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Purification and Characterization of Methionine Gamma Lyase-Deaminase (Mgld) from the Oral Pathogenic Organism *Porphyromonas gingivalis*

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Abstract

Porphyromonas gingivalis is an oral pathogenic bacterium that causes halitosis (bad breath) and periodontitis in humans. Halitosis manifests due to volatile sulfur compounds such as methylthiol, generated from methionine degradation by methionine gamma lyase-deaminase (Mgld). In this report, we cloned and expressed *Porphyromonas gingivalis mgld* using a bacterial expression system, and the purified homotetrameric enzyme was characterized by a novel isotope assay using L-[1-^{14}C]-methionine as a substrate. Mgld is a PLP-dependent L-methionine C-S lyase-deaminase that cleaves the gamma-carbon-sulfur bond of L-methionine to methylthiol and forms the deaminated overall product α-ketobutyrate. We find that exogenous ^{3}H-L-2-aminobutyrate is not deaminated into ^{3}H-α-ketobutyrate under conditions of enzyme excess, supporting efficient transfer of bound intermediate(s) during L-methionine catabolism. The overall reaction to form α-ketobutyrate from L-methionine exhibits K_m of 1.0 mM, V_{max} of 5.27 μmol min^{-1} mg^{-1}, and a monomeric k_{cat}/K_m of 3729.3 M^{-1} s^{-1}. Mgld exhibits optimal activity above pH 8 and in a temperature range of 37°-50°C. Several compounds were tested for Mgld inhibition. The natural product DL-propargylglycine stands out as the most effective Mgld inhibitor and thus may be useful for the control of halitosis. Mgld has almost no activity on N-formylmethionine, confirming the requirement of a free α-amino-nitrogen on the substrate to form a Schiff base with the enzyme.

Keywords: Methionine degradation; Oral pathogen; Volatile sulfur compounds; Methionine metabolism; Methionine gamma lyase

Abbreviations: CBL: Cystathionine β-lyase; CBS: Cystathionine β-synthase; DPP: Dipeptidyl-peptidase; DTNB: 5'-Dithio-bis (2-nitro benzoic Acid; MGL: Methionine γ-lyases; Mgld: L-Methionine γ-Lyase-L-Deaminase; PG: DL-Propargylglycine; PLP: Pyridoxal 5' phosphate; SAM: S-Adenosyl methionine; TLC: Thin layer chromatography; VSCs: Volatile sulfur compounds

Introduction

Porphyromonas gingivalis (*P. gingivalis*) is a gram-negative, anaerobic pathogenic bacterium that causes halitosis and periodontitis [1,2]. Greater than 47% of American adults are affected from infectious periodontitis [3,4]. *P. gingivalis* is an asaccharolytic organism which uses protein as an energy source. A variety of dipeptidyl-peptidases (DPPs) that form free amino acids from dipeptides have been identified from *P. gingivalis* [5]. Once individual amino acids are formed they can be processed for metabolism. Aspartate for example, is deaminated by aspartate-ammonia-lyase and the resulting fumarate can serve as a terminal electron acceptor of the respiratory chain. Free methionine released from DPP5 activity can be metabolized through carbon-sulfur lyase and deaminase enzymatic actions to form methylthiol, ammonia and α-ketobutyrate [6]. As part of bacterial energy metabolism, α-ketobutyrate is further converted to propionyl CoA and eventually to the TCA cycle metabolite oxaloacetate. Both methylthiol and ammonia are highly odoriferous compounds that cause oral malodor. Two molecules of methylthiol can non-enzymatically oxidize and condense to form dimethyl disulfide [7]. Under fermentative conditions, methylthiol reacts with methanol to produce dimethyl sulfide. Furthermore, methylthiol is enzymatically oxidized with molecular oxygen to hydrogen peroxide, hydrogen sulfide and formaldehyde by methylthiol oxidase [methylthiol: oxygen oxidoreductase] [8]. Methylthiol, dimethylsulfide, dimethyldisulfide, and hydrogen sulfide are collectively called volatile sulfur compounds (VSCs), molecules that can lead to inflammation, oxidative damage to the oral mucosa, and eventual periodontitis. The production of methylthiol by *P. gingivalis* appears to be important for infectivity [9,10,14].

In mammals, methionine can be re-synthesized by methionine synthase using homocysteine, the methyl group from 5-methyl-tetrahydrofolate and the coenzyme cobalamin [11-14]. Methionine is then enzymatically activated into S-adenosylmethionine (SAM), the universal methyl group donor, by ATP-dependent methionine adenosyltransferase [11,12]. Demethylation of SAM yields S-adenosylhomocysteine which is subsequently converted into free homocysteine. The sulfur moiety of homocysteine condenses with serine to form cystathionine with the elimination of H_2O, catalyzed by the pyridoxal 5-phosphate (PLP)-dependent cystathionine β-synthase (CBS) [13]. Cystathionine β-lyase (CBL), a trans-sulfurylase also using PLP, cleaves cystathionine at the β-position to release cysteine and the rest of the moiety is deaminated to form β-ketobutyrate and ammonia [14]. However, the direct cleavage of methionine is absent in mammals.

