Biosynthesis of Phosphoserine in the *Methanococcales*  
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*Methanococcus maripaludis* and *Methanocaldococcus jannaschii* produce cysteine for protein synthesis using a tRNA-dependent pathway. These methanogens charge tRNA^Cys^ with 1-phosphoserine, which is also an intermediate in the predicted pathways for serine and cystathionine biosynthesis. To establish the mode of phosphoserine production in *Methanococcales*, cells extract of *M. maripaludis* were shown to have phosphoglycerate dehydrogenase and phosphoserine aminotransferase activities. The heterologously expressed and purified phosphoglycerate dehydrogenase from *M. maripaludis* had enzymological properties similar to those of its bacterial homologs but was poorly inhibited by serine. While bacterial enzymes are inhibited by micromolar concentrations of serine bound to an allosteric site, the low sensitivity of the archaeal protein to serine is consistent with phosphoserine's position as a branch point in several pathways. A broad-specificity class V aspartate aminotransferase from *M. jannaschii* converted the phosphohydroxypyruvate product to phosphoserine. This enzyme catalyzed the transamination of aspartate, glutamate, phosphoserine, alanine, and cysteate. The *M. maripaludis* homolog complemented a *serC* mutation in the *Escherichia coli* phosphoserine aminotransferase. All methanogenic archaea apparently share this pathway, providing sufficient phosphoserine for the tRNA-dependent cysteine biosynthetic pathway.

Many methanogenic archaea have a remarkable tRNA-dependent pathway for cysteine biosynthesis. In these organisms, an unusual class II aminoacyl-tRNA synthetase catalyzes the aminoacylation of tRNA^Cys^ with 1-phosphoserine (Sep) (38). This Sep-tRNA^Cys^ is then sulfurylated to produce Cys-tRNA^Cys^ and Biochemistry, University of Texas at Austin, 1 University Station A5300, Austin, TX 78712. Phone: (512) 471-4491. Fax: (512) 471-8696. E-mail: degraham@mail.utexas.edu.

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FIG. 1. Biosynthesis of L-phosphoserine in Methanococcales. PGDH catalyzes the oxidation of D-3-phosphoglycerate to produce phosphohydroxypyruvate, concomitant with the reduction of NAD\(^+\). A broad-specificity aminotransferase (AspAT) catalyzes the transamination reaction from L-glutamate to produce L-3-phosphoserine and α-ketoglutarate. Phosphoserine phosphatase produces l-serine for protein synthesis and glycine production. In a reaction with homocysteine (Hcy), phosphoserine produces cystathionine (50). Alternatively, Sep can be used to aminocatalyze tRNACys, which can be converted to Cys-tRNACys\(^{\text{5S}}\), which can be converted to Cys-tRNACys\(^{\text{5S}}\) by a sulfide transferase enzyme (38).

> canonical phosphoserine transaminase (EC 2.6.1.52) catalyzes amino group transfer from L-glutamate to phosphohydroxypyruvate to produce L-phosphoserine. However, no ortholog of the M. barkeri serC gene was found in the genome of M. maripaludis or M. jannaschii (4, 17). Therefore, this reaction has been a missing step in metabolic reconstructions (4, 41). Phosphoserine phosphatase (EC 3.1.3.3) is conserved in all methanogen genomes, and the M. jannaschii protein has been characterized biochemically and crystallized (47).

We demonstrated that M. maripaludis contains phosphoglycerate dehydrogenase and phosphoserine aminotransferase activities through in vitro assays. Heterologously expressed and purified phosphoglycerate dehydrogenase from M. maripaludis catalyzed the reversible formation of PHP but was poorly inhibited by L-serine. In the second step, a class V aspartate aminotransferase (AspAT) gene from M. maripaludis complemented a serC mutation in E. coli, permitting growth without exogenous serine. A homologous AspAT gene from M. jannaschii was heterologously expressed at high levels, and the purified protein catalyzed the transamination of aspartate, glutamate, phosphoserine, cysteine, and alanine. Together, these results confirm the prediction that methanogens use a phosphorylated serine pathway, and they suggest that the route to phosphoserine for tRNA-dependent cysteine biosynthesis evolved early in the euryarchaeal lineage.

