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Purification and Properties of Serine Hydroxymethyltransferase from *Sulfolobus solfataricus*

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Serine hydroxymethyltransferase (SHMT) catalyzes the reversible cleavage of serine to glycine with the transfer of the one-carbon group to tetrahydrofolate to form 5,10-methylenetetrahydrofolate. No SHMT has been purified from a nonmethanogenic *Archaea* strain, in part because this group of organisms uses modified folates as the one-carbon acceptor. These modified folates are not readily available for use in assays for SHMT activity. This report describes the purification and characterization of SHMT from the thermophilic organism *Sulfolobus solfataricus*. The exchange of the α-proton of glycine with solvent protons in the absence of the modified folate was used as the activity assay. The purified protein catalyzes the synthesis of serine from glycine and a synthetic derivative of a fragment of the natural modified folate found in *S. solfataricus*. Replacement of the modified folate with tetrahydrofolate did not support serine synthesis. In addition, this SHMT also catalyzed the cleavage of both *allo*-threonine and β-phenylserine in the absence of the modified folate. The cleavage of these two amino acids in the absence of tetrahydrofolate is a property of other characterized SHMTs. The enzyme contains covalently bound pyridoxal phosphate. Sequences of three peptides showed significant similarity with those of peptides of SHMTs from two methanogens.

Serine hydroxymethyltransferase (SHMT) catalyzes the reversible interconversion of serine and glycine with tetrahydrofolate (H_4PteGlu) serving as the one-carbon acceptor and forming 5,10-methylenetetrahydrofolate (CH_2-H_4PteGlu) (9). SHMT has been purified from both procaryotic and eucaryotic cells, and the cDNA sequences for more than 20 different forms are present in the SwissProt database. However, only limited information on SHMT from a member of the *Archaea* is available, in part because these organisms use modified folates as a coenzyme (13). These modified folates are not commercially available, making it difficult to assay for folate-requiring enzymes.

The *Archaea* represent a third kingdom separate from the *Eukarya* and *Bacteria*. Within the *Archaea* are three distinct groups: the methanogens, the halophiles, and the hyperthermophiles. The halophiles appear to use folates, like members of the *Eukarya* and *Bacteria*. The methanogens use methanopterin, a dimethylated pteridine (methyl groups at C-7 and C-9) connected to 1-(4-aminophenyl)-1-deoxy-D-ribitol (APDR), a common structural element found in all the currently known modified folates (Fig. 1) (13, 14). The hyperthermophile *Sulfolobus solfataricus* contains a modified folate which has structural features between those of the methanogenic modified folates and folate. It contains the above-described core structure but has a nonmethylated pterin attached to the APDR moiety (Fig. 1) (16). The structure of a group attached to the APDR moiety has yet to be determined.

Without the pterin cofactor to accept the one-carbon group, it is not possible to assay in vitro the conversion of serine to glycine and thus to monitor the purification of the enzyme. In *Eukarya* and *Bacteria*, the cleavage of serine to glycine and CH_2-H_4PteGlu is an important source of one-carbon groups for the biosynthesis of thymidine, methionine, and purines (9). It is not known if serine is the major source of biosynthetic one-carbon groups in the *Archaea*. Clearly, in methanogens, acetate and CO_2 are the major sources of the one-carbon units used in methanogenesis (2).

A unique property of SHMT purified from both procaryotic and eucaryotic cells is that it cleaves both *allo*-threonine and β-phenylserine to glycine and either acetaldehyde or benzaldehyde, respectively, in the absence of H_4PteGlu (10). The enzyme also catalyzes the exchange of the α-proton of glycine with solvent protons (3). This exchange reaction is accelerated several orders of magnitude by the addition of H_4PteGlu in those SHMTs that have been studied in detail (9). We observed *allo*-threonine cleavage activity in *S. solfataricus*, *Halobacterium salinarium*, *Haloferax volcanii*, and *Pyrococcus furiosus*. This study reports on the purification of the enzyme that catalyzes the cleavage of *allo*-threonine and the exchange of the α-proton of glycine in *S. solfataricus.*

