine were drastically changed, while those of the other isotype remained unaltered [35, 40].

In *P. putida* MGL, chemical modification with 2-nitrothio-cyanobenzoic acid and labeling with a PLP analog, N-(bromoa-cetyl)pyridoxamine phosphate, suggested the catalytic importance of Cys116[41]. The substitution of this cysteine to histidine caused a drastic increase or decrease in the activity of MGL toward cysteine or methionine, respectively; their catalytic efficiency (kcat/Km) for cysteine increased by 16.2 fold, while that for methionine decreased by 552 fold, mainly due to the reduction in kcat[38]. Similar changes in the activity were also observed for methionine and cysteine analogs[38].

The crystal structure revealed that the cysteine residue is located in the proximity of a tyrosine residue[26], which attacks the γ-position of the substrate[42].

However, direct interaction between the cysteine residue of MGL and methionine, as a substrate, was not observed. Thus, the structures of MGL/methionine intermediates at various reaction stages should be resolved to elucidate how the cysteine residue is involved in γ-elimination of methionine.

Physiological functions

Association with Anaerobic Metabolism

Anaerobic bacteria and parasitic protozoa that possess MGL, rely on glycolysis and amino acid degradation for energy generation[43, 44]. In those anaerobic organisms, for example, an-aerobic

Fig.5: A proposed reaction process of α,γ-elimination of methionine by MGL

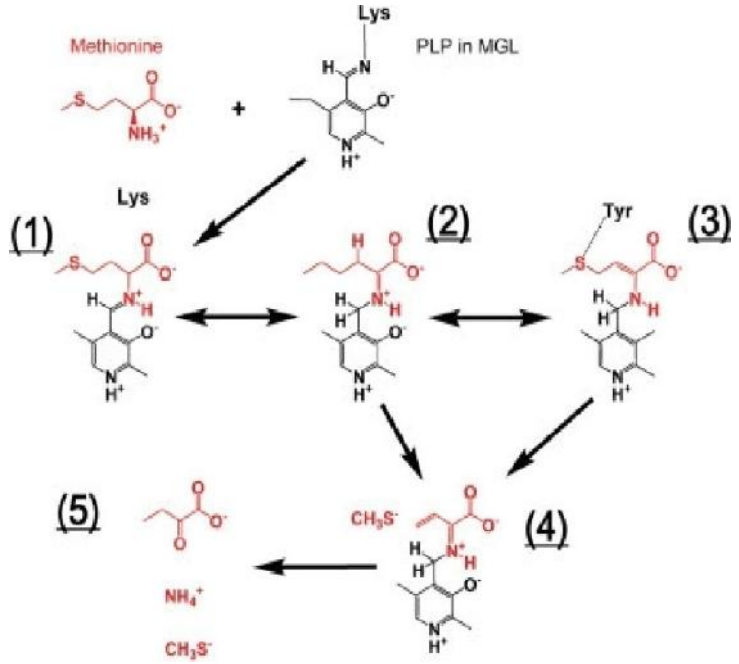
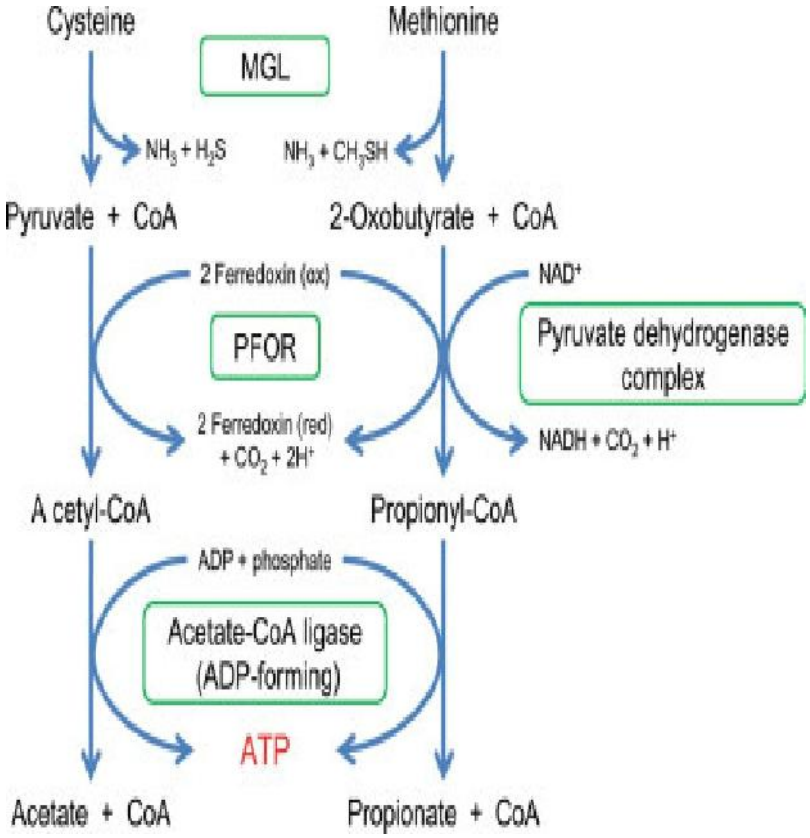