A variety of bacteria, parasitic protozoa, and plants harbor the ability to catabolize methionine to α-keto acids, ammonia, and methylthiol [14]. This activity, carried out by the enzyme methionine gamma lyase-deaminase (Mgld), monitors intracellular methionine levels and feeds

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substrates to the TCA cycle, nitrogen metabolism, and redox cycling. Mgld degrades L-methionine in an α,γ-elimination that requires the cofactor PLP. The *P. putida* crystal structure revealed two monomers associating to construct a catalytic dimer that further arranges as an active homotetramer [15,16]. This quaternary arrangement is also observed for other gamma-family PLP enzymes including *E. coli* cystathionine β-lyase and cystathionine γ-synthase. One molecule of PLP covalently binds to one Mgld monomer via a Schiff-base to a conserved lysine, dislodging the substrate primary amino group. A five-step mechanism ensues in which a tyrosine acid-catalyst removes the substrate γ-thiol releasing α-keto acid and ammonia. Cys116 in *P. putida* Mgld appears to distinguish the Mgld family from other PLP γ-family enzymes such as the cystathionine lyases and synthases. Physiologically, Mgld has been implicated as important for seed germination in *A. thaliana*, anaerobic energy production in parasitic protozoa and bacteria, and the pathogenicity of *P. gingivalis*. Deletion of *mgld* markedly reduced *P. gingivalis* infectivity as evidenced by increased survival in mice (7.7% to 36%) [10].

In this paper, we report the purification and characterization of recombinant methionine gamma lyase-deaminase (Mgld) from *Porphyromonas gingivalis*. To accurately measure enzymatic activity, we developed a novel assay system that quantifies the non-volatile product ^{14}C-α-ketobutyrate, which is formed through the lyase and hydrolytic deaminase activities of the enzyme. Interestingly, Mgld does not convert exogenous ^{3}H-L-2-aminobutyrate to ^{3}H-α-ketobutyrate, suggesting the enzyme bound transfer of putative intermediates to final product. Formylmethionine is also not a substrate for Mgld, suggesting that the modification of the amino group of Met might help stabilize intracellular levels of free methionine. DL-propargylglycine severely inhibits Mgld activity and shows negligible toxic effects on oral epithelial cell lines (Venkatachalam et. al., unpublished), suggesting that this Mgld inhibitor could be a useful mouth wash ingredient for controlling halitosis and periodontitis.

Materials and Methods

Materials

Radionuclides L-[1-^{14}C]-methionine and ^{3}H-L-2-aminobutyrate were purchased from Moravek. Oligonucleotides were synthesized at the DNA core facilities of Johns Hopkins University. Taq DNA polymerase and reagents for cloning were purchased from Invitrogen Inc. Thin-layer chromatography plates were from E. Merck (Darmstadt, Germany). AKTA FPLC, immobilized metal affinity columns (IMAC), and size exclusion columns were from GE Healthcare Inc. The SDS-PAGE MW standards (Unstained Precision Plus) were obtained from BioRad. All other compounds, solvents and reagents were purchased from Sigma-Aldrich.

Cloning and expression of full length Mgld

The ORF corresponding to *P. gingivalis* Mgld (UNIPROT entry C3VMV9_PORGN) was amplified by PCR using genomic DNA from *P. gingivalis* W83 with forward (5'-CAGACA GCTAGCATGCGTAGTGGCTTTG-3') and reverse (5'-GAACTCGAATTCTTAGATCAGGC TGTCCAGAC-3') primers designed to introduce an NheI site preceding the ATG start codon and an EcoRI site downstream from the stop codon, and ligated into pGEM-T vector (Promega). The *mgld* insert was subcloned from pGEM-T by digestion with NheI and EcoRI and ligated into a pET28a vector (Novagen) harboring an N-terminal 6X-His Tag followed by a TEV protease cleavage site. The resultant vector, pET-Mgld, was transformed into *E. coli* BL21 (DE3). A 2 liter culture containing pET-Mgld was grown with continuous shaking at 37°C in Luria broth supplemented with kanamycin (40 μg/ml) until mid-log phase (A_{600} of 0.4-0.6). Protein expression was then induced with 1 mM IPTG and the cultures were incubated at 37°C with shaking for 10-12 h. Cells were harvested at 5500 *g* and resuspended in 40 mM Tris-HCl buffer (pH 7.5), 250 mM NaCl, and 5 mM imidazole (buffer A) prior to freezing at -20°C.

Protein purification

Resuspended frozen cells were thawed on ice, and DNase I (10 μg/ml), phenylmethylsulfonyl fluoride (PMSF) (0.1 mM) and Complete Mini EDTA free protease inhibitor tablets (Roche) were added. Cells were lysed using a French Press at 20,000 PSI and the homogenate was clarified by centrifugation at 21,000 *g* for 40 min at 4°C. The supernatant was then purified through a 5 ml Ni-NTA column. Peak fractions, containing purified Mgld, were treated with TEV protease to remove the N-terminal His-tag. His-tag free protein was re-applied to a nickel column. Then flow through was then applied to a Superdex 200 size exclusion column at a rate of 1 ml/min in 40 mM Tris-HCl (pH 7.5), 1 mM DTT, 250 mM NaCl, 2 mM EDTA and 10% glycerol (storage buffer). The peak fractions from the S-200 column were concentrated on an Amicon ultracentrifugal filter (Millipore). Protein purity was monitored by 8-25% SDS-PAGE PhastGels (GE Healthcare) using the unstained Precision Plus polypeptide standards (Biorad).

