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Serine Hydroxymethyltransferase from Mung Bean (*Vigna radiata*) Is Not a Pyridoxal-5′-Phosphate-Dependent Enzyme[1]

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ABSTRACT

Serine hydroxymethyltransferase from mammalian and bacterial sources is a pyridoxal-5′-phosphate-containing enzyme, but the requirement of pyridoxal-5′-phosphate for the activity of the enzyme from plant sources is not clear. The specific activity of serine hydroxymethyltransferase isolated from mung bean (*Vigna radiata*) seedlings in the presence and absence of pyridoxal-5′-phosphate was comparable at every step of the purification procedure. The mung bean enzyme did not show the characteristic visible absorbance spectrum of a pyridoxal-5′-phosphate protein. Unlike the enzymes from sheep, monkey, and human liver, which were converted to the apoenzyme upon treatment with L-cysteine and dialysis, the mung bean enzyme similarly treated was fully active. Additional evidence in support of the suggestion that pyridoxal-5′-phosphate may not be required for the mung bean enzyme was the observation that pencillamine, a well-known inhibitor of pyridoxal-5′-phosphate enzymes, did not perturb the enzyme spectrum or inhibit the activity of mung bean serine hydroxymethyltransferase. The sheep liver enzyme upon interaction with *O*-amino-D-serine gave a fluorescence spectrum with an emission maximum at 455 nm when excited at 360 nm. A 100-fold higher concentration of mung bean enzyme-*O*-amino-D-serine complex did not yield a fluorescence spectrum. The following observations suggest that pyridoxal-5′-phosphate normally present as a coenzyme in serine hydroxymethyltransferase was probably replaced in mung bean serine hydroxymethyltransferase by a covalently bound carbonyl group: (a) inhibition by phenylhydrazine and hydroxylamine, which could not be reversed by dialysis and or addition of pyridoxal-5′phosphate; (b) irreversible inactivation by sodium borohydride; (c) a spectrum characteristic of a phenylhydrazone upon interaction with phenylhydrazine; and (d) the covalent labeling of the enzyme with substrate/product serine and glycine upon reduction with sodium borohydride. These results indicate that in mung bean serine hydroxymethyltransferase, a covalently bound carbonyl group has probably replaced the pyridoxal-5′-phosphate that is present in the mammalian and bacterial enzymes.

SHMT[3] (EC 2.1.2.1) is the first enzyme in the pathway for the interconversion of folate coenzymes (2). This enzyme, in addition to providing one carbon unit for the biosynthesis of purines, thymidylate, and other metabolites, plays an important role in photorespiration in plants (21). Detailed investigations carried out on SHMT from bacterial and mammalian sources (for review, see ref. 19), have demonstrated the absolute requirement of PLP for the reaction:

$$\text{L-serine} + \text{H}_4\text{-folate} \overset{PLP}{\rightleftharpoons} \text{glycine} + 5,10\text{-CH}_2\text{-H}_4\text{-folate}$$

where H_4-folate is DL-tetrahydrofolate, and 5,10-CH_2-H_4-folate is 5,10-methylenetetrahydrofolate.

Relatively few investigations have been described on the isolation and characterization of SHMT from plants. Although SHMT from spinach (28), maize (13), soybean nodules (15), tobacco (16), castor endosperm (5), carrot, pea leaves, wheat (4), cauliflower (14), and *Lemna minor* (27) have been studied in some detail, the requirement of PLP for catalysis has not been clearly established. Earlier work from our laboratory on the homogeneous mung bean SHMT (18) had suggested that this enzyme may not require PLP for catalysis. In this communication, we describe experiments carried out to establish that mung bean SHMT is not a PLP-dependent enzyme and that a carbonyl moiety covalently linked to the protein probably carries out the function of PLP in catalysis.

MATERIALS AND METHODS

The following biochemicals were obtained from Sigma Chemical Company, St. Louis, MO: L-serine, 2-ME, EDTA, PLP, Tris, 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide, folic acid, phenylhydrazine hydrochloride, hydroxylamine hydrochloride, sodium borohydride, sodium pyruvate, α-ketoglutaric acid, and pencillamine. Sepharose CL-4B, AH-Sepharose, and phenyl-Sepharose were from Pharmacia, Uppsala, Sweden. L-[-3-^{14}C]Serine, [U-^{14}C]serine, [U-^{14}C]glycine, [U-^{14}C]tryptophan, [U-^{14}C]aspartate, and U[^{14}C]lysine were from Amersham, Buckinghamshire, UK. *O*-Amino-D-serine was prepared according to the method of Klosterman (10). All other chemicals were of analytical grade.