MATERIALS AND METHODS

Chemicals. PHP (hydroxyproprionic acid dimethylketal) was produced from the hydroxyproprionic acid dimethylketal cyclohexylammonium salt by acid hydrolysis according to the manufacturer’s instructions (Sigma). The PHP concentration was determined by analysis of inorganic phosphate in samples treated with bacterial alkaline phosphatase (Fermentas), correcting for inorganic phosphate according to the manufacturer’s instructions (Sigma). The PHP concentration was determined by analysis of inorganic phosphate in samples treated with bacterial alkaline phosphatase (Fermentas), correcting for inorganic phosphate according to the manufacturer’s instructions (Sigma). The PHP concentration was determined by analysis of inorganic phosphate in samples treated with bacterial alkaline phosphatase (Fermentas), correcting for inorganic phosphate according to the manufacturer’s instructions (Sigma).

Cloning of serA and aspC/serC genes. Genes were amplified from chromosomal DNA of Methanococcus maripaludis S2 or Methanocaldococcus jannaschii JAL-1 by using PCR. The M. maripaludis serA gene (MMP1588; GenBank accession no. NP_987511) was cloned between Nhel and BamHII sites of the modified plasmid pET-15b (Novagen) to produce vector pDG304. The Nhel-BamHI fragment containing MMP1588 was subcloned into pET-11a (Novagen) to produce vector pDG3384. The M. maripaludis aspC/serC gene (MMP0391; GenBank accession no. NP_987511) was cloned between Nhel and BamHII sites of plasmid pET-20b (Novagen). The M. jannaschii aspC/serC gene (MJ0959; GenBank accession no. NC_000909) was cloned between Nhel and BamHII sites of plasmid PET-19b (Novagen) to produce vector pDG245. Plasmids were propagated in E. coli DH5α or DH10B (Invitrogen). The oligodeoxynucleotide primers used for PCR were MJ0959Fwd (5′-GGCGAATTTTATTTGCTAACATTTGTAAGACAGG-3′), MJ0959Rev (5′-GCGGATCTCATTTCCATATTGAACTCTTTTGC-3′), MJ0911F (5′-GATCCCCGATGAGTGGATGACTCAAAAAAGCTG-3′), MJ0911R (5′-CTTGTGATGATTGAATCTTTTCGAGC-3′), MMP1588N (5′-ATTCATTAGGGTCTCAGTAGCATGAAAACTTATAACTGACCCGC-3′), and MMP1588B (5′-CTTCGCCCGAGAATTTAGGATTCATTAGTTGT-3′). Dideoxynucleotide sequencing confirmed the sequences of inserts in recombinant plasmids.

Complementation of E. coli serC mutation. The MMP0391 gene was cloned into EcoRI and HindIII restriction sites of plasmid vector pKQV4 to express the gene under the control of a tac promoter (42). The recombinant plasmid was transformed into the serC mutant E. coli KL285 (C5SC 4130) (5). The pKQV4 plasmid was used as an empty vector control, and the E. coli serC gene cloned into pKQV4 was used as a positive control in complementation experiments. A single colony of each transformant was picked from LB agar plates containing ampicillin and grown in liquid M9 minimal medium containing 0.2% (wt/vol) d-glucose and amino acids. Amino acids were used at a concentration of 10 μg ml\(^{-1}\), except for serine, which was used at 50 μg ml\(^{-1}\). All media contained 100 μg ml\(^{-1}\) ampicillin. Plates were grown for 36 h at 30°C. Digital images of plates were inverted and transformed using Photoshop software (Adobe) to enhance contrast.

Protein expression and purification. E. coli BL21(DE3) (Novagen) transformed with expression vectors was grown in Luria broth containing ampicillin (100 μg ml\(^{-1}\)) at 37°C with shaking at 250 rpm. When cultures reached an optical density at 600 nm (OD\(_{600}\)) of 0.6 to 0.7, protein expression was induced by the addition of 0.3% (vol/vol) L-β-mercaptoethanol and 0.5 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) at 37°C for 4 h. After 4 h of incubation with the inducer, cells were harvested by centrifugation and stored at −20°C.