Methionine γ-lyase and deaminase (Mgld) overall activity assay

The overall reaction of α-ketobutyrate formation was performed in a total volume of 24 μl. A typical reaction contained 4 μl of reaction buffer [150 mM Tris-HCl (pH 8.0)], 4 μl of 1 mM pyridoxal phosphate (PLP), 6 μl of storage buffer, and 4 μl Mgld (0.1 μg/μl), gently mixed and incubated at 37°C for 5 min. Each reaction was started by adding L-[1-^{14}C]-methionine (6 μl; different ratios of a 7.3 mM stock and H_2O were mixed to get appropriate substrate concentrations) and was incubated at 37°C for 15 min. The reaction was stopped by boiling for 1 min, cooled on ice for 2 min and centrifuged at 3575 *g* for 7 min. A 2μl aliquot was chromatographed on a silica gel plate using 25% 20 mM phosphate buffer (pH 7) and 75% acetonitrile. Following chromatography, the TLC plates were dried, and exposed to X-ray film for 36-48 h (Eastman-Kodak Co). The respective spots of substrate and products were cut out and radioactivity determined by liquid scintillation.

L-2-Aminobutyrate deaminase (2-ABD) assay

The deamination reaction of ^{3}H-2-aminobutyrate was performed in a total volume of 24 μl. The reaction contained 4 μl of reaction buffer [150 mM Tris-HCl (pH 8)], 4 μl of 1 mM pyridoxal phosphate (PLP), 6 μl of storage buffer, and 4 μl of Mgld (1.0 μg/μl)]. The solution was gently mixed and incubated at 37°C for 5 min. The reaction was started by adding ^{3}H-2-aminobutyrate (4.25 nmol) and the contents were incubated at 37°C for 15 min. Further processing, chromatography, and analysis were performed as described for the overall activity assay.

Competition experiment with substrate analogs for Lyase-deaminase activity

For each competition reaction, 4 μL of 150 mM Tris-HCl (pH 8), 4 μl of 1 mM pyridoxal phosphate (PLP), and 2 μL of either 10 mM L-cystathionine, D-methionine, DL-propargylglycine, O-succinyl-L-homoserine, L-methionine or L-cysteine (i.e., one competitor per tube) were mixed. Next, 6 μL of storage buffer and 4 μL of 0.1 μg/μL purified Mgld were added to each reaction and gently mixed. The contents were pre-incubated at 4°C for 10 min for binding and then 4 μL of L-[1-^{14}C]-methionine was added to each tube (1 mM final concentration). The

contents were gently mixed and incubated at 37°C for 15 min for overall activity measurements (i.e. ^{14}C-α-ketobutyrate formation). Further processing, chromatography and analysis of products were performed as described above for the lyase-deaminase activity assay.

Comparison of Mgld activity on fMet and L-Met (Lyase half reaction)

N-formyl-L-methionine and L-methionine were compared for the lyase half reaction using the DTNB assay. Briefly, a 1 mL assay containing 50 mM potassium phosphate buffer (pH 7.2), 1 mM L-Met, 0.02 mM PLP, 0.025 mM DTNB and 10 µL of Mgld (~5 µg total enzyme) in storage buffer were mixed quickly, layered with two drops of mineral oil and incubated at 27°C for 30 min. The absorbance was measured at 410 nm using UV/VIS spectrophotometry. Methylthiol formation was calculated using the observed absorbance and the extinction coefficient of 14,150 M^{-1} cm^{-1}.

Results

Cloning, expression, and purification of Mgld

Using genomic DNA of the oral pathogenic organism *Porphyromonas gingivalis* W83 as a template, cDNA corresponding to the full length coding sequence of *mgld* was PCR amplified, cloned into pGEM-T, and subcloned into pET28a-TEV bacterial protein expression system in-frame with an N-terminal 6X-His Tag. The nucleotide sequence revealed an open reading frame of 1182 bp and the deduced amino acid sequence comprised 393 amino acid residues. The *mgld* cDNA sequence and the deduced protein sequence have been deposited in GenBank (nucleotide I.D. FJ875028, protein I.D. ACO94451.1). *P. gingivalis* Mgld possesses highly conserved motifs for PLP binding (K206) as well as other putative active site residues such as Y53, R55, and Y108 found among various β and γ carbon-sulfur lyases (Figure 1A).