Buffer A was Tris buffer (0.05 M) (pH 8.5), containing 10% glycerol, 2-ME (10 mM), EDTA (1 mM), serine (2 mM), and 0.01% PMSF. Buffer B was Tris buffer (0.025 M) (pH 8.5), containing 10% glycerol, 2-ME (10 mM), EDTA (1 mM), serine (2 mM), and 0.01% PMSF. Blue Sepharose was prepared according to the method of Heyns and de Moor (7).

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[2] Since deceased.

[3] Abbreviations: SHMT, serine hydroxymethyltransferase; PLP, pyridoxal-5′-phosphate; OADS, *O*-amino-D-serine; AH, aminohexyl.

OADS-AH-Sepharose

To swollen AH-Sepharose (1 g), 10 mg OADS dissolved in 1 mL of water was added. The pH of the mixture was adjusted to 6, and 20 mg of 3-(3-dimethylaminopropyl)-1-ethocarbodiimide was added with constant stirring. The slurry was kept at room temperature (22 ± 5°C) in an end-over-end mixer for 1 h and the pH was readjusted to 6. Stirring was continued overnight at 4°C. The gel was washed with 100 mL of cold (0–4°C) sodium acetate (0.2 M) (pH 4), followed by 100 mL of sodium bicarbonate buffer (0.2 M) (pH 8.3) and 100 mL 0.5 M NaCl. Finally, the gel was washed with distilled water and packed onto a column. The column was then equilibrated with the desired buffer before use.

Enzyme Assay

Mung bean SHMT activity was monitored essentially as described by Taylor and Weissbach (23), with a slight modification. The assay was carried out in 0.025 M Tris buffer (pH 8.5) containing 10 mM 2-ME and 1 mM EDTA instead of sodium phosphate buffer. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol formaldehyde/min at 37°C and pH 8.5. The specific activity was expressed as units/mg protein. Protein was estimated by the method of Bradford (3) using crystalline BSA as the standard. Sheep liver SHMT activity was monitored by the method of Manohar *et al.* (12). The concentration of SHMT from all the sources used in this study was calculated assuming a mol wt of 200,000.

Purification of Mung Bean SHMT

In the Absence of PLP

Mung bean seedlings (250 g) germinated for 48 h at 37°C were cooled to 4°C and homogenized in buffer A (1.2 mL/g of seedlings) in a Waring Blendor. The homogenate was passed through two layers of cheesecloth and centrifuged in an RC-5B Sorvall centrifuge at 10,000*g* for 20 min at 4°C. The supernatant (crude extract step, Table I) was passed through glass-wool to remove lipids, and solid $(NH_4)_2SO_4$ was added to 40% saturation. After keeping for 20 min at 4°C, the mixture was centrifuged at 10,000*g* for 20 min at 4°C, and the pellet was discarded. The supernatant was raised to 80% $(NH_4)_2SO_4$ saturation by a further addition of solid $(NH_4)_2SO_4$. After 20 min, the suspension was centrifuged at 10,000*g* for 30 min at 4°C. The precipitate was dissolved in a minimal amount of buffer A (50 mL) and dialyzed against buffer B (1000 mL) for 6 h at 4°C (40–80% $(NH_4)_2SO_4$ step, Table I).

The dialyzed enzyme extract was then loaded onto a phenyl-Sepharose column (1 × 30 cm) equilibrated with buffer B containing 10% $(NH_4)_2SO_4$. The unadsorbed fraction containing SHMT activity was dialyzed at 4°C against 4 liters of buffer B for 48 h with six changes (phenyl-Sepharose step, Table I). The enzyme was then adsorbed on a Blue Sepharose column (1 × 20 cm) equilibrated with buffer B. After extensive washing with buffer B (until *A* of the eluate at 280 nm was less than 0.05), the enzyme was eluted from the column with buffer B containing 1 M KCl. Fractions (2 ml) containing SHMT activity were pooled and dialyzed against buffer B to remove KCl.