Fig.6: A proposed reaction scheme of energy generation from 2-oxobutyrate. 2-Oxobutyrate, generated from methionine by MGL, serves as a substrate for PFOR or pyruvate dehydrogenase complex, which leads to the ATP generation. ‘‘ox’’ and ‘‘red’’ are the oxidized and reduced form of ferredoxin, respectively



amitochondrial parasites, pyruvate is converted to acetate via acetyl-CoA (Fig. 6). The conversion proceeds in two sequential reactions catalyzed by pyruvate:ferredoxin oxidoreductase (PFOR, EC. 1. 2. 7. 1) and acetate-CoA ligase (ADP-forming) (EC. 6. 2. 1. 13). In this process, one ATP is generated from one pyruvate. As 2-oxobutyrate, generated from methionine by MGL, is condensed with CoA to form propionyl-CoA, which is in turn decomposed by acetate-CoA ligase (ADP-forming) with a concomitant ATP generation, the process might contribute to energy metabolism.

Utilization of mgl for the treatment of cancers

MGL for Cancer Treatment

Many cancer cells have an absolute requirement for plasma methionine, whereas normal cells are relatively resistant to the restriction of exogenous methionine[45]. Methionine depletion has a broad spectrum of antitumor activities[46]. Under methionine depletion, cancer cells were arrested in the late S-G2 phase due to the pleiotropic effects and underwent apoptosis. Thus, therapeutic exploitation of P. putida MGL to deplete plasma methionine has been extensively investigated[47]. Growth of various tumors such as Lewis lung carcinoma[48], human colon cancer lines[49], glioblastoma[50], and neuro-blastoma[51] was arrested by MGL. MGL in combination with anticancer drugs such as cisplatin, 5-fluorouracil, nitrosourea, and vincristine displayed synergistic antitumor effects on rodent and human tumors in mouse models[52, 53, 54]. It was also reported that MGL introduced by adenovirus vector inhibited the growth of tumors *in vitro*. MGL, when combined with selenomethionine, a suicide prodrug substrate of MGL, inhibited tumor growth in rodents and prolonged their survivals[55]. Methaneselenol produced by decomposition of selenomethionine, was oxidized to methylseleninic acid, which in turn oxidized protein sulfhydryls and generated reactive oxygen species, and was then reduced back to selenol by glutathione[56]. In addition to the synergistic effects noted above, the advantage of anticancer therapy using MGL is its wide range of target tumors, including those resistant to the conventional chemotherapeutics and radiation. Taken together, MGL treatment will provide a novel paradigm for cancer therapy.

Modifications of MGL to Reduce its Side Effects

It was reported that administration of MGL caused anaphylactic shock in macaque monkeys[57]. To overcome this problem, polyethylene glycol-conjugated MGL (PEG-MGL) was constructed. PEG-MGL reduced immunogenicity; the IgG titers decreased by 10 to 10,000-fold, depending on the binding rate of PEG and MGL, compared to naked MGL. The half life and depletion time of MGL in the mouse plasma was improved by PEG conjugation. The enzymatic activity of PEG-MGL was detected for 72 h, while that of unconjugated MGL was undetectable after 24 h, and the half life of PEG-MGL increased by 20 times (38 h), compared to unconjugated MGL (2 h)[58]. Simultaneous coadministration of pyridoxal 5^0-phosphate and oleic acid, or dithiothreitol treatment also strengthened effectiveness of PEG-MGL in the rodent model [59, 60].

Other applications of MGL

Elevated blood and serum homocysteine is known as a notorious risk factor for cardio-vascular diseases, dementia, and Alzheimer's disease[61]. It was reported that the administration of the combination of vitamins (folic acid, vitamins B_6, and B_{12}) decreased homocysteine concentrations, but did not significantly reduce the risk of death from cardiovascular diseases[62, 63, 64]. Thus, therapeutic interventions by directly lowering homocysteine by the administration of MGL may be worth attempting.

The unique enzymological property of MGL was applied to clinical examination of homocysteine, cysteine, and PLP. These examination methods utilizing MGL with sufficient sensitivities are suitable for mass screening and, thus, can be an economical alternate of the expensive HPLC-dependent method.

Perspectives

Although MGL has been explored to be an ideal drug target against microbial infections and also for the treatment of cancers, its reaction mechanism and physiological functions remain to be fully elucidated. Recently structural analyzes of wild type and mutant MGLs have been reported[32, 38, 30], which disclose the substrate recognition and reaction mechanisms. In addition, the tertiary structures of MGLs were resolved[36], including the complex with inhibitors. To further elucidate the reaction mechanisms, the tertiary structures of various stages of the MGL-substrate/prodrug/inhibitor

complex need to be resolved. This should lead to a further fine adjustment of anti-infective agents targeting MGL and anti-cancer drugs exploiting MGL.

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