Protein purification. Polystyrene-tagged proteins were purified and separated from native E. coli proteins by Ni\(^{2+}\)-affinity chromatography. E. coli cells containing heterologously expressed protein were suspended in cold binding buffer containing 20 mM sodium phosphate (pH 7.4) and 0.5 M NaCl. Cells were lysed by passage through a French pressure mini cell at 8,000 lb/in\(^2\) (Thermo Electron) and then sonicated on ice for 2 min, using a Sonifier 450 with a microtip (15 W, 30% duty) (Branson) to reduce viscosity. Lysates were clarified by centrifugation (15,000 × g for 10 min at 4°C), and the cell extract was applied to a 5-mL HisTrap column (GE Healthcare) equilibrated with binding buffer. Chromatography was performed using an AKTApriune system (GE Healthcare) at a flow rate of 5 ml min\(^{-1}\). Protein was eluted from the column with a linear gradient to 100% elution buffer over 20 min. Elution buffer contained 20 mM sodium phosphate (pH 7.4), 0.5 M NaCl, and 0.5 M imidazole. Fractions containing the target protein were identified by their absorbance at 280 nm, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and activity assay.

Fractions containing the His\(_{6}\)-MMP1588 protein were pooled and concentrated in a stirred ultrafiltration cell (Amicon) with a 10-kDa molecular-size cutoff filter (Pall) under N\(_2\). The retentate was desalted using a 5-mL HiTrap Sephrose G-25 column (GE Healthcare) equilibrated in 50 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES)-KOH (pH 7.5). Fractions containing the His\(_{6}\)-MJ0959 protein were dialyzed at 4°C against buffer containing 50 mM HEPES-KOH (pH 7.4) and 5 mM MgCl\(_2\). Protein was concentrated inside dialysis tubing, using polyethylene glycol.

Small-scale purifications were carried out using spin columns containing Ni-
Separose resin (QIAGEN). Cell extract was applied to columns equilibrated with binding buffer, followed by centrifugation (200 × g for 1 min at room temperature). Columns were washed with binding buffer, and protein was eluted with 200 μl elution buffer. Total protein concentration was determined using the Bio-Rad protein assay or the BCA assay (Pierce), with bovine serum albumin (fraction V) as a standard.

Cell extracts were prepared from 85 mg M. maripaludis cells grown in minimal medium under a H2-CO2 atmosphere in sealed serum vials at 37°C (29, 370, 242). Methane was used for chemical ionization in the positive mode, and a mass range of 100 to 600 units was scanned, with an approximate scan time of 0.59 s. Using this method, the N-ethylcarbonyl trifluoroethyl ester derivatives had the following retention times and mass spectral data. The molecular ion (MH+) is shown first, if observed, followed by the base peak (in italics), and characteristic fragment ions are listed in decreasing order of intensity: 2-oxoglutarate, 4.51 min (311, 183, 211, and 291) and 5.45 min (183 and 211); alanine, 4.59 min (244, 116, 170, 224, and 144); phosphoserine, 4.62 min (242, 142, 222, 170, and 114); aspartate, 5.62 min (370, 242, 270, and 350); and glutamate, 6.04 min (284 and 256).

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Kinetics of oxaloacetate decarboxylation. Rates of oxaloacetate decarboxylation were measured by UV absorbance spectroscopy (22). Reaction mixtures contained 0.3 to 2.3 mM freshly prepared oxaloacetate added to 300 μl of solution containing 50 mM HEPES-KOH (pH 7.5) and 80 mM KCl at 50°C. Absorbance of the oxaloacetate enolic tautomer was measured at 260 nm in a quartz cuvette at 50°C. The rate constant was calculated by linear regression of the measured initial rates at various oxaloacetate concentrations.