The enzyme was finally loaded onto an OADS-AH-Sepharose column (1 × 10 cm) equilibrated with buffer B and washed extensively with the same buffer (500 mL). The enzyme was eluted with buffer B containing 0.2 M KCl and 1 mM α-ketoglutarate. The active fractions (2.0 mL) were pooled, dialyzed against buffer B, and concentrated by dialysis against solid sucrose (OADS-AH Sepharose step, Table I).

Purification of Mung Bean SHMT in the Presence of PLP

The purification was carried out essentially as described above, except that all the buffers contained PLP (2 mM). Since PLP precluded binding of the enzyme to OADS-AH-Sepharose, it was removed from the enzyme extract by the addition of 10 mM L-cysteine, followed by dialysis against buffer B not containing PLP and then loaded on to the OADS-Sepharose column. The enzyme was eluted with buffer B containing 2 mM PLP and 0.2 M KCl and concentrated as described above. The activity at each step of purification is given in Table I.

Table I. *Purification of Mung Bean SHMT in the Presence and in the Absence of PLP*

Fraction	Activity[a]		Specific Activity × 10^3	
	+PLP (2 mM)	−PLP	+PLP (2 mM)	−PLP
	units			
Crude	16.1	17.8	4	6
40–80% $(NH_4)_2SO_4$	8.0	7.3	8	8
Phenyl Sepharose	10.6	13.5	12	16
Blue Sepharose	2.4	2.3	168	116
OADS-AH Sepharose	0.86	1.9	860	1266

[a] One unit of activity is defined as the amount of enzyme required to form 1 μmol of HCHO/min at 37°C at pH 8.5.

Purification of Sheep, Monkey, and Human Liver SHMT

The procedures described by Baskaran *et al.* (1), Ramesh and Rao (17), and Vijayalakshmi (24) were used to prepare mammalian SHMT. All of these enzyme preparations were homogeneous (1, 17, 24).

Removal of PLP Bound to Enzyme by L-Cysteine Treatment

The mung bean SHMT (5 μM) in 0.025 M Tris buffer, pH 8.5, was incubated with L-cysteine (10 mM) at 4°C for 24 h. The mixture was then dialyzed extensively against buffer B to remove excess L-cysteine and the thiazolidone complex formed. The enzyme activity was monitored before and after dialysis. The dialyzed enzyme was also assayed after preincubation with PLP (1 mM) for 10 min at 37°C.

Visible Absorbance Spectra

Visible *A* spectra of mung bean SHMT (10 μM) in 0.025 M Tris buffer (pH 8.5) and sheep liver SHMT (5 μM) in 0.4 mM sodium phosphate buffer (pH 7.2) were recorded between 300 and 500 nm on a Shimadzu UV-Vis 240 spectrophotometer.

Enzyme-Penicillamine Interaction

The *A* spectrum of mung bean SHMT (10 μM) in 0.025 M Tris buffer (pH 8.5) was recorded from 300 to 500 nm before and after incubation (for 30 min at 37°C) with penicillamine (10 mM). In a control experiment, the spectrum of sheep liver SHMT (1 μM) similarly treated was recorded.

Inhibition of the Enzyme Activity by Penicillamine

Mung bean SHMT (0.5 μM) and sheep liver SHMT (1.0 μM) were incubated separately with pencillamine (1 mM) for 10 min at 37°C. At the end of the incubation period, a 10-μL aliquot from each reaction mixture was withdrawn for monitoring residual enzyme activity.

Fluorescence Spectra of Enzyme-OADS Complex

Mung bean SHMT (7.5 μM) was mixed with OADS (100 μM), and the fluorescence emission spectrum of the complex after excitation at 360 nm was recorded using a Hitachi Fluorescence Spectrophotometer. This spectrum was compared with a fluorescence emission spectrum similarly recorded for sheep liver SHMT (0.075 μM) incubated with OADS (10 μM).

Inhibition of Enzyme Activity by Carbonyl Reagents

Mung bean SHMT (0.5 μM) in 0.025 M Tris buffer (pH 8.5) was incubated separately with phenylhydrazine, sodium borohydride, and hydroxylamine (0.25–10 mM) for 30 min at 37°C. An aliquot (10 μL) was withdrawn for monitoring residual enzyme activity. The enzyme treated with the carbonyl reagents was dialyzed against buffer B extensively to remove excess reagent and any dialyzable complex formed. Residual activity of the enzyme (0.05 μM) was then monitored as described above in the absence and in the presence of PLP (1 mM). A similar experiment was carried out using sheep liver SHMT.