MATERIALS AND METHODS

Materials. Amino acids, Superdex 200, thiols, and buffers were purchased from Sigma, St. Louis, Mo. The pterin cofactor was synthesized as described elsewhere (14). Matrex Red A and Matrex Green affinity columns were from Amicon, Danvers, Mass. Fractogel TMAE was purchased from Merck (EM Separations Technology, Gibbstown, N.J.). The UnoQ anion-exchange column was obtained from Bio-Rad (Hercules, Calif.). NaB^3H_4 (718 mCi/mmol) was purchased from ICN, Costa Mesa, Calif.

Enzyme assays. SHMT activity was determined by observing the rate of exchange of the α-hydrogen of [2-^{3}H]glycine (10 to 40 Ci/mmol; ICN). The commercial solution was purified by adding 0.5 mCi of [2-^{3}H]glycine in 10 mM HCl to a 1- by 3-cm Dowex-50 column equilibrated with 10 mM HCl. The column was washed with 10 mM HCl until the eluted radioactivity had fallen to negligible

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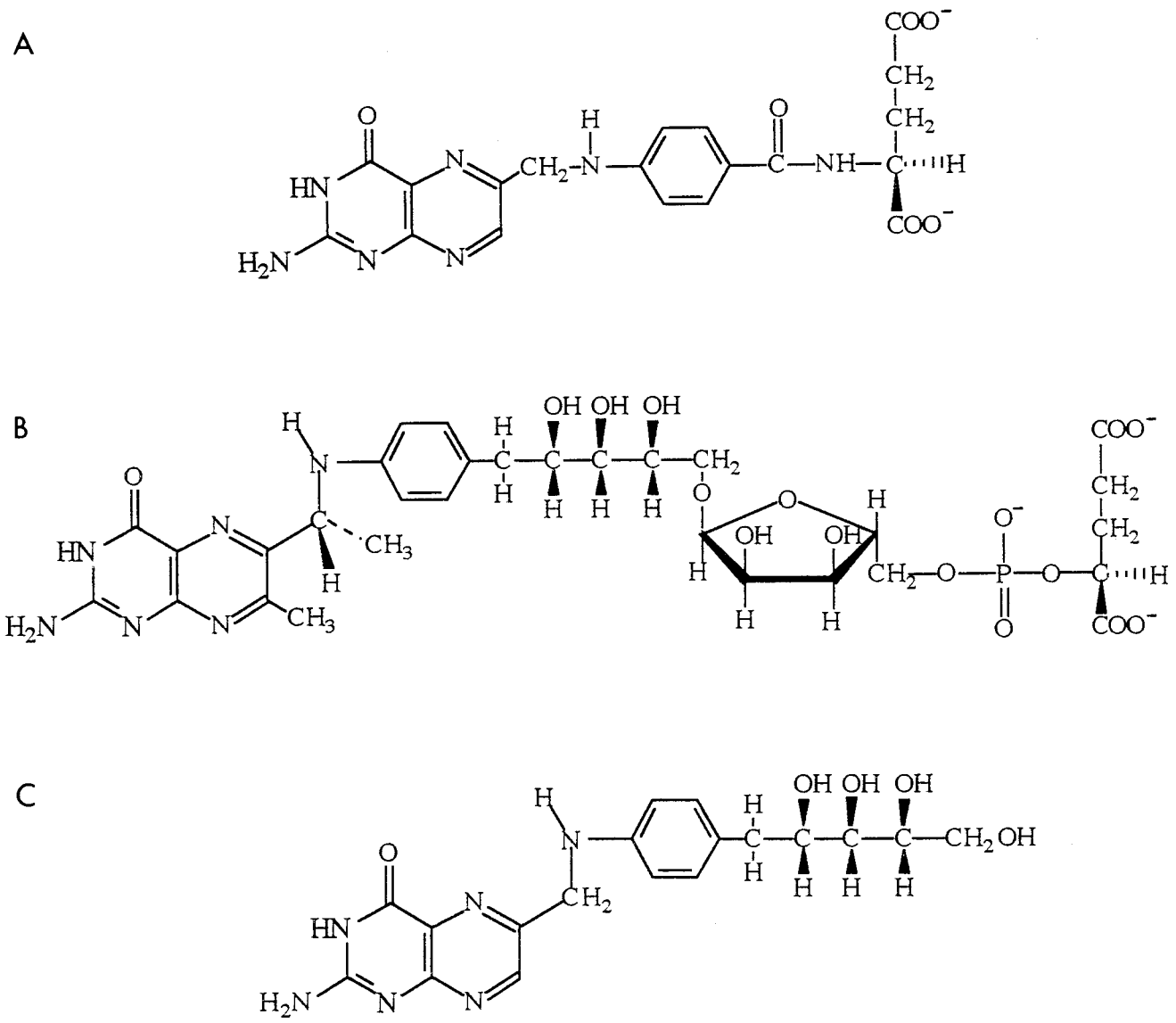