RESULTS

M. maripaludis uses the phosphorylated pathway for serine biosynthesis. To establish that M. maripaludis expresses enzymes required for the phosphorylated pathway for serine biosynthesis, enzyme activities were measured in cell extracts. Reaction mixtures containing cell extract, 123 μM PHP, and 150 μM NADH produced 3-Phosphoglycerate and NAD+, with a specific activity of 11 mU mg−1 protein. Reaction mixtures containing extract, 40 μM PHP, and 5 mM l-aspartate or l-glutamate completely converted PHP to phosphoserine (Fig. 2). These phosphoglycerate dehydrogenase and phosphoserine aminotransferase activities confirmed the presence of the phosphorylated pathway to produce phosphoserine and serine in M. maripaludis. Similar activities were detected in extracts from M. jannaschii. Alternatively, these methanogens could phosphorylate serine directly to produce phosphoserine if they express a serine kinase enzyme. A cell extract of M. maripaludis (44 μg) was incubated with 10 mM l-serine and 5 mM Mg-ATP for 30 min at 30°C in a 100-μl reaction mix to test for serine kinase activity. No phosphoserine was detected in the reaction product (<2 μM).

Characterization of M. maripaludis 3-Phosphoglycerate dehydrogenase. The M. maripaludis PGDH (MPM1588) amino acid sequence is similar to those of bacterial and eukaryotic homologs. These proteins share a carboxy-terminal ACT domain (an amino acid allosteric binding site) that is not found in other members of the 2-hydroxyacid dehydrogenase family (2). Residues identified in the active site of E. coli PGDH are highly conserved in methanogenic orthologs, except for Asn108, which is replaced with serine in the nicotineamide binding site (39). A homology model of the MMP1588 protein was produced by the SWISS-MODEL server, based on PGDH crystal structure models deposited in the Protein Data Bank (40). The

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The oxidation of 1 mM D-3-phosphoglycerate. In reaction mixtures containing 40 μM PHP, 5 mM L-aspartate, and 44 μg M. maripaludis cell extract were incubated for 30 min at 30°C. The CBI derivatives of the amino acids were separated by reversed-phase HPLC and detected by their fluorescence. All of the PHP in the reaction mix was converted to phosphoserine, based on comparison of the phosphoserine-CBI peak area to an external standard. The asparagine-CBI peak is off scale in these overlaid chromatograms.

model included no significant clashes, emphasizing the high degree of conservation in this protein.

To test the predicted function of MMP1588 in phosphoserine biosynthesis, we cloned the gene and expressed the protein, fused to an amino-terminal hexahistidine tag, heterologously in E. coli. Purified His₆-MMP1588 had an apparent mass of 65 kDa, as determined by SDS-polyacrylamide gel electrophoresis (Fig. 3). This value is close to the protein’s expected mass of 59 kDa. Analytical size exclusion chromatography identified large aggregates of protein that eluted in the column void volume as well as smaller forms with Stokes radii of 49 and 35 Å, corresponding to tetrameric and dimeric forms of the protein, respectively.

The purified enzyme catalyzed the NAD⁺-dependent oxidation of D-3-phosphoglycerate, with a specific activity of 55 mU mg⁻¹ protein, corresponding to a rate of 3.2 min⁻¹. No significant activity was detected using NADP⁺ instead of NAD⁺ (<0.005 U mg⁻¹). The enzyme had maximal catalytic activity at 45°C, with no activity observed at 60°C. However, preincubation of the protein at 45°C for 15 min reduced its activity by 50%, while it retained 80% activity after 30 min of incubation at 37°C. Therefore, standard assays were performed at 37°C. The His₆-MMP1588 enzyme did not require Mg²⁺ or thiol reductants for full activity: neither 20 mM MgCl₂ nor 1 mM DTT stimulated activity.

No NAD⁺ reduction was observed using 1 mM D-malate, DL-lactate, glycolate, DL-glycerate, or 2-hydroxyisocaproate as an alternative substrate, nor did these 2-hydroxyacids inhibit the oxidation of 1 mM D-3-phosphoglycerate. In reaction mixtures containing various concentrations of D-3-phosphoglycerate and 0.2 mM NAD⁺, the enzyme had an apparent Kₘ equal to 370 ± 110 μM and a turnover number of 3.7 ± 0.29 min⁻¹. No inhibition was detected in reaction mixtures containing 1 to 3 mM L-serine.