Spectra of Enzyme-Hydrazone

The phenylhydrazone of pyruvate was prepared according to the method of Riley and Snell (20). The enzyme-phenylhydrazone in 0.025 M Tris buffer (pH 8.5) was prepared by incubation of mung bean SHMT (5 μM) with phenylhydrazine (10 mM) for 30 min at 37°C and the reaction mixture dialyzed extensively to remove unreacted phenylhydrazine. The spectrum of the dialyzed enzyme-phenylhydrazone was recorded on a UV-Vis 240 Shimadzu Spectrophotometer. Pyruvoylhydrazone spectrum was also recorded for comparison. Sheep liver SHMT (10 μM) was treated with phenylhydrazine according to the method of Wada and Snell (25) and the spectrum of the enzyme-pyridoxalhydrazone was recorded.

Covalent Labeling of Enzyme with Radioactive Amino Acids

Mung bean SHMT (5 μM) and sheep liver SHMT (5 μM) in 200 μL were separately incubated with the following ligands: [U-^{14}C]serine (165 mCi/mmol), 12 nmol; [U-^{14}C]glycine (108 mCi/mmol), 21 nmol; [U-^{14}C]tryptophan (46 mCi/mmol), 21 nmol; [U-^{14}C]aspartate (87 mCi/mmol), 22.9 nmol; and [U-^{14}C]lysine (99 mCi/mmol), 21 nmol at 37°C for 20 min. This was followed by the addition of sodium borohydride (10 mM) at pH 8.5 and incubated for 20 min at 37°C. The enzyme was precipitated with 20% TCA and transferred onto a glass fiber filter. The filter was then washed successively with 100 mL of 20, 10, and 5% TCA followed by ethanol:ether (1:1, v/v). The filter was dried under an IR lamp, suspended in 5 mL of Liquosorb LKB scintillation fluid, and radioactivity was measured on a RackBeta LKB spectrometer. As a control, in experiments with mung bean SHMT, sodium borohydride (10 mM) was added before the addition of amino acids. The binding was expressed as a percentage of the expected theoretical binding. The theoretical binding was calculated assuming four binding sites per mole of the enzyme. Earlier work with mung bean (18) and sheep liver (11) enzymes indicated that SHMT is a homotetramer.

RESULTS

Requirement of PLP for the Activity of Mung Bean SHMT

It is evident from Table I that the total activity and specific activity of the enzymes at the different steps of purification were similar both in the absence and in the presence of PLP. An increase in the total activity of the enzyme on passage of the $(NH_4)_2SO_4$ fraction through a phenyl-Sepharose column was due to the removal of a protein-bound inhibitor present in the crude extracts of mung bean seedlings (our unpublished results). The specific activity at the final step of purification carried out in the presence and absence of PLP was 860 and 1266 units, respectively, and the enzyme was found to be homogeneous by Phast Gel isoelectric focusing.

Effect of L-Cysteine Treatment on the Enzyme Activity

Effect of L-cysteine on the activity of SHMT from mung bean seedlings, monkey (17), sheep (12), and human liver (24) is given in Table II. It is evident that the enzyme from

Table II. *Effect of L-Cysteine on the Activity of Mung Bean, Human, Monkey, and Sheep Liver SHMT*

Mung bean SHMT (5 μM) in 0.025 M Tris buffer (pH 8.5) was incubated with L-cysteine (10 mM) at 4°C for 24 h. An aliquot (10 μL) of this reaction mixture was assayed for residual activity (24) before dialysis and after dialysis for 24 h against buffer B. An aliquot (10 μL) of the dialyzed enzyme was also assayed for residual activity after incubation with PLP (1 mM) for 10 min at 37°C.