FIG. 1. Structures of pteridine coenzymes found in eucaryotes and procaryotes (folate) (A), methanogens (methanopterin) (B), and *S. solfataricus* (sulfopterin fragment) (C).

amounts. The bound [2-^{3}H]glycine was then eluted with 1 M NH_4OH. Fractions containing radioactivity were pooled and lyophilized to dryness. The residue was dissolved in 1 ml of 20 mM potassium phosphate (KPi) (pH 7.3) containing 20 mM 2-mercaptoethanol. This solution contained about 5×10^5 cpm per μl, which represents the amount of ^{3}H in both the $2H_R$ and $2H_S$ positions of glycine. This stock solution was stored at 4°C.

Typical assays were performed with 50 to 70 μl of 20 mM KPi (pH 7.3)–3 μl of [2-^{3}H]glycine–1 to 20 μl of protein solution in a 0.7-ml Eppendorf tube. The reaction solutions were placed in a 75°C water bath for 10 min, after which they were placed on ice, and the reactions were stopped by the addition of 15 μl of 10% trichloroacetic acid. If visible amounts of precipitated protein were present, the tubes were centrifuged for 1 min. The clear supernatants were added to a 1-cm-long column of Dowex-50 equilibrated with 10 mM HCl in a 1-ml plastic syringe. After absorption of the bound glycine, the columns were washed with three 200-μl aliquots of 10 mM HCl, the eluate was collected in scintillation vials containing 7 ml of ScintiVerse (Fisher), and radioactivity was counted.

Cleavage of *allo*-threonine to glycine and acetaldehyde was performed as follows. Extracts of *S. solfataricus* (5 to 25 μl) were added to 100-μl reaction tubes containing 20 mM KPi (pH 7.3) and 20 mM DL-*allo*-threonine. The tubes were incubated at 75°C for 10 min. The reactions were stopped by placing the tubes on ice for 3 min. The amount of acetaldehyde produced was determined by adding 100 μl of reaction solution to a cuvette containing 800 μl of 20 mM KPi (pH 7.3) and 300 μM NADH. A baseline absorbance at 340 nm was recorded, and the reaction was initiated by the addition of 10 μl of a 2-mg/ml solution of alcohol dehydrogenase. The total decrease in absorbance at 340 nm was used to calculate the amount of acetaldehyde in the solution by use of an ε_{340} of 6,240 M^{-1} cm^{-1} for NADH. This assay was linear with time and enzyme concentration in the range of 10 to 100 nmol of acetaldehyde.

Cleavage of β-phenylserine to glycine and benzaldehyde was determined by addition of the partially purified *S. solfataricus* SHMT to 900 μl of KPi (pH 7.3). A baseline absorbance at 290 nm was determined, and the reaction was initiated by the addition of 20 μl of a 100 mM stock solution of *threo*-β-phenylserine in ethanol. The increase in the amount of benzaldehyde formed was monitored at A_{290}.