Operating in the reverse direction, His₆-MMP1588 catalyzed the reduction of PHP, with a specific activity of 34 U mg⁻¹ protein (33 s⁻¹). No significant activity was detected with 2-oxoglutarate, oxaloacetate, pyruvate, glyoxylate, or formate in place of PHP (<0.03 U mg⁻¹). However, the enzyme did reduce sulfopyruvate, a PHP analog, with a specific activity of 0.1 U mg⁻¹. His₆-MMP1588 had optimal catalytic activity (reducing PHP) at pH 5.3 but retained 75% activity from pH 4.5 to 9. Potassium chloride stimulated activity, as reaction mixtures containing 100 mM KCl produced twofold more NAD⁺ than did reaction mixtures without added salt. Apparent kinetic parameters for PHP reduction were as follows: Kₘ, 49 ± 5.3 μM; and kₐₘₖ, 47 ± 2.4 s⁻¹.

The ACT domains of several bacterial D-3-phosphoglycerate dehydrogenases bind the effector L-serine cooperatively, causing allosteric inhibition (39). For example, Mycobacterium tuberculosis PGDH has an Iₐ₅₀ of 30 μM, and E. coli PGDH has an Iₐ₅₀ of 2 to 4 μM L-serine (7), where Iₐ₅₀ is the inhibitor concentration that causes 50% maximal activity. Although M. maripaludis PGDH contains a carboxy-terminal ACT domain, it is poorly inhibited by L-serine. Preincubation with 1 to 3 mM L-serine reduced the rate of PHP reduction by 27 to 46%. Similar incubations with 3 mM L-glutamate reduced activity by 38%. D-Serine, L-alanine, DL-phosphoserine, DL-cysteine, and 2-oxoglutarate did not significantly inhibit PHP reduction at a 3 mM concentration. Bacillus subtilis PGDH becomes desensitized to serine inhibition during incubation without DTT (37). Therefore, we incubated M. maripaludis PGDH with 20 mM DTT and 10 mM EDTA at 4°C for 24 h. The DTT-treated enzyme was no more sensitive to inhibition: L-serine (1 to 3 mM) inhibited PHP reduction by 43 to 51%.

Although the polyhistidine tag on the MMP1588 protein enabled facile separation of the heterologous protein from the native E. coli PGDH, it is possible that the tag could interfere with protein subunit assembly and the formation of the regulatory domain. Therefore, the MMP1588 protein was expressed without a fusion tag in E. coli BL21(DE3)/pDG304. SDS-PAGE analysis of a cell extract from that lactose-induced strain showed a soluble protein corresponding to MMP1588, which had an apparent mass of 55 kDa and accounted for 30% of the total Coomassie blue-stained protein. The extract had a protein (33 s⁻¹). No significant activity was detected with 2-oxoglutarate, oxaloacetate, pyruvate, glyoxylate, or formate in place of PHP (<0.03 U mg⁻¹). However, the enzyme did reduce sulfopyruvate, a PHP analog, with a specific activity of 0.1 U mg⁻¹. His₆-MMP1588 had optimal catalytic activity (reducing PHP) at pH 5.3 but retained 75% activity from pH 4.5 to 9. Potassium chloride stimulated activity, as reaction mixtures containing 100 mM KCl produced twofold more NAD⁺ than did reaction mixtures without added salt. Apparent kinetic parameters for PHP reduction were as follows: Kₘ, 49 ± 5.3 μM; and kₐₘₖ, 47 ± 2.4 s⁻¹.

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The MJ0959 protein is 70% identical to the MMP0391 protein, and 91% of the aligned amino acid residues are similar. The His6-MJ0959 protein was expressed heterologously as a soluble protein with an apparent mass of 45.4 kDa, which is close to its predicted mass of 45.2 kDa. The protein was purified by Ni2+-affinity chromatography and desalted by dialysis. From 250 ml of E. coli expressing the heterologous protein, we obtained 17 mg of purified protein. This protein preparation was at least 95% pure, as judged by SDS-polyacrylamide gel electrophoresis (Fig. 3) and analytical size exclusion chromatography. The protein was stable during heating at 70°C for 10 min.

Analytical size exclusion chromatography showed that the protein forms a high-molecular-weight complex with a 57-Å Stokes radius, which corresponds to a 330,000-Da apparent mass. Therefore the protein probably forms an octameric complex. No peaks corresponding to tetrameric, dimeric, or monomeric proteins were observed. The high-molecular-weight species had absorbance maxima at 278, 331, and 416 nm, consistent with a PLP internal aldimine (45). Based on published molar absorption coefficients for PLP enzymes, 70% of the purified MJ0959 protein is bound to PLP (27).