Source	Specific Activity			
	Holoenzyme	Holoenzyme + L-cysteine (10 mM)	Post-dialysis −PLP	Post-dialysis + +PLP (1 mM)
	μmol/min/mg protein			
Mung bean	0.8	0	0.8	0.8
Human liver	3.6	0	0.1	3.2
Monkey liver	3.4	0	0.1	3.5
Sheep liver	6.1	0	0.1	5.7

all these sources was completely inhibited by L-cysteine (10 mM). Upon dialysis of the L-cysteine-treated enzyme, the activity was fully regained in the case of mung bean SHMT, and the addition of PLP (1 mM) did not enhance the activity of the enzyme. Although the activity of sheep (12), monkey (17), and human liver (24) SHMT were also inhibited by L-cysteine, the activity was not regained upon dialysis but was restored fully upon the addition of PLP (1 mM) (Table II).

Visible *A* Spectrum of the Enzyme

The mung bean SHMT did not show distinctive *A* maximum in the visible region (Fig. 1, curve 1). On the other hand, sheep liver SHMT showed an *A* peak at 425 nm (Fig. 1, curve 2), characteristic of PLP-proteins.

Enzyme-Penicillamine Interaction

The interaction of penicillamine with PLP-dependent enzymes resulted in the disappearance of the characteristic *A* at 425 nm and appearance of a new peak at 330 nm (22). It is evident from Figure 2 that the characteristic *A* of sheep liver SHMT at 425 nm (curve 2A) disappeared and a new peak appeared at 330 nm (curve 2B) upon interaction of the enzyme with pencillamine. However, no such *A* change was observed when pencillamine was added to mung bean SHMT (Fig. 2, curve 1A, 1B).

Mung bean (0.5 μM) and sheep liver (1 μM) SHMT were separately preincubated with penicillamine, and the residual enzyme activity was monitored as described in "Materials and Methods." Mung bean SHMT was not inhibited by penicillamine (1 mM) treatment, whereas the sheep liver SHMT lost 65% of its activity.

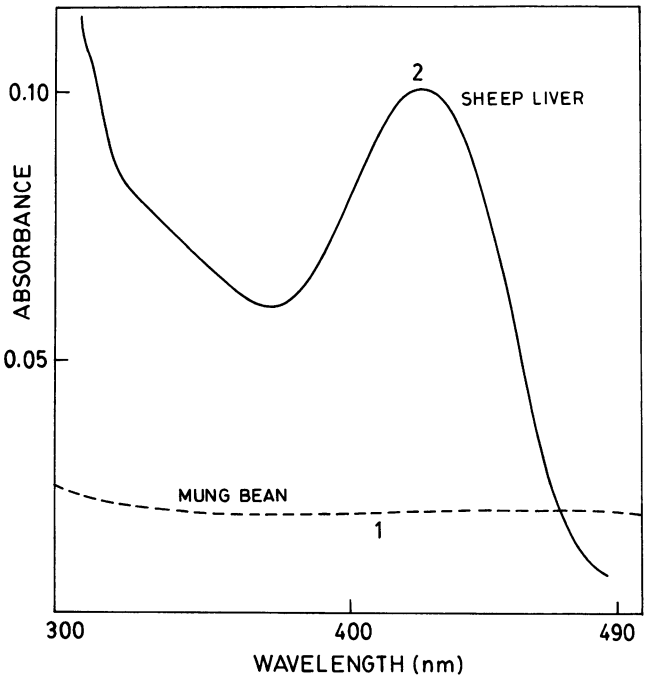

Figure 1. Visible *A* spectra of mung bean SHMT (10 μM) (curve 1) in 0.025 M Tris buffer (pH 8.5) and sheep liver SHMT (5 μM) (curve 2) in 0.4 mM sodium phosphate buffer (pH 7.2) recorded against the corresponding buffers in a Shimadzu spectrophotometer.