Conversion of glycine to serine was performed by adding partially purified SHMT to 800 μl of 20 mM KPi (pH 7.3)–glycine (24 μM)–formaldehyde (24 μM)–modified methylenetetrahydrofolate analog (60 μM). Norvaline (24 μM) was included as an internal amino acid standard. The solution was incubated at 75°C, and the reactions were stopped by the addition of trifluoroacetic acid (30 μl of a 0.1% solution) to a 200-μl aliquot of the reaction solution. The solution was boiled for 1 min; after centrifugation, the supernatant was dried under vacuum. The residue was resuspended in 20 mM sodium borate (pH 9), and an aliquot was analyzed on an amino acid analyzer. Controls that did not contain either the modified folate or CH_2-H_4PteGlu were treated in the same way.

Molecular weight determination. The M_r for the subunit of SHMT was determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis

(PAGE). The native M_r was determined from chromatography on a size exclusion column of Superdex 200 (1 by 58 cm). The buffer was 50 mM KPi (pH 7.5) containing 100 mM NaCl, 5 mM 2-mercaptoethanol, and 0.2 mM EDTA. A flow rate of 0.8 ml/min was maintained by a peristaltic pump. The column was calibrated with bovine rabbit liver cytosolic SHMT (M_r, 212,000), *Escherichia coli* SHMT (M_r, 94,000), serum albumin (M_r, 66,000), ovalbumin (M_r, 45,000), and a viral core protein (M_r, 26,000).

Isolation and sequencing of peptides. About 200 μg of the most highly purified preparation of SHMT was incubated in 20 mM potassium BES (pH 7.3) containing 10 μM PLP for 30 min at 30°C. To this solution was added about 5 μl of an NaB^3H_4 solution (10^6 cpm per μl) in 0.1 M NaOH. After 10 min of incubation at room temperature, this procedure was repeated. The solution was then dialyzed extensively overnight against only the potassium *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonate buffer. The protein contained 1.6×10^6 cpm covalently linked to the protein as the secondary amine of the reduced pyridoxal 5′-phosphate. A small portion of this solution was analyzed by SDS-PAGE. The gel was stained with Coomassie blue and then transferred to a polyvinylidene fluoride membrane by electroblotting. The stained bands were cut from the gel, and radioactivity was counted in 6 ml of ScintiVerse in a scintillation spectrometer.

The remaining NaB^3H_4-labeled protein was denatured with urea and digested with trypsin, and peptides were separated by reverse-phase high-pressure liquid chromatography and sequenced as described previously (5).

RESULTS AND DISCUSSION

Purification of SHMT. Purification was performed on the extract from multiple 45- or 100-g portions of wet cell paste. All buffers contained 5 mM 2-mercaptoethanol, 0.2 mM EDTA, and 20 μM PLP. All purification steps were performed at room temperature, except for dialysis overnight, which was done at 4°C.

(i) Homogenate and ammonium sulfate fractionation. Frozen cells (100 g) were thawed and suspended in 700 ml of 20 mM KPi (pH 7.3). The cells were disrupted by passage through a cell disrupter (Avestin, Ottawa, Ontario, Canada) at 15,000 to 20,000 lb/in^2. The homogenate was centrifuged at 12,000 × *g* for 30 min, and the resulting pellet was discarded. An ammonium sulfate fraction between 45% (277 g/liter) and 70% (171 g/liter) saturation was isolated by centrifugation. The pellet was dissolved in 20 mM KPi (pH 7.3) and extensively dialyzed against 20 mM KPi (pH 7.7).

(ii) DEAE-Sephadex chromatography. A DEAE-Sephadex column (10 by 25 cm) was equilibrated with 20 mM KPi (pH 7.7) that did not contain PLP. The dialyzed supernatant was added to the column, which was washed with equilibration buffer until the A_{280} was below 0.3. Protein was eluted with a linear gradient of 1 liter of equilibration buffer and 1 liter of 50 mM KPi (pH 6.4) containing 0.5 M NaCl. Fractions containing activity were pooled, and the protein was precipitated by the addition of ammonium sulfate to 70% (472 g/liter) saturation. After centrifugation at 12,000 × *g* for 30 min, the pellet was dissolved in 20 mM KPi (pH 7.3) and dialyzed extensively against this buffer. The next morning, the dialysis buffer was changed to 20 mM KPi (pH 6.5), and dialysis was continued for another 4 h.

