cDNA cloning, overexpression in *Escherichia coli*, purification and characterization of sheep liver cytosolic serine hydroxymethyltransferase

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A sheep liver cDNA clone for the cytosolic serine hydroxymethyltransferase (SHMT) was isolated and its nucleotide sequence determined. The full-length cDNA of SHMT was placed under the control of T7 promoter in pET-3C plasmid and expressed in *Escherichia coli*. The overexpressed enzyme, present predominantly in the soluble fraction, was catalytically active. The recombinant SHMT was purified to homogeneity with a yield of 10 mg/l bacterial culture. The recombinant enzyme was capable of carrying out tetrahydrofolate-dependent and tetrahydrofolate-independent reactions as effectively as the native enzyme. The $K_m$ values for serine (1 mM) and tetrahydrofolate (0.82 mM) were similar to those of the native enzyme. The recombinant enzyme had a characteristic visible spectrum indicative of the presence of pyridoxal 5'-phosphate as an internal aldimine. The apoenzyme obtained upon removal of the cofactor was inactive and could be reconstituted by the addition of pyridoxal 5'-phosphate demonstrating that the recombinant SHMT was functionally very similar to the native SHMT. This overexpression of eukaryotic tetrameric SHMT in *E. coli* and the purification and characterization of the recombinant enzyme should thus allow studies on the role of specific amino acids and domains in the activity of the enzyme.

*Keywords.* Sheep serine hydroxymethyltransferase; cDNA clone; overexpression; recombinant enzyme.

Serine hydroxymethyltransferase (SHMT) is a pyridoxal-5'-phosphate(pyridoxal-P)-dependent enzyme catalyzing the reversible conversion of serine and tetrahydrofolate (H$_4$-folate) to glycine and 5,10-methylene-H$_4$-folate. This enzyme is a component of thymidylate cycle, along with thymidylate synthase and dihydrofolate reductase. The latter two enzymes are not only targets for cancer chemotherapy but also have served as model systems to study the structure/function relationships.

Earlier studies from our laboratory on the mechanism of interaction of substrates and substrate analogues with sheep liver SHMT has led to the design of active-site-directed inhibitors of SHMT [1–5]. The chemical modification studies with the sheep liver enzyme, indicated that histidine, cysteine and arginine residues were essential for activity [6], and the involvement of Arg269 in the binding of H$_4$-folate was demonstrated [7]. In spite of the successful isolation of the gene from several eukaryotic systems [8–12], the overexpression of this gene has not been reported. However, for understanding the structure/function relationship of the enzyme, it would be necessary to have an overexpressing clone of SHMT. In this communication, cloning, sequencing, and overexpression of the cytosolic sheep liver SHMT gene in *E. coli* is reported. We also describe the purification and characterization of the recombinant SHMT.

**EXPERIMENTAL PROCEDURES**

*Materials.* [$\alpha$-32P]dATP (3000 Ci/mmol), [$\alpha$-32P]dCTP (3000 Ci/mmol) were obtained from Board of Radiation and Isotope Technology, Unit of Department of Atomic Energy, Government of India, Bombay, India. [3$^5$S]dATP [$\alpha$-$S$] (600 Ci/mmol), 1-[3$^14$C]serine (55 mCi/mmol), were obtained from Amersham International. Restriction endonucleases, DNA-modifying enzymes were purchased from New England Biolabs, Perkin-Elmer Cetus and Amersham International. Cibacron Blue-agarose was from Bethesda Research Laboratories. CM-Sephadex C-50, Sephacryl S-200 were obtained from Pharmacia Fine Chemicals. All other biochemicals used in this study were obtained from Sigma Chemical Company. H$_4$-Folate was prepared by the method of Hatfeti et al. [13]. Sheep liver cDNA 5'stretch (SWAJ-2) library was purchased from Clonetech Laboratories, Inc. The oligonucleotide primers were synthesized by Oligonucleotide Synthesis Facility, Centre for Genetic Engineering, Indian Institute of Science, Bangalore, India and National Biosciences, Plymouth MN, USA.

*Bacterial strains and growth conditions.* *E. coli* strain DH5$a$ (Bethesda Research Laboratories) was the recipient for the plasmids used in subcloning and sequencing. Strain LE392 (obtained from Clonetech laboratories) was the recipient strain for plating the plasmid. BL21(DE3) [14] strain was used for the bacterial expression of pET-SHMT construct.
E. coli strains containing the plasmids were grown in Luria-Bertani medium or in terrific broth as described by Sambrook et al. [15].

**Plasmid and DNA isolation, DNA modifications and bacterial transformation.** Plasmid preparations were by the alkaline lysis procedure as described by Sambrook et al. [15]. DNA was isolated by the plate lysate method [15] with the following modifications. Before poly(ethylene glycol) precipitation, the phage particles was released upon proteinase-K treatment. Restriction endonuclease digestions, nuclease digestions and ligations were carried out according to the manufacturer’s instructions. The preparation of competent cells and transformation were carried out by the method of Alexander et al. [16].