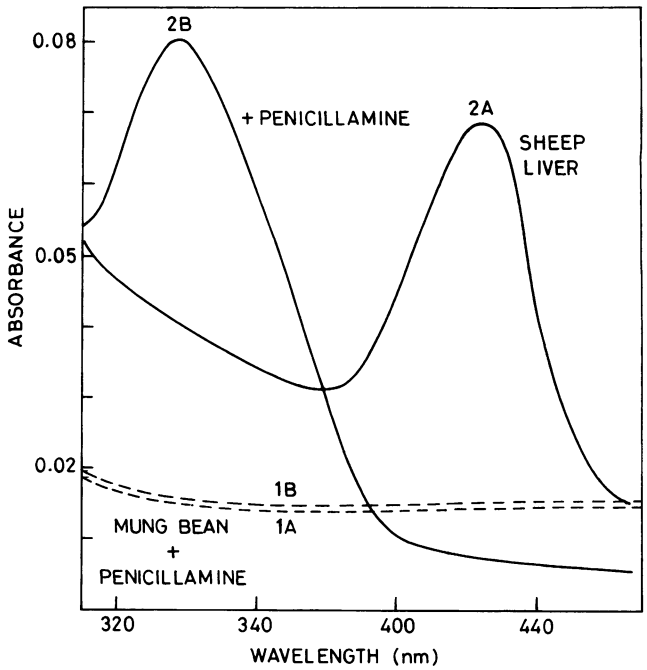

Figure 2. Interaction of penicillamine with mung bean and sheep liver SHMT. Mung bean SHMT (10 μM) in 0.5 mL of 0.025 M Tris buffer (pH 8.5) was incubated with pencillamine (10 mM) for 30 min at 37°C. The spectra were recorded on a Shimadzu spectrophotometer before (curve 1A) and after penicillamine treatment (curve 1B). Sheep liver SHMT (1 μM) in 0.4 mM sodium phosphate buffer (pH 7.2) was treated with penicillamine (10 mM) as described above and spectra recorded before (curve 2A) and after treatment (curve 2B).

Fluorescence Spectrum of the Enzyme-OADS Complex

OADS-PLP complex had a fluorescence excitation maximum at 360 nm and emission maximum at 455 nm (1). Mung bean SHMT (7.5 μM) mixed with 100 μM OADS did not show the fluorescence spectrum (Fig. 3, curve 2) characteristic of PLP-OADS complex (Fig. 3, curve 3). The sheep liver SHMT (0.075 μM) at 1/100 the concentration of mung bean SHMT showed a fluorescence spectrum with an emission maximum at 455 nm (Fig. 3, curve 1).

The Inhibition of Mung Bean SHMT by Carbonyl Reagents

Mung bean SHMT was treated with phenylhydrazine, hydroxylamine, and sodium borohydride as described in "Materials and Methods." It is evident from Table III that the carbonyl reagents effectively abolished enzyme activity. Dialysis did not restore the activity. Addition of PLP (1 mM) also did not reverse the inhibition (Table III). In the case of sheep liver SHMT, inhibition by phenylhydrazine and hydroxylamine was overcome effectively upon dialysis and addition of PLP (Table III). Inhibition by sodium borohydride, as expected, was not relieved by dialysis and addition of PLP.

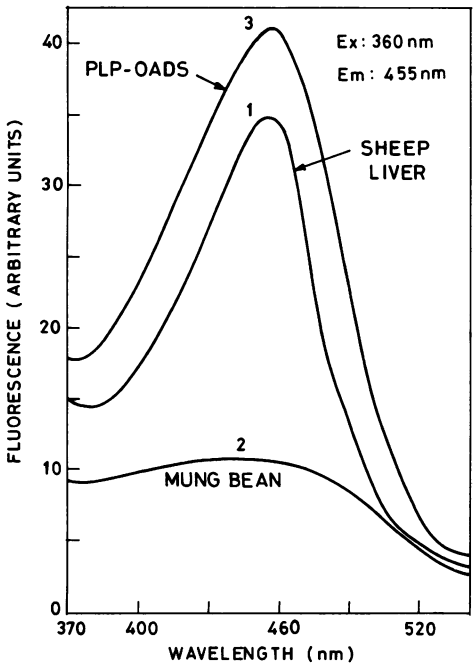

Figure 3. Fluorescence spectra of enzyme-OADS complex. Mung bean SHMT (7.5 μM) in 0.025 M Tris (pH 8.5) was mixed with OADS (100 μM) in the same buffer and the fluorescence emission spectrum of the complex upon excitation at 360 nm was recorded (curve 2) in a Hitachi Fluorescence Spectro-photometer. Sheep liver SHMT (0.075 μM) in 0.4 mM sodium phosphate buffer (pH 7.2) was incubated with OADS (10 μM) and fluorescence spectra of the complex recorded as described before (curve 1). The spectrum of the PLP (4 μM)-OADS (10 μM) complex is shown as curve 3.