For Mgld purification, clarified bacterial cell lysate supernatant was purified using nickel column chromatography. The eluted peak fractions contained mainly His-tag recombinant protein with minor impurities of lower molecular weight as evidenced by SDS-PAGE (Figure 1C). The His-tag moiety was cleaved using TEV protease, and the tag fragments and protease were removed using a 5 mL His-trap column. The His-tag-free recombinant protein was concentrated and further purified using a Superdex 200 gel filtration column (Figure 1B). Peak fractions from the sizing column contained a nearly homogeneous protein with a MW of ~43 kDa as evidenced by SDS-PAGE (Figure 1C). On the

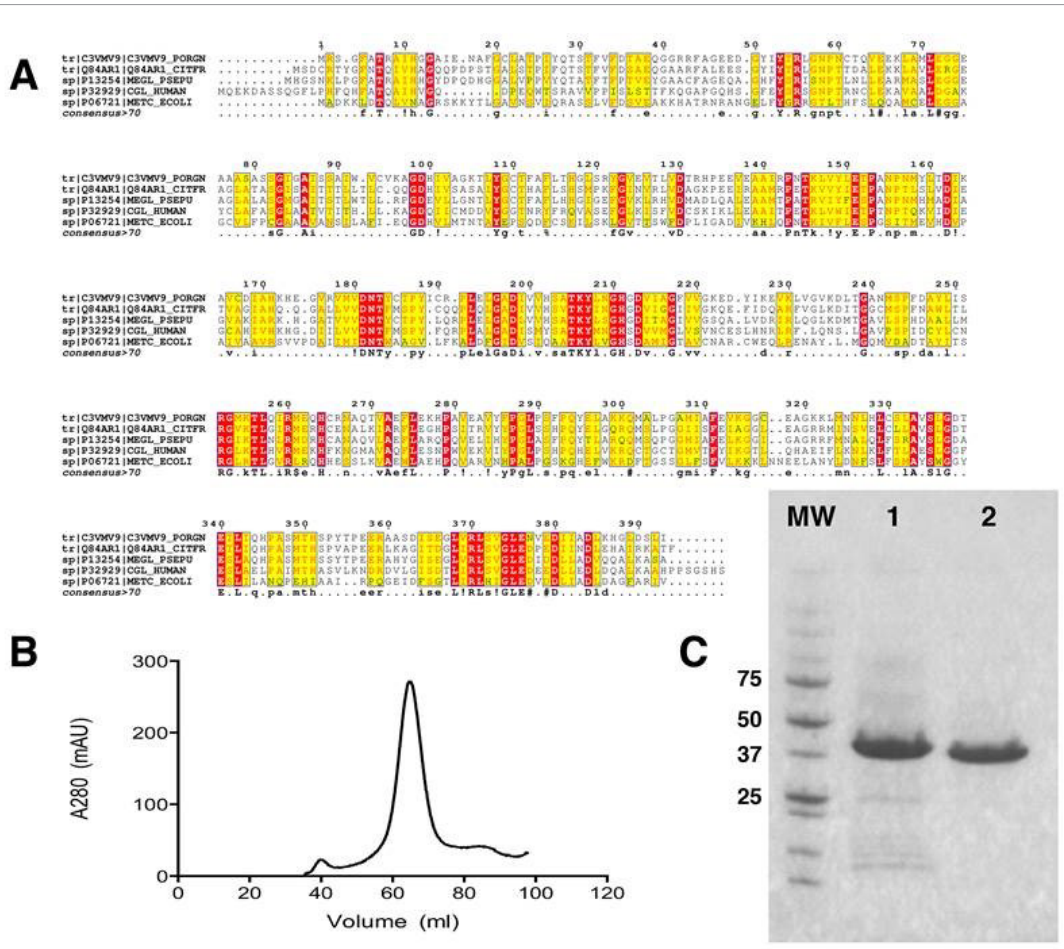

Figure 1: Sequence features and purification of Mgld. (A) Sequence of *Porphyromonas gingivalis* Mgld (UNIPROT entry C3VMV9_PORGN) compared to a diverse set of homologs illustrates the level of sequence conservation between MGL enzymes and the related gamma subfamily of PLP enzymes (MGL from *Citrobacter freundii* and *Pseudomonas putida*, human cystathionine γ-lyase, and *E. coli* cystathionine β-lyase). Sequence based structure alignment was carried out using T-COFFEE, and sequence similarity is highlighted using ESPRIPT. Sequences are from UniProt, and accession numbers are indicated on the figure. (B) Elution profile of Mgld by gel filtration column chromatography on a 120 ml Superdex column (HiLoad S-200 16/60 run at 1 ml/min). Mgld elutes as a tetramer, based on a standard calibration curve. (C) Purification of Mgld. Commassie stained SDS gel (8-25% gradient Phastgel, GE Healthcare) showing 3 µg aliquots of recombinant Mgld from the S200 peak fraction with the His-tag (lane 1), and His-tag free (lane 2). The location and size (kDa) of polypeptide standards (MW lane) are indicated.

sizing column, Mgld elutes with a native MW of ~160 kDa (based on a standard calibration curve) and is likely tetrameric in solution like other MGL family lyases.