**PCR amplification of SHMT gene-specific cDNA fragment.** Based on the amino acid sequence of sheep cytosolic SHMT determined by Usha et al. [17], two degenerate oligonucleotides DSH1 (5’-CAGAGRGTSGGCTGAGSACATCG-CYACGAGAAACTT-3’) and DSH2 (5’-GCGCTGAGGATTTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGGTTGGG
Protein sequencing. The recombinant SHMT was analyzed on SDS/PAGE [28], transferred to Immobilon™P membrane (Millipore), stained with Ponceau-S and the appropriate band was cut out. The cut membrane containing the protein was destained and loaded on to a Shimadzu gas-phase sequenator, (Millipore), stained with Ponceau-S and the appropriate band

RESULTS

Isolation of a cDNA clone for sheep liver SHMT. A cDNA fragment of 0.65 kb was amplified by PCR using primers DSH1 and DSH2. DSH1 has a partly degenerate sequence coding for the amino acids, QRDEELIASENFY, residues 43–55 in the sheep liver cytosolic SHMT [17], while DSH2 is also partly degenerate, codes for HVVVTTTKTLRG, residues 248–256 of the sheep liver SHMT. The PCR product was cloned in TpBluescript KS(+) vector. Plasmid (pPSH) containing the insert was identified and sequenced from both the ends. The predicted amino acid sequence encoded by insert DNA was homologous to the sheep cytosolic SHMT amino acid sequence [17]. The sheep liver cDNA library (70,000 plaques) was screened using the 650-bp PCR product and 10 positive clones were identified after three successive rounds of screening. The maximum size of the insert from all these clones was only 1.2 kb instead of the expected 1.45 kb for the full-length clone. The pPSH contained additional 135 bp at the 5‘ end compared to the 1.2-kb partial clones. This 135-bp fragment was used to screen the cDNA library (300,000 plaques) to identify full-length clones. 12 positive clones were identified after four successive rounds of screening and the inserts from these positive clones were in the range of 1.5–1.7 kb.

Subcloning and sequencing. The inserts were released from the λ clones by partial EcoRI and complete XbaI digestion and cloned in pBluescript at EcoRI and XbaI sites. The plasmid clones with inserts of more than 1.2 kb were selected. The clones pSH1, pSH2 and pSH8 of 1.5 kb and pSH11 (1.7 kb) were used for further characterization. ExoIII/S1 nuclease deletions were performed using pSH11 in both directions and all the deletion clones were sequenced. pSH11 has an open reading frame (ORF) with 1452 bp and a 20-bp 5‘ non-coding sequence, whereas, the pSH2 clone indicated the presence of a 26-bp non-coding sequence in addition to ORF. Sheep cDNA library was constructed by cloning the cDNA at EcoRI and XbaI sites of λSWA1-2 vector without protecting the internal XbaI sites. Due to the presence of an internal XbaI site at the stop codon, none of the clones contained the 3‘ non-coding sequence. The complete nucleotide sequence of sheep cytosolic SHMT obtained from pSH11 is shown in Fig. 1. The deduced amino acid sequence showed 98% identity with sheep liver cytosolic SHMT sequence [17], 90.5% identity with rabbit [10, 30] and human [11] SHMT.

Overexpression of sheep SHMT. The cDNA encoding SHMT, from Met1 through the TAG stop codon, was placed under the control of a strong T7 promoter (for details, see Experimental Procedures and Fig. 2). To avoid errors in the PCR amplification, we have used Vent DNA polymerase which has a 5–15-fold more efficient proof-reading activity compared with Taq polymerase [31, 32]. The bacterial cells containing the SHMT expression plasmid (PET-SHMT) were induced with isopropyl-1-thio-β-D-galactopyranoside for various time periods (0–5 h) and analyzed on SDS/PAGE. There was no further increase in the level of expression beyond 2 h of isopropyl-1-thio-β-D-galactopyranoside induction (data not shown). Densitometric scanning of the gel (Fig. 3) showed that 22% of the total cellular protein was due to the overexpression of SHMT. More than 90% of this protein was present in the soluble fraction.

Purification and characterization of the recombinant SHMT. The specific activity of the recombinant protein in the crude extract was 0.6 U (μmol · HCHO · min⁻¹ · mg⁻¹) compared with the value of 0.02 U in sheep liver a 0.03 U in E. coli cells [BL21(DE3) strain]. The recombinant enzyme was purified by ammonium sulfate fractionation, Cibacron Blue agarose chromatography and Sephacryl S-200 gel filtration (Table 1 and Fig. 3). This procedure resulted in a 5.5-fold purification and 27% recovery. The recombinant enzyme eluted on a Sephacryl S-200 column at the same position as the native enzyme suggesting that it is a tetramer like the native SHMT (data not shown). The enzyme was found to be homogeneous on SDS/PAGE (Fig. 3) and had a subunit molecular mass of 53 kDa. The single band obtained was transferred onto a poly(vinylidene difluoride) membrane and the N-terminus was sequenced. The N-terminal sequence of the first 14 amino acids, AAPVNKAPRDLW was identical to the deduced sheep cytosolic SHMT sequence (Fig. 1). The kinetic
characterization of recombinant SHMT revealed that the enzyme had a $K_m$ of 1 mM for serine, 0.82 mM for H$_4$-folate and a $V_{max}$ of 4.6 pmol HCHO min$^{-1}$ mg$^{-1}$ protein.