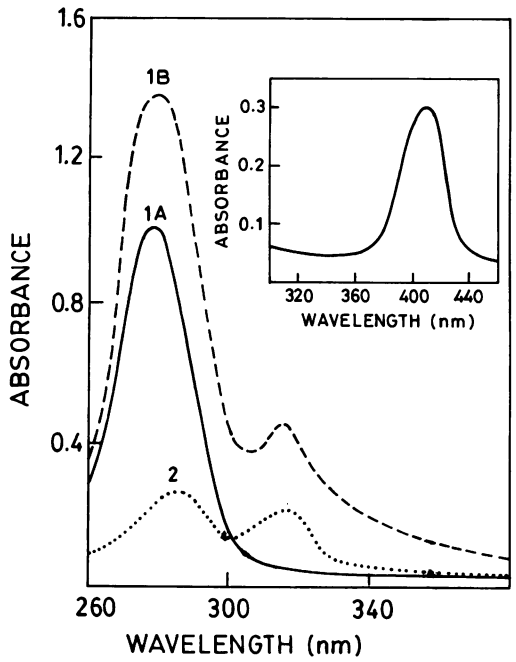

Figure 4. Enzyme-hydrazone spectrum. Mung bean SHMT (10 μM) in 0.025 M Tris buffer (pH 8.5) was incubated with phenylhydrazine (10 mM) for 30 min at 37°C. The spectrum of the reaction mixture after extensive dialysis against buffer B was recorded (curve 1B) between 260 and 340 nm. The spectrum of Mung bean SHMT (10 μM) in 0.025 M Tris buffer (pH 8.5) was recorded before treatment with phenylhydrazine (curve 1A). Spectrum of pyruvoylhydrazone prepared by the method of Riley and Snell (20) was also recorded as above (curve 2). The spectrum of pyridoxalhydrazone obtained after treatment of sheep liver SHMT (10 μM) with phenylhydrazine according to the method of Wada and Snell (25) was also recorded (inset).

Table III. *Effect of Carbonyl Reagents on the Activity of Mung Bean SHMT*

Mung bean SMHT (0.5 μM) in 0.025 M Tris buffer (pH 8.5) was incubated with different concentrations (as indicated below) of phenylhydrazine, hydroxylamine, and sodium borohydride for 10 min at 37°C. An aliquot (10 μL) was withdrawn at the end of the incubation period and assayed for residual activity.

Carbonyl Reagent	Mung bean SHMT[a]						Sheep liver SHMT[b]		
	Pre-dialysis				Post-dialysis		Pre-dialysis, 10.0 mM	Post-dialysis	
	0.25 mM	0.5 mM	1.0 mM	10.0 mM	−PLP	+PLP		−PLP	+PLP
	%								
Phenylhydrazine	15	50	67	100	100	100	97	98	7
Hydroxylamine	32	66	70	100	100	100	95	94	3
Sodium borohydride	90	95	100	100	100	100	97	97	96

[a] Mung bean SHMT (0.5 μM) was treated with phenylhydrazine (10 mM), hydroxylamine (10 mM), and sodium borohydride (10 mM) and dialyzed against buffer B as described in "Materials and Methods." An aliquot (10 μL) was assayed for activity before and after incubation with 1 mM PLP in 0.025 M Tris buffer (pH 8.5) for 10 min at 37°C.
[b] A similar experiment was carried out using sheep liver SHMT (0.5 μM).

Spectrum of the Enzyme-Phenylhydrazone

The mung bean SHMT, which showed a simple protein spectrum (Fig. 4, curve 1A) before phenylhydrazine treatment, showed a new peak at 315 nm after phenylhydrazine treatment (Fig. 4, curve 1B). Pyruvoylhydrazone showed an *A* maximum at 315 (Fig. 4, curve 2). Sheep liver SHMT treated with phenylhydrazine showed a typical pyridoxalhydrazone spectrum with an *A* maximum at 410 nm (Fig. 4, inset).

Evidence for a Covalently Linked Carbonyl Group in Mung Bean SHMT

It is evident that serine and glycine, which are substrate and product, respectively, can form covalent complexes with mung bean SHMT as indicated by the significant amount of radioactivity present in the TCA precipitate (Table IV). Amino acids that were not substrate(s) or product(s) like aspartate, tryptophan, and lysine did not bind to the enzyme efficiently. As expected, very little radioactivity was observed in the TCA precipitate in the case of sheep liver SHMT (Table IV).