Enzymatic characterization of Mgld – Overall activity assay of Mgld

The purified protein was tested for overall activity on the substrate

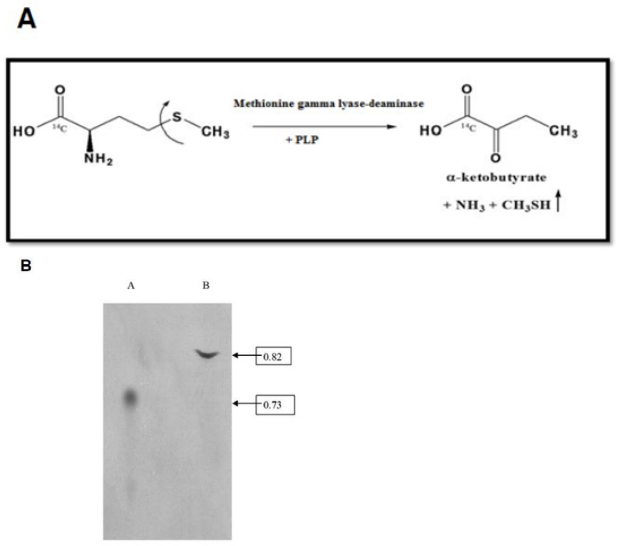

Figure 2: Mgld overall reaction. (A) The substrate methionine was labeled (^{14}C) at the carboxyl carbon i.e. (L-[1-^{14}C]-methionine). Cleavage and deamination of the substrate yield labeled [^{14}C]-α-ketobutyrate, undetected unlabeled volatile ammonia, and methylthiol. (B) TLC separation of 2μL aliquots of the reactions. Lane A: reaction that contained all the ingredients except Mgld shows substrate L-[1-^{14}C]-methionine spot (Rf=0.73), minor impurity below and no product. Lane B: reaction that contained Mgld shows only product [^{14}C]-α-ketobutyrate spot (Rf=0.82).

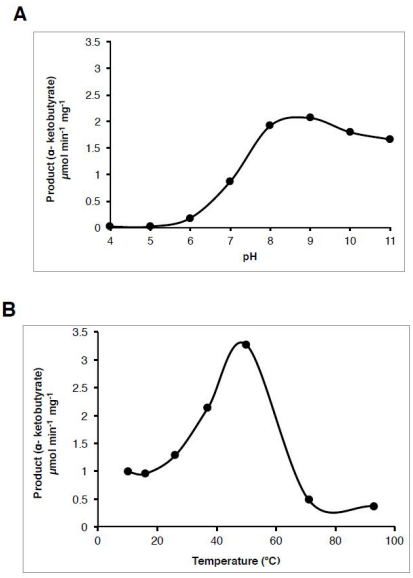

Figure 3: Characterization of Mgld overall activity using L-[1-^{14}C]-methionine. (A) Effect of pH on Mgld activity. Reactions were performed for 15 min at 37°C, using reaction buffers at various pHs [sodium citrate (pH 4, 5, and 6); phosphate (pH 6 and 7); Tris-HCl (pH 8 and 9), and glycine-NaOH (pH 9, 10, and 11)]. (B) Effect of temperature on Mgld activity. Reaction at each temperature was performed for 15 min at pH 8 with 0.4 μg of enzyme according to the standard assay procedures described in Materials and Methods.

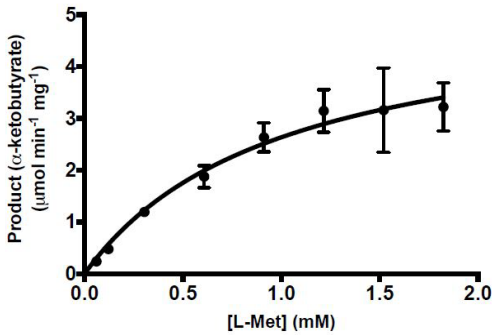

Figure 4: Kinetic analysis of Mgld using L-methionine substrate. Purified enzyme was assayed against varying concentrations of L-methionine. Formation of the product α-ketobutyrate was measured as described in Materials and Methods. Data points represent two independent experiments. Km and Vmax values were determined by non-linear regression using GraphPad PRISM (GraphPad Software).

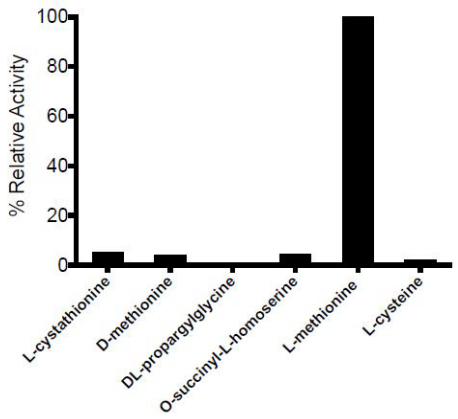

Figure 5: Formation of α-ketobutyrate by Mgld is inhibited by a variety of methionine analogs and related compounds. Mgld lyase-deaminase activity was measured using L-[1-^{14}C]-Methionine at 37°C, after intial binding of the respective substrate analogs with Mgld for 10 min at 4°C. L-Met was used as the substrate control. The overall reaction was measured by quantitating the amounts of ^{14}C-α-ketobutyrate formed and plotted as % relative activity.