The enzyme had an absorbance maximum at 423.5 nm, a characteristic feature of pyridoxal-P present as an internal aldimine (Fig. 4). The bound pyridoxal-P could be removed by incubating the enzyme with D-alanine (200 mM) for 3 h at 37°C followed by dialysis to remove the pyridoxamine phosphate and pyruvate formed in the reaction. The apoenzyme had no spectrum in the visible region (Fig. 4) and was enzymically inactive. The apoenzyme could be reconstituted into the holoenzyme by the addition of pyridoxal-P (100 pM) as evidenced by the absorption spectrum with a maximum at 423.5 nm (Fig. 4) and the regaining of enzyme activity. The specific activity of the reconstituted enzyme was 3.0 pmol min$^{-1}$ mg$^{-1}$ compared with the value of 3.2 pmol min$^{-1}$ mg$^{-1}$ of the enzyme before removal of the pyridoxal-P.

**DISCUSSION**

Although extensive biochemical investigations on the mechanism of action of mammalian SHMT have been carried out previously [33, 34], a major limitation for a further understanding of the structure/function relationship has been the non-availability of an overexpressing clone of the eukaryotic SHMT. The results presented in this communication demonstrate the overexpression of an eukaryotic SHMT (from sheep liver) in E. coli. An SHMT overexpression vector (pET-SHMT) was constructed in which the sheep liver cytosolic SHMT encoding cDNA fragment was placed under the control of a strong T7 promoter, inducible by isopropyl-1-thio-p-D-galactopyranoside. The enzyme was not only expressed at high levels, i.e., 20-fold above the basal level in E. coli but was also present predominantly in the soluble fraction (Fig. 3). This protein constituted 22% of the total protein in the cell extracts. In contrast, the expression of rabbit liver cytosolic SHMT in COS-1 cells was only marginally above the basal level [10].

The recombinant enzyme was purified to homogeneity with a yield of 10 mg/l bacterial culture (Table 1). The native and subunit molecular mass of the recombinant enzyme was...
213 kDa and 53 kDa, respectively. Thus the recombinant SHMT is also a homotetramer like the cytosolic sheep SHMT [26]. However the recombinant enzyme from E. coli was shown to be a dimer [35, 36], whereas, the wild-type enzyme was a tetramer [37]. It would be interesting to examine the differences in the nature of subunit interactions that lead to different states of assembly of subunits in the E. coli and sheep liver recombinant enzymes. The N-terminal sequence of the recombinant enzyme was identical to the deduced sequence although, the N-terminal methionine was lacking. This is not surprising if we consider that the N-terminal methionine in the recombinant SHMT is followed by alanine. It has been observed that in 96% of the cytosolic E. coli proteins, in which the second residue was alanine, the N-terminal methionine was excised [38]. A comparison of the deduced amino acid sequence of sheep cytosolic SHMT clone with the primary structure of the sheep liver cytosolic SHMT [17] revealed that there were differences in the amino acid sequence at 10 positions as indicated in Fig. 1. Those differences might be attributable to the differences in the genetic variety of sheep used for preparing cDNA library and for the isolation of the enzyme. In addition a few residues (A37, P44, W111/F, V115/Y, G136/A, P171/A and R196/K were misidentified in the protein sequence and have now been corrected. Despite small sequence differences between the enzyme purified from sheep liver and the recombinant enzyme, we did not detect any differences in the properties of the two enzyme preparations. The expressed SHMT effectively interacted with antibodies raised against sheep liver cytosolic SHMT indicating that the antigenic epitopes are well retained in the recombinant enzyme (data not shown) The enzyme was capable of using serine or β-phenylserine as the substrate, demonstrating its ability to carry out H2-folate-dependent or independent reaction. The predicted secondary structure of the recombinant enzyme was identical to that of the native enzyme (data not shown). The purified recombinant enzyme had the characteristic visible absorption spectrum indicative of the presence of pyridoxal-P as an internal aldimine which could be removed and reconstituted in a manner similar to that of native sheep liver SHMT. The only difference in the properties of the recombinant enzyme and the native enzyme is the ability to bind CM-Sephadex which could have arisen from the absence of acetylation at the N-terminus or replacement of a few charged residues. The amino acid residues essential for activity are unchanged in the recombinant protein [7, 17]. The results presented in this study convincingly demonstrated that the recombinant SHMT was functionally identical to the native enzyme. The availability of a homogeneous and active recombinant SHMT in abundant amounts has laid the foundation for critical studies aimed at elucidating the role of the amino acid residues involved in the catalysis and binding of the substrates and substrate analogues.

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