Purified MJ0959 catalyzed the transamination of 2-oxoglutarate from L-aspartate to produce glutamate and oxaloacetate. Glutamate was identified by GC-MS, as the N-ethoxycarbonyl trifluoroethyl ester, and by HPLC, as the fluorescent CBI derivative. The enzyme catalyzed glutamate formation with a specific activity of 19 U mg⁻¹, corresponding to a rate of 14 s⁻¹. In the reverse reaction, the transamination of oxaloacetate from L-glutamate, the enzyme had a lower specific activity, i.e., 0.3 U mg⁻¹ (0.2 s⁻¹). After a 2-h incubation with excess enzyme (11 μg) at 50°C, 5 mM L-aspartate and 5 mM 2-oxoglutarate were converted to 2.6 mM glutamate and 2.2 mM alanine. Alanine formation was confirmed by GC-MS and coelution of an alanine-CBI derivative during HPLC.

A reaction mix containing enzyme and 5 mM aspartate with no 2-oxoacid produced only 0.2 mM alanine. Therefore, alanine is probably formed through the enzyme-catalyzed transamination of pyruvate produced by the spontaneous decomposition of oxaloacetate. The rate of nonenzymatic oxaloacetate decarboxylation was measured under similar conditions, and the reaction was found to be of the first order with respect to oxaloacetate, with a rate constant of 2.5 × 10⁻⁴ s⁻¹. This measured rate constant is similar to those reported previously for uncatalyzed oxaloacetate decarboxylation (11), and it is sufficiently high to account for the observed alanine. However, we cannot rule out alanine formation as a β-decarboxylation side reaction catalyzed by MJ0959 without further experiments.

This enzyme also catalyzed the transamination of PHP, using either L-aspartate or L-glutamate as an amino group donor (Fig. 5). In reaction mixtures containing 5 mM L-aspartate and 1.2 mM PHP, the enzyme catalyzed phosphoserine production, with a specific activity of 1.5 U mg⁻¹ (1.1 s⁻¹). A small amount of alanine was produced in this reaction as well. L-Glutamate could also serve as an amino group donor for the transamination of PHP, and no alanine was detected in these reaction mixtures. The reverse reaction, using phosphoserine as a donor, produced aspartate and glutamate from oxaloacetate and 2-oxoglutarate, respectively.

FIG. 4. The M. maripaludis MMP0391 gene complements the serC mutation of E. coli KL285. (A) M9 minimal medium with glucose containing all 20 amino acids supports the growth of E. coli KL285 containing (clockwise from top) pKQV4 (E. coli serC), empty pKQV4 vector, and pKQV4 (MMP0391). (B) Minimal medium containing 19 amino acids (without serine) and 1 mM IPTG. All media contain 100 μg ml⁻¹ ampicillin.

Specific activity of 15 U mg⁻¹ in standard PGDH activity assays measuring the NADH-dependent reduction of PHP. In the presence of 3 mM L-serine, the extract had a specific activity of 12 U mg⁻¹, while reaction mixtures containing 3 mM L-cysteine or DL-phosphoserine demonstrated somewhat lower activities, of 10 and 8.5 U mg⁻¹, respectively. The native E. coli PGDH did not contribute significantly to this activity, as a cell extract of E. coli BL21(DE3)/pET-11a contained <0.25 U mg⁻¹ activity (the background rate of PHP-independent NADH oxidation). Therefore, the presence of an amino-terminal polyhistidine tag does not account for the insensitivity of M. maripaludis PGDH to allosteric inhibition by serine.

Broad-specificity aspartate aminotransferase catalyzes phosphoserine production. The M. maripaludis MMP0391 protein was annotated as an aspartate aminotransferase (AspAT) by the genome sequencing project (17). Because a homologous AspAT from Methanobacterium thermautotrophicus was reported to catalyze the transamination of phosphoserine (43, 44), we proposed that a single enzyme could catalyze both reactions in the Methanococcales. The MMP0391 gene was cloned into plasmid pKQV4 downstream from a tac promoter. In the presence of the isopropyl-β-D-thiogalactopyranoside (IPTG) inducer, this gene complements the serC mutation of E. coli KL285 (5) (Fig. 4). The E. coli KL285/pKQV4 control strain did not grow on serine-free medium. Slight growth of E. coli KL285/pKQV4(MMP0391) in the absence of IPTG may be caused by leaky expression from the tac promoter.