L-[1-^{14}C]-methionine by monitoring and analyzing the final product ^{14}C-α-ketobutyrate. The overall reaction scheme is depicted in Figure 2A. As shown, L-methionine is ^{14}C labeled at the carboxyl carbon and after cleavage and deamination, the product α-ketobutyrate would bear the label on its carboxyl carbon. The volatile ammonia and methylthiol products would be unlabeled and thus not interfere with this assay. The isotopic substrate L-[1-^{14}C]-methionine and the final product ^{14}C-α-ketobutyrate were separated by silica gel thin layer chromatography (TLC) using 75% acetonitrile and 25% 20 mM phosphate buffer. Figure 2B shows a typical autoradiography of the TLC plates exposed onto film. Lane B shows the quantitative conversion of the substrate (1 mM) into the final product ^{14}C-α-ketobutyrate (Rf 0.82) in the presence of all the components of the reaction mixture plus the purified enzyme (1 μg) incubated at 37°C for 15 min. The reaction without the enzyme showed no conversion of substrate into any product as evidenced by the presence of the L-[1-^{14}C]-methionine spot (Rf 0.73; Lane A, Figure 2B). To our knowledge, this is the first report of an assay that can quantitatively measure the non-volatile overall product (^{14}C-α-ketobutyrate) from the lyase-deaminase activities of Mgld.

Previous methods have relied only on the half activity (lyase

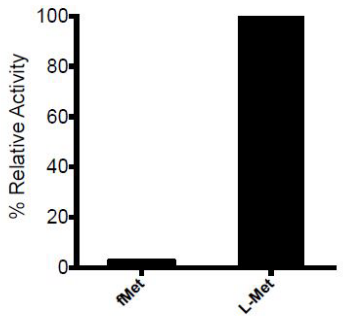

Figure 6: C-S Lyase activity of Mgld on fMet. Mgld was tested for C-S lyase half reaction activity on substrates fMet and L-Met using the DTNB assay as described in Materials and Methods. Mgld had only ~2% relative activity with fMet as compared to the L-Met substrate.

reaction) where volatile methylthiol formation is monitored by its reaction with DTNB, and subsequent measurement of absorbance of TNB (2-nitro-5-thiobenzoate) at 410 nm. The DTNB assay is a very convenient approach for comparing relative lyase activity of Mgld with various non-labeled analogs with its activity on L-methionine. Nevertheless, the DTNB assay should be considered less quantitative than our isotope assay due to the possible loss of the methylthiol product. In our lab, we carried out Mgld characterization using the DTNB assay in which the cuvettes were overlaid with mineral oil. Without the mineral oil overlay, we could detect the pungent sulfur odor from the lyase reaction by its smell. However, with our mineral oil overlay technique, there was a considerable reduction in the odor, indicating minimal loss of volatile methylthiol.

The overall lyase-deaminase activity was measured by titrating increasing amounts of Mgld ranging from 0.2 to 1 µg for 15 min. Using 0.2 µg of protein, the reaction was linear with time. For routine assays, 0.4 µg of Mgld was used with a reaction time of 15 min at 37°C, which ensured substrate availability and fairly optimal reaction conditions. Mgld was poorly active below pH 6, and activity was optimal at pH 8 and above (Figure 3A). In terms of temperature dependence, the enzyme exhibited higher activity between 37°-55°C (Figure 3B).

Kinetic analysis

Purified enzyme preparations were used for determining kinetic parameters with varying concentrations of L-methionine. The overall reaction measured by the isotope assay for the formation of α-ketobutyrate from L-methionine exhibited a K_m of 1.0 mM, V_{max} of 5.27 µmol·min^{-1} mg^{-1} and a monomeric k_{cat}/K_m of 3729.3 M^{-1} s^{-1} (Figure 4). The K_m and V_{max} were determined using non-linear regression fit of the data assuming standard Michaelis-Menten kinetics.

Effects of inhibitor compounds on Mgld overall activity

Various substrate analogs were tested as potential Mgld inhibitors. For these experiments, Mgld was pre-incubated with each unlabeled inhibitor on ice for 10 min. Mgld was then tested for overall activity in each case by incubating with isotopically L-[1-^{14}C] labeled L-methionine substrate for 15 min at 37°C and then measuring the amount of ^{14}C-α-ketobutyrate product formed. Incubation of Mgld with unlabeled L-methionine prior to the isotope assay served as a control. The results in Figure 5 show that pre-incubation of Mgld with any of the inhibitors/competitors resulted in a markedly decreased overall activity, relative to the L-Met control. The decrease in overall (lyase-deaminase) activity is consistent with each of these compounds competing with L-Met for the substrate binding site. DL-propargylglycine was the most potent inhibitor of Mgld overall activity, perhaps forming an allene adduct (suicide adduct) that knocks out overall lyase-deaminase activity.

Comparison of activity on fMet and L-Met

Mgld was also tested for C-S lyase half reaction by the DTNB assay with fMet and L-Met as substrates. When using fMet as substrate, less than 2% of relative lyase activity is observed as compared to the L-methionine substrate (Figure 6).

Discussion

To characterize the MGL family enzyme from *Porphyromonas gingivalis*, full length cDNA corresponding to *mgld* was obtained by PCR amplification using *P. gingivalis* W83 genomic DNA as a template. The sequenced cDNA revealed an open reading frame of 1182 bp and the nucleotide sequence was deposited in GenBank, (nucleotide I.D. FJ875028). Mgld protein sequence contains highly conserved motifs for PLP binding and shares the same putative active site residues as seen in β and γ carbon-sulfur lyases [17-21]. Given the high conservation within the MGL family of PLP dependent lyases (Figure 1A), *P. gingivalis* Mgld is expected to adopt a three domain architecture composed of an N-terminal substrate entry domain, a large PLP binding domain, and a carboxyl terminal deaminase domain. Since Mgld is tetrameric like other MGL, CBS, and CBL family enzymes [17-21], it is likely that it shares similar dimer and tetramer catalytic active site interfaces. Based on this, key residues of *P. gingivalis* Mgld predicted to participate in PLP coenzyme interactions are Y53, R55, G83, Y108, D181, T205 and K206. The C-terminal residues V369, R370, L371 are also conserved among various deaminases, and we suggest that these Mgld residues may participate in the deaminase half reaction.