Table IV. *Covalent Labeling of Mung Bean and Sheep Liver SHMT with Radiolabeled Amino Acids*

Mung bean SHMT (5.0 μM) in 0.025 M Tris-HCl (pH 8.5) and sheep liver SHMT (5.0 μM) in 0.4 mM sodium phosphate buffer (pH 7.2) were incubated with the following radioactive ligands separately for 20 min at 37°C: [U-^{14}C]serine (165 mCi/mmol), 12.0 nmol; [U-^{14}C] glycine (108 mCi/mmol), 21 nmol; [U-^{14}C]aspartate (87 mCi/mmol), 22.9 nmol; [U-^{14}C]lysine (99 mCi/mmol), 21 nmol; and [U-^{14}C]tryptophan (46 mCi/mmol), 21 nmol. At the end of the incubation period, sodium borohydride (10 mM) was added and incubated for 20 min at 37°C. The enzyme was then precipitated with 10% TCA transferred on to a glass fiber filter and washed on a sampling manifold (Millipore) with 100 mL of 20, 10, and 5% TCA successively followed by ethanol:ether (1:1 v/v). The filter was dried and counted for radioactivity and the binding efficiency expressed as a percentage of the expected theoretical value. This was calculated on the rationale that the tetrameric enzyme (four active centers per mole of the enzyme) would facilitate the binding of 4 moles of the ligand per mole of the enzyme (assuming a stoichiometric reaction between ligand and enzyme).

Radiolabeled Amino Acid	Binding to Mung Bean SHMT	Binding to Sheep Liver SHMT
	%	%
Serine	80	7.4
Glycine	37	1.7
Aspartate	0.6	0.93
Lysine	4.6	0.13
Tryptophan	1.5	Not done

DISCUSSION

We present evidence in this study to indicate that PLP is not required for the activity of the mung bean SHMT, whereas this coenzyme was required for the activity of this enzyme from many other sources (19). Whereas the monkey liver enzyme (17) isolated in the absence of PLP had very little activity, the mung bean enzyme isolated in the presence and in the absence of PLP was fully active (Table I). Additional evidence for the non-involvement of PLP in the reaction catalyzed by mung bean SHMT was the absence of the characteristic PLP-Schiff's base spectrum in the mung bean enzyme (Fig. 1), and the effect of penicillamine and OADS on the *A* and fluorescence properties of the enzyme (Figs. 2, 3).

Riley and Snell (20) reported that histidine decarboxylase (EC 4.1.1.22) from mammalian sources was a PLP protein, whereas the enzyme from *Lactobacillus* 30.a.IV had a pyruvoyl moiety at the active site of the enzyme. Similarly, pyruvate has been shown to replace PLP in proline reductase (EC 1.4.4.1) (8, 9), uroconase (EC 4.2.1.49) (6), and *S*-adenosyl methionine decarboxylase (EC 4.1.1.50) (26). To explore the possible presence of a carbonyl group in mung bean SHMT, interaction of the enzyme with carbonyl reagents was examined. Phenylhydrazine, sodium borohydride, and hydroxylamine irreversibly and completely abolished the enzyme activity (Table III). Furthermore, the addition of PLP did not relieve the inhibition (Table III), confirming our suggestion that PLP probably had no role to play in the catalytic activity of mung bean SHMT, whereas in the case of sheep liver SHMT the inhibition was overcome by PLP. In addition, mung bean SHMT treated with phenylhydrazine showed an *A* peak at 315 nm (Fig. 4), whereas sheep liver SHMT showed an *A* peak at 410 nm (Fig. 4, inset), indicating the formation of a carbonylhydrazone (6, 20) and pyridoxalhydrazone (25), respectively. Results presented in Table IV showed that whereas mung bean SHMT could be labeled with the substrate/product serine and glycine efficiently, other amino acids were not covalently carried into the TCA precipitate. The sheep liver SHMT, as expected of a PLP protein, did not show significant labeling with any of the amino acids. This suggested that the carbonyl function was probably an integral part of the mung bean SHMT primary sequence.

The observations reported in this communication clearly point out that mung bean SHMT did not require PLP for its activity and instead a covalently linked carbonyl group probably replaced PLP involved in catalysis. It would be interesting to determine whether this is a common feature of plant enzymes or unique to mung bean SHMT.

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