The MMP0391 protein was fused to a carboxy-terminal polyhistidine tag and expressed in E. coli. The heterologously expressed protein had an apparent mass of 44.3 kDa, similar to the expected mass of 42.9 kDa. However, most of the expressed protein was found in the insoluble portion of cell lysate. Protein solubilized from inclusion bodies had no detectable aminotransferase activity (data not shown). Therefore, the orthologous protein from M. jannaschii, MJ0959, was chosen for biochemical characterization.
Sulfopyruvate is an analog of oxaloacetate and an intermediate in methanogenic coenzyme M biosynthesis (14). When incubated with aspartate or glutamate in enzymatic reaction mixtures, sulfopyruvate was transaminated to form cysteate. Reaction mixtures containing oxaloacetate or 2-oxoglutarate and L-cysteate acid produced aspartate and glutamate, respectively. Other 2-oxoacid substrates for the purified enzyme included glyoxylate and pyruvate. However, glycine, L-alanine, and L-serine were poor amino group donors for the reverse reaction.

Vertical inheritance of class V aspartate aminotransferases in methanogenic archaea. All of the methanogenic euryarchaea have orthologs of the archaeal aspartate aminotransferase (Fig. 6). Phylogenetic analysis showed that this gene was vertically inherited among the euryarchaea, even the heterotrophic archaea *Archaeoglobus fulgidus* and *Halobacterium* sp., which probably evolved from a methanogenic ancestor. In contrast, the deeply diverging heterotrophic euryarchaea, such as *Pyrococcus* spp. and *Thermoplasma* spp., have no close homolog. The *Thermococcales* do have homologs of an uncharacterized aminotransferase family that includes crenarchaeal homologs, 2-aminoethylphosphonate aminotransferase from *Salmonella enterica* serovar Typhimurium, and human serine-pyruvate aminotransferase. Finally, several clostridia and most cyanobacteria also contain uncharacterized homologs that were likely acquired by horizontal gene transfer and then vertically inherited within modern bacterial lineages. The last group includes plant peroxisomal and α-proteobacterial serine-glyoxylate aminotransferases.

**DISCUSSION**

The *M. maripaludis* phosphoglycerate dehydrogenase has similar structural and kinetic properties to those of the well-characterized *E. coli* and *M. tuberculosis* PGDH proteins (Table 1). The *M. tuberculosis* protein has been called the link between *E. coli* and mammalian PGDHs, due to its extended carboxy-terminal domain, substrate inhibition kinetics, substrate specificity, and ionic strength requirements (7). *M. maripaludis* PGDH has a carboxy-terminal extension similar to that of *M. tuberculosis* PGDH and requires 100 to 200 mM KCl for maximal activity. Although gel filtration experiments did not resolve the size of native *M. maripaludis* PGDH, the results suggest that it forms a very-high-molecular-weight complex that can dissociate into tetrameric and dimeric forms. However, *M. maripaludis* PGDH is more similar to the *B. subtilis* and mammalian enzymes in its low sensitivity to inhibition by serine. The structural basis for this discrepancy remains to be determined, but the regulatory properties are consistent with the methanogen’s requirement for phosphoserine to make cysteine and cystathionine. In *E. coli*, cysteine is made from free serine, so phosphoserine levels need only be controlled by the serine concentration. This linear pathway is well regulated through allosteric inhibition by the end product. In the *Methanococcales*, phosphoserine appears to be a branch point (Fig. 1). It is required for serine, cysteine, and cystathionine biosynthesis, so canonical feedback regulation of phosphoglycerate dehydrogenase by serine would decrease cysteine and cystathionine production, regardless of their levels. Similar branch points, such as the aspartokinase reaction, are regulated either by isoenzymes or by allosteric inhibition of down-
stream enzymes. The mode of regulation of these pathways is currently unknown for the Methanococcales.