Methionine γ-lyases (MGL; EC 4.4.1.11) use PLP as a coenzyme to cleave L-methionine between the γ-carbon and sulfur of the thioether bond to form methylthiol and the purported intermediate L-2-aminobutyrate, which is then converted to α-ketobutyrate and ammonia [6]. In most reports, this enzyme has been characterized by the lyase half reaction measuring the formation of methylthiol (we have used the more accurate nomenclature of methylthiol instead of methanethiol for CH_3SH), using the DTNB spectrophotometric method [22]. The DTNB assay provides a convenient approach for measuring the rate of the lyase half reaction. Nevertheless, since methylthiol is highly volatile (boiling point of 4.5°C), kinetic parameters measured using the DTNB assay need to be used with caution. In addition, interference from PLP absorption at 412 nm contributes to a high background, especially with the large amounts of protein that are used for such assays. Using a mineral oil overlay and taking care to perform appropriate protein background subtraction during data analysis appears to improve results from the DTNB the assay.

In this article, we report a novel isotope assay in which methionine is labeled at the carboxyl carbon and the overall reaction is quantified by measuring the formation of ^{14}C-α-ketobutyrate using silica gel TLC separation. Our assay is very specific, and no background product spots corresponding to α-ketobutyrate were seen in absence of the enzyme. In the presence of purified Mgld protein, we observe quantitative formation of product and no intermediate 2-aminobutyrate, as shown by autoradiography and measured by liquid scintillation. Thus, Mgld is a lyase-deaminase that forms α-ketobutyrate from L-methionine without any accumulation of intermediates in an *in vitro* reaction under excess substrate and/or excess protein concentrations. Since deaminase is a type of a hydrolase we propose to study this enzyme further using isotopically labeled water and reclassify the enzyme with a new EC number.

Purified Mgld protein was assayed by the isotope assay described in the methods and it exhibited K_m of 1.0 mM for methionine, V_{max} of 5.27 μmol min^{-1} mg^{-1} and monomeric k_{cat}/K_m of 3729.3 M^{-1} s^{-1}. This K_m value for *P. gingivalis* Mgld is close to the physiological concentration of methionine (~0.6-0.8 mM) reported for bacteria [14]. Numerous reports [23-27] have measured much higher K_m values (based on the lyase half reaction) that reach up to 90 mM. One reason for this discrepancy may have been the loss of volatile products when employing the DTNB assay, either directly into the atmosphere or indirectly via the reaction of VSCs with oxygen to form disulfides that are not reactive with DTNB, and thus underestimation of the product formed. In addition, the K_m value for the overall reaction (lyase-deaminase) might be distinctly different than the lyase half reaction alone.

The rate of Mgld catalysis (k_{cat}/K_m of 3729.3 M^{-1} s^{-1}) is in the mid-range of typical enzymatic rates. Mgld activity at temperatures between 72-93°C was very minimal, making it unlikely that the mechanism of ammonia release to form α-ketobutyrate is non-enzymatic. Exogenous ^{3}H-2-aminobutyrate incubated with excess Mgld (4 μg) did not form any α-ketobutyrate. On the other hand, with the L-methionine substrate and lesser amounts of Mgld (1 μg), the reaction was quantitative in forming α-ketobutyrate. This suggests a direct reaction of the enzyme bound substrate to form α-ketobutyrate from L-methionine via lyase-deaminase activity without any detectable accumulation of intermediates. The rate limiting step for the overall reaction could be the deaminase activity since the lyase half reaction is much faster as judged by the DTNB mineral oil assay. We have thus revised the name of this bifunctional lyase-deaminase enzyme as Mgld.

In conclusion, *Porphyromonas gingivalis* Mgld is an enzyme with lyase and deaminase activity. Catalysis is facilitated by the coenzyme PLP. We have developed a L-[1-^{14}C]-methionine isotope assay that provides a quantitative way to measure overall lyase-deaminase activity. DL-propargylglycine is a potent inhibitor of *P. gingivalis* Mgld and blocks the formation of methylthiol, and is thus a promising therapeutic agent to control diseases caused by *P. gingivalis*. Mgld has almost no activity on N-formylmethionine, confirming the Mgld's requirement of a free α-amino-nitrogen on the substrate for catalysis.