(iii) Matrex Red A affinity column. The dialyzed enzyme solution was added to a Matrex Red A column (3.5 by 14 cm) that had been equilibrated with 20 mM KPi (pH 6.5). The column was washed with equilibration buffer until the A_{280} was below 0.1. The enzyme was eluted with a linear gradient of 300 ml of equilibration buffer and 300 ml of equilibration buffer containing 1 M NaCl. Activity appeared in the fractions near the beginning of the gradient. Fractions containing activity were pooled, and the protein was precipitated with ammonium sulfate and centrifuged as described for the eluate from the DEAE column. The protein pellet was dissolved in 20 mM KPi (pH 7.3) and dialyzed overnight against 2 liters of this buffer. The next morning, the dialysis buffer was changed to 10 mM KPi (pH 7.7), and dialysis was continued for another 4 h.

(iv) Fractogel TMAE column. The enzyme was added to a Fractogel TMAE column (2.5 by 11 cm) equilibrated with pH 7.7 KPi buffer. The column was washed with equilibration buffer until the A_{280} was below 0.1. The enzyme was then eluted with a linear gradient of 300 ml of equilibration buffer and 300 ml of 10 mM KPi (pH 7.7) containing 400 mM NaCl. Fractions containing activity were pooled and concentrated by the addition of ammonium sulfate to 70% saturation as described above. The dissolved pellet was dialyzed against 20 mM KPi (pH 7.3) overnight and then against 20 mM KPi (pH 6.5) for 4 h.

TABLE 1. Purification of *S. solfataricus* enzyme from 100 g of cell paste

Step	Vol (ml)	Total protein (mg)	Total U[a] (cpm, 10^6)	Sp act (U/mg, 10^3)	Fold purification	% Yield
Homogenate	775	13,500	3.4	0.25	1	100
$(NH_4)_2SO_4$	100	3,800	2.4	0.63	2.5	70
DEAE-Sephadex	98	912	1.02	1.1	4.4	30
Matrex Red A	24	716	0.63	3.6	14.2	19
Fractogel TMAE	150	63	0.42	6.8	27.1	12.3
Matrex Green	1.1	4.3	0.12	27.5	109	3.5

[a] One unit is defined as the amount of enzyme that yields 1,000 cpm under standard assay conditions.

(v) Matrex Green affinity column. The enzyme solution was added to a Matrex Green column (1 by 8 cm) and washed with pH 6.5 KPi buffer until the A_{280} in the eluate was below 0.1. The protein was eluted with a linear gradient of 75 ml of equilibration buffer and 75 ml of equilibration buffer containing 1 M NaCl. The enzyme activity eluted near the end of the gradient. After being concentrated by 70% ammonium sulfate precipitation, the enzyme solution was dialyzed against 1 liter of 50 mM KPi (pH 7.5). At this point, we had so little protein left that it was pooled with multiple samples purified through the same procedure. A number of columns were used to try to improve on the final purification. Many of these did not work. The final two steps reported below were performed without knowledge of the original amount of cell paste used, resulting in a lack of knowledge about yields and purification. However, the purification was not greater than a few fold for any column, since only a few bands were present on SDS-polyacrylamide gels of the protein eluted from the Matrex Green column. The results of the purification through the Matrex Green column are shown in Table 1.

(vi) Superdex 200 chromatography. The enzyme from several previous purification steps was chromatographed on a Superdex 200 column (1 by 58 cm) as described in Materials and Methods. Active fractions were pooled and concentrated with 70% ammonium sulfate. The pellet was dissolved in a minimal amount of buffer and dialyzed against 1 liter of 20 mM KPi (pH 8.0) that did not contain PLP.