Based on their structural similarity, the euryarchael aspartate aminotransferases belong to the large superfamily of PLP-dependent transferases and the cystathionine synthase-like family of proteins (31). These aspartate aminotransferases have been classified as either subgroup IV aminotransferases (25, 43) or class V aminotransferases (33). This classification distinguishes them from the canonical bacterial/eucaryal aspartate aminotransferases, which belong to subgroup I or class I, and reflects their separate evolutionary history. However, these classifications are not sufficient to predict the catalytic activities and physiological roles of the enzymes (13, 41). A recent study used o-phthalaldehyde derivatization and HPLC separation with fluorescence detection to study the activities of 20 aminotransferases from *Corynebacterium glutamicum* (23). Affinity purification of the heterologously expressed proteins reduced the possibility of contamination by other aminotransferases. This high-throughput, generic approach offers an unprecedented amount of information about an organism’s aminotransferase complement but cannot screen all possible substrate combinations. We used analogous derivatization chemistry to characterize the class V AspAT from *M. jannaschii*.

The broad-specificity AspAT from *M. jannaschii* is an ortholog of the AspAT that was previously purified, cloned, and characterized from *Methanothermobacter thermautotrophicus* SF-4 (formerly *Methanobacterium thermautotrophicus*) (43, 44). These proteins are probably orthologs of the AspAT purified from *Methanothermobacter thermophilus* (53). All three proteins formed high-molecular-weight complexes, which were probably tetramers and octamers. While these enzymes have the highest specific activities for catalyzing the transamination of aspartate and 2-oxoglutarate, they also have appreciable activities with alanine and phosphoserine. We showed that *M. maripaludis* AspAT complemented a serC mutation in *E. coli*; it remains to be determined whether AspAT is also the primary alanine aminotransferase in these methanogens.

Our finding that AspAT catalyzes cysteate transamination may be important for understanding the biosynthesis of coenzyme M in the Methanosarcinales. These methanogens have no homologs of the first three enzymes identified in the coenzyme M biosynthetic pathway of *M. jannaschii* but do have a functional sulfopyruvate decarboxylase enzyme (S. Namboori and D. E. Graham, unpublished data). Therefore, they must have another means to produce sulfopyruvate, such as the transamination of cysteate. Additional studies are required to determine the relevance of this pathway and the mechanism of cysteate formation in these strict anaerobes.

Separate archaeal lineages have evolved at least three different aspartate aminotransferases. The class V aminotransferases evolved in the euryarchaea after the divergence of the Thermococcales and Thermoplasmatales. Members of the Methanosarcinales also have homologs of the Thermus thermophilus bacterial subgroup IV aspartate aminotransferase (19). Although the Thermococcales and crenarchaea have homologs of the subgroup IV aminotransferases, the functions of these proteins are not known. *Pyrococcus furiosus* has a broad-specificity class I aspartate aminotransferase (48) as well as a homolog of the class I aspartate aminotransferase found in the crenarchaeon *Sulfolobus solfataricus* (6). The diverse substrate specificities of the class IV aminotransferases and the apparent convergent evolution of aspartate aminotransferase activity in at least three different classes of aminotransferases demonstrate the difficulty in predicting enzyme function for highly similar members of a protein family (51).

The evolution of the methanogenic class V aminotransferase family parallels the evolution of the tRNA-dependent cysteine biosynthetic pathway. Most methanogenic euryarchaea that carry this AspAT also have homologs of the O-phosphoseryl-tRNA synthetase and the Sep-tRNA:Cys-tRNA synthase (38). Therefore, these enzymes for phosphoserine and tRNA-dependent cysteine biosynthesis were encoded in the genome of the euryarchaeal methanogen ancestor. The Methanosarcinales acquired canonical cysteine biosynthesis enzymes (phosphoserine transaminase, serine acetyltransferase, O-acetylseryl sulhydrolase, and cysteinyl-tRNA synthetase) by horizontal gene transfer, making the tRNA-dependent pathway redundant (3, 21, 26). Yet these organisms have retained the class V AspAT, suggesting that this multifunctional enzyme plays several roles in cell metabolism.

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