References

1. Nakano Y, Yoshimura M, Koga T (2002) Correlation between oral malodor and periodontal bacteria. Microbes Infect 4: 679-683.
2. Morita M, Wang HL (2001) Association between oral malodor and adult periodontitis: a review. J Clin Periodontol 28: 813-819.
3. Rosenberg M (2002) The science of bad breath. Sci Am 286: 72-79.
4. Tangerman A (2002) Halitosis in medicine: a review. Int Dent J 3: 201-206.
5. Ohara-Nemoto Y, Rouf SM, Naito M, Yanase A, Tetsuo F, et al. (2014) Identification and characterization of prokaryotic dipeptidyl-peptidase 5 from Porphyromonas gingivalis. J Biol Chem 289: 5436-5448.
6. Kreis W, Hession C (1973) Isolation and purification of L-methionine-alpha-deamino-gamma-mercaptomethane-lyase (L-methioninase) from Clostridium sporogenes. Cancer Res 33: 1862-1865.
7. Loesche WJ, Kazor C (2002) Microbiology and treatment of halitosis. Periodontol 2000 28: 256-279.
8. Suylen GM, Large PJ, Van Dijken JP, et al. (1987) Methyl Mercaptan Oxidase, a key enzyme in the metabolism of methylated sulphur compounds by hyphomicrobium EG. J Gen Microbiol 133: 2989-2997.
9. Fukamachi H, Nakano Y, Okano S, et al. (2005) High production of methyl mercaptan by L-methionine-alpha-deamino-gamma-mercaptomethane lyase from Treponema denticola. Biochem Biophys Res Commun 331: 127-131.
10. Yoshimura M1, Nakano Y, Yamashita Y, Oho T, Saito T, et al. (2000) Formation of methyl mercaptan from L-methionine by Porphyromonas gingivalis. Infect Immun 68: 6912-6916.
11. Catoni GI (1953) S-Adenosylmethionine; a new intermediate formed enzymatically from L-methionine and adenosinetriphosphate. J Biol Chem 204: 403-416.
12. Cantoni GI, Durell J (1957) Activation of methionine for transmethylation. II. The methionine-activating enzyme; studies on the mechanism of the reaction. J Biol Chem 225: 1033-1048.
13. Foster MA, Dilworth Mj, Woods Dd (1964) Cobalamin And The Synthesis Of Methionine By Escherichia Coli. Nature 201: 39-42.
14. Sato D, Nozaki T (2009) Methionine gamma-lyase: the unique reaction mechanism, physiological roles, and therapeutic applications against infectious diseases and cancers. IUBMB Life 61: 1019-1028.
15. Motoshima H, Inagaki K, Kumasaka T, Furuichi M, Inoue H, et al. (2000) Crystal structure of the pyridoxal 5'-phosphate dependent L-methionine gamma-lyase from Pseudomonas putida. J Biochem 128: 349-354.
16. Kudou D, Misaki S, Yamashita M, et al. (2007) Structure of the antitumour enzyme L-methionine gamma-lyase from Pseudomonas putida at 1.8 Å resolution. J Biochem 141: 535-544.
17. Percudani R, Peracchi A (2003) A genomic overview of pyridoxal-phosphate-dependent enzymes. EMBO Rep 4: 850-854.
18. Clausen T, Huber R, Laber B, Pohlenz HD, Messerschmidt A (1996) Crystal structure of the pyridoxal-5'-phosphate dependent cystathionine beta-lyase from Escherichia coli at 1.83 Å. J Mol Biol 262: 202-224.
19. Eliot AC, Kirsch JF (2004) Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annu Rev Biochem.73: 383-415.
20. Sridhar V, Xu M, Han Q (2000) Crystallization and preliminary crystallographic characterization of recombinant L-methionine-alpha-deamino-gamma-mercaptomethane lyase (methioninase). Acta Crystallogr D Biol Crystallogr 56: 1665-1667.
21. Nikulin A, Revtovich S, Morozova E, Nevskaya N, Nikonov S, et al. (2008) High-resolution structure of methionine gamma-lyase from Citrobacter freundii. Acta Crystallogr D Biol Crystallogr 64: 211-218.
22. Dias B, Weimer B (1998) Purification and characterization of L-methionine gamma-lyase from brevibacterium linens BL2. Appl Environ Microbiol 64: 3327-3331.
23. Sato D, Yamagata W, Harada S, Nozaki T (2008) Kinetic characterization of methionine gamma-lyases from the enteric protozoan parasite Entamoeba histolytica against physiological substrates and trifluoromethionine, a promising lead compound against amoebiasis. FEBS 275: 548-560.
24. Tokoro M, Asai T, Kobayashi S, Takeuchi T, Nozaki T, et al. (2003) Identification and Characterization of Two Isoenzymes of Methionine γ-Lyase from Entamoeba histolytica: A key enzyme of sulfur-amino acid degradation in an anaerobic parasitic protist that lacks forward and reverse trans-sulfuration pathways. J Biol Chem 278: 42717-42727.
25. McKie AE, Edlind T, Walker J, Mottram JC, Coombs GH (1998)The primitive protozoon Trichomonas vaginalis contains two methionine gamma-lyase genes that encode members of the gamma-family of pyridoxal 5'-phosphate-dependent enzymes. J Biol Chem 273: 5549-5556.
26. Coombs GH, Mottram JC (2001)Trifluoromethionine, a prodrug designed against methionine gamma-lyase-containing pathogens, has efficacy in vitro and in vivo against Trichomonas vaginalis. Antimicrob Agents Chemother 45: 1743-1745.
27. Lockwood BC, Coombs GH (1991) Purification and characterization of methionine gamma-lyase from Trichomonas vaginalis. Biochem J 279: 675-682.