(vii) UnoQ anion-exchange chromatography. The enzyme was added to a UnoQ column (7 by 35 mm) in a fast protein liquid chromatography system equilibrated with 20 mM KPi (pH 8.0) that did not contain PLP. After being washed with equilibration buffer, proteins were eluted with a linear gradient of 20 ml of 20 mM KPi (pH 8.0) and 20 ml of the same buffer with 250 mM NaCl. An SDS-polyacrylamide gel of protein samples after several of the purification steps is shown in Figure 2. A pH stability study showed that the enzyme is most stable between pHs 6.5 and 8.0.

Determination of the size of SHMT. The retention volume of the SHMT activity from the Superdex 200 column and comparison with the elution volumes of several proteins of

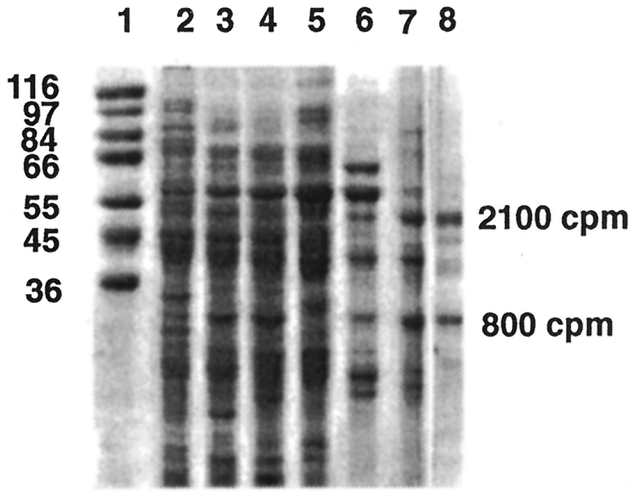

FIG. 2. SDS-PAGE of proteins from *S. solfataricus* after several steps of purification. Lanes: 1, molecular mass standards (in kilodaltons); 2 to 8, ammonium sulfate, DEAE-Sephadex, Matrex Red A, Fractogel TMAE, Matrex Green, Superdex 200, and UnoQ fractions, respectively. The protein used in lane 8 was reduced with NaB^3H_4 before being subjected to electrophoresis. After the gel was stained, it was transferred to a polyvinylidene fluoride membrane. The major bands were cut from the membrane, and counts were determined in a liquid scintillation spectrometer. The numbers on the right for the two major bands represent the counts per minute above background levels.

known molecular sizes suggested that the SHMT activity was associated with an 80-kDa protein.

The subunit size of the protein was determined from an SDS-polyacrylamide gel with molecular mass standards. Figure 2, lane 8, shows that the major protein band was about 53 kDa. However, minor protein bands were present in the 40-kDa region, and another major band was present at about 30 kDa. Since the protein was not pure, it is not clear if either of the major bands was the protein that exhibited the glycine α-proton exchange activity. It is also possible that the activity resided in one of the minor bands. To determine which band exhibited the activity, we took advantage of the ability of NaB^3H_4 to rapidly reduce the aldimine bond of all PLP enzymes to a stable secondary amine with the incorporation of a tritium atom on the 4′ carbon of PLP. Reduction of our partially purified preparation resulted in stable counts being incorporated into the extensively dialyzed protein solution, with a concomitant loss of enzyme activity. After transfer to a polyvinylidene fluoride membrane by electroblotting, the two major bands and two minor bands were cut from the membrane and added to separate vials of scintillation fluid for counting. Only the two major bands, which migrated at 53 and 30 kDa, showed incorporation of significant numbers of counts (listed beside lane 8 in Fig. 2). About two-thirds of the counts were associated with the major band migrating at 53 kDa, and one-third of the counts were associated with the protein migrating at 30 kDa.

In the elution of the protein from the UnoQ column, each tube was analyzed for protein by SDS-PAGE and for activity in the glycine tritium exchange reaction. The elution of the major band at 53 kDa followed the activity profile, but much of the 30-kDa protein eluted prior to the elution of this activity. This result suggests that the major band at 53 kDa is responsible for the catalytic activity in the glycine α-proton exchange reaction.

There can be considerable error in the estimation of protein sizes by both SDS-PAGE and molecular sieve chromatography. Each is based on the Stoke's radius of the protein rather than on molecular mass. However, the results suggested that the putative SHMT in *S. solfataricus* is a homodimer with a subunit mass in the 50-kDa region. This is the same approximate size and quaternary structure as in *E. coli* SHMT (3). The eucaryotic SHMTs have similar subunit sizes but are homotetramers.

Evidence that PLP is a cofactor. Several additional studies were performed to further establish that PLP is the coenzyme for *S. solfataricus* SHMT. The addition of $NaCNBH_3$ resulted in complete inactivation of the enzyme activity, and this activity could not be restored by the addition of PLP. This result is in agreement with studies of other PLP enzymes in which this reagent reduces the internal aldimine of PLP to the catalytically inactive and stable secondary amine.

Both the eucaryotic and procaryotic SHMTs are inhibited by L-cysteine, which forms a thiazolidine complex with the active-site PLP (9). The thiazolidine complex can be separated from the enzyme by chromatography on a Sephadex G-25 column equilibrated with 1 M ammonium sulfate, resulting in an inactive apoSHMT. The addition of PLP completely reactivates the apoSHMT to holoSHMT. This experiment was repeated with partially purified *S. solfataricus* SHMT. L-Cysteine inhibited the catalytic activity, and its removal on the Sephadex G-25 column resulted in a greater than 80% loss of catalytic activity. The addition of PLP completely restored the activity (data not shown).

Substrate specificity. Evidence that the partially purified activity that catalyzed the solvent exchange of [2-^{3}H]glycine was SHMT was demonstrated by showing that glycine and formaldehyde were converted to serine in the presence of the synthetic modified folate derivative of the naturally occurring folate analog. Figure 3 shows that serine was formed only in the presence of the modified folate. The coenzyme used in this study contained the important pteridine ring and the ADPR moiety but lacked an unknown extension of the ADPR portion of the molecule (Fig. 1). The *S. solfataricus* enzyme did not cleave serine to glycine in either the presence or the absence of H_4PteGlu, suggesting that H_4PteGlu cannot replace the modified folates

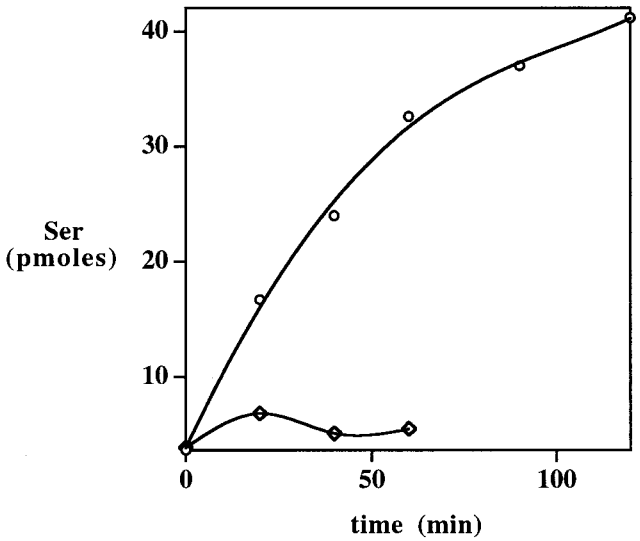

FIG. 3. Synthesis of serine in the presence (○) and absence (◇) of a partial structure of the modified folate found in *S. solfataricus*. Reactions were performed as described in Materials and Methods. Aliquots were removed over time, and the formation of serine was determined by amino acid analysis. Replacement of the partial modified folate found in *S. solfataricus* with H_4PteGlu produced no increase in the amount of serine formed over that in the control.

TABLE 2. Alignment of *S. solfataricus* sequenced peptides with putative peptides from *M. jannaschii* and *M. thermoautotrophicum* glycine hydroxymethyltransferases[a]

Organism	Peptide sequence
S. solfataricus	LE-TINLIPSENVM
	:| :|||| |||:
M. thermoautotrophicum	MESSINLIASENIT
	| || |||||||
M. jannaschii	^{22}RE-SIKLIASENIT^{34}
S. solfataricus	FVTLGGSLYLFP
	:: :||||:|||
M. thermoautotrophicum	IILFGGSLFLFP
	:||||||| ||
M. jannaschii	^{165}LILFGGSLFPFP^{176}
S. solfataricus	AVIAPVQAGAHVSHT
	| | ||||
M. thermoautotrophicum	ARIM-YD-GAHVLGL
	|:| || ||||||
M. jannaschii	^{191}AKIA-YD-GAHVLGL^{203}

[a] In the SwissProt database, SHMT is listed as glycine hydroxymethyltransferase.

used by this organism. In eucaryotic and procaryotic cells, an extension of polyglutamate residues to the pteridine and benzoylglutamate moieties increased coenzyme affinity for folate-requiring enzymes but had little effect on activity (11). The unknown part of the structure of the modified folate sulfopterin may play a similar role.

The substrate specificity of the enzyme was tested in comparison with the properties of both eucaryotic and procaryotic SHMTs. The *S. solfataricus* enzyme was able to cleave both *allo*-threonine and β-phenylserine to glycine and either acetaldehyde or benzaldehyde, respectively. These reactions occurred with microgram amounts of protein, showing that these two amino acids are good substrates for the *S. solfataricus* enzyme.

Only a single report of putative purified SHMT activity from a member of the *Archaea* has been made (4). This activity, from a methanogen, was believed to be associated with a single band on an SDS-polyacrylamide gel. From a molecular sieve column, a molecular mass of 102 kDa was determined, but the size of each subunit was not determined. The enzyme activity required PLP, Mg^{2+}, and the cofactor methanopterin. The requirement for Mg^{2+} is unusual for SHMTs purified from other sources. No sequence information for the protein was provided.

Isolation of peptides. Enzyme that had been labeled with NaB^3H_4 was denatured and digested with trypsin. A radioactive peptide from the digest was isolated and sequenced. Unfortunately, the peptide was not pure, and a few amino acids were released at each sequencing step. A unique characteristic of most other SHMTs is the active-site sequence of -T-T-H-K(PLP)-T-L- (8). The SHMT from *Methanococcus jannaschii* has the sequence -G-S-T-H-K^{226}-T-F- (1). Lys-226 is in the correct location for binding PLP, as judged from the *E. coli* SHMT with an active site Lys-229 (3, 8). The radiolabeled peptide from the *S. solfataricus* preparation had a possible sequence of V-T-T-H-X-X-L. The first unknown residue could be the labeled Lys with the attached reduced PLP. No definitive sequence could be ascribed to the *S. solfataricus* enzyme from these studies because the peptide was not pure, but the sequence is supportive in that the correct amino acids were present.

Three other tryptic peptides from the *S. solfataricus* protein digest were found to be pure, and their sequences are shown in Table 2, along with the corresponding sequences of putative peptides from the *M. jannaschii* and *Methanobacterium thermoautotrophicum* SHMTs (1, 12). The SHMTs from these two methanogens have not been purified, and their putative sequences were obtained from sequence homology of an open reading frame in the genome. The homology and similar size of the predicted proteins relative to other SHMTs strongly suggest that this open reading frame is for SHMT in these two organisms.

The similarity in the size and properties of the *S. solfataricus* SHMT relative to procaryotic and eucaryotic SHMTs suggests that the cleavage of serine may have a similar function as an important source of one-carbon groups for the biosynthesis of thymidylate and methionine in this organism. The fragment of the modified folate in *S. solfataricus* has been shown to function in thymidylate (7) and methionine (15a) biosynthesis. It is not clear if purine synthesis in *S. solfataricus* also requires folate transfer of a formyl group, as is required in eucaryotes and procaryotes. Recent studies have shown that in *M. thermoautotrophicum*, purines appear to be synthesized without the aid of a modified folate coenzyme (15). Also, an enzyme that forms formylglycinamide ribonucleotide without participation of a folate coenzyme has been purified from and characterized for *E. coli* (6).

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