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Properties of human and rabbit cytosolic serine hydroxymethyltransferase are changed by single nucleotide polymorphic mutations ☆

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Abstract

Serine hydroxymethyltransferase (SHMT) is a key enzyme in the formation and regulation of the folate one-carbon pool. Recent studies on human subjects have shown the existence of two single nucleotide polymorphisms that may be associated with several disease states. One of these mutations results in Ser394 being converted to an Asn (S394N) and the other in the change of Leu474 to a Phe (L474F). These mutations were introduced into the cDNA for both human and rabbit cytosolic SHMT and the mutant enzymes expressed and purified from an *Escherichia coli* expression system. The mutant enzymes show normal values for k_{cat} and K_m for serine. However, the S394N mutant enzyme has increased dissociation constant values for both glycine and tetrahydrofolate (tetrahydropteroylglutamate) and its pentaglutamate form compared to wild-type enzyme. The L474F mutant shows lowered affinity (increased dissociation constant) for only the pentaglutamate form of the folate ligand. Both mutations result in decreased rates of pyridoxal phosphate addition to the mutant apo enzymes to form the active holo enzymes. Neither mutation significantly affects the stability of SHMT or the rate at which it converts 5,10-methenyl tetrahydropteroyl pentaglutamate to 5-formyl tetrahydropteroyl pentaglutamate. Analysis of the structures of rabbit and human SHMT show how mutations at these two sites can result in the observed functional differences.

Keywords: Serine hydroxymethyltransferase; Gene polymorphism; Single nucleotide polymorphism; One-carbon metabolism; Tetrahydrofolate; B6 enzyme; Folic acid; Homocysteine; Folate binding affinity; Site-directed mutagenesis

Recent evidence shows that defects leading to mild elevations in blood hCys[1] levels are potential long-term risk factors for many pathogenic conditions [1–3]. Such defects may be associated with polymorphisms in key enzymes [4]. Currently, the connection between hyperhomocysteinemia and the polymorphism of genes encoding enzymes involved in one-carbon metabolism has been established in patients with neural tube defects, cardio vascular disease and pregnancy complications [5–7]. One of the genes investigated is serine hydroxymethyltransferase (SHMT; EC 2.1.2.1), which catalyzes the principal pathway for incorporation of one-carbon units into folate requiring one-carbon metabolism.

SHMT reversibly converts serine and $H_4PteGlu_n$ to glycine and 5,10-CH_2-$H_4PteGlu_n$ and plays a significant

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[1] *Abbreviations used:* hCys, homocysteine;SHMT, serine hydroxymethyltransferase; hcSHMT and rcSHMT refer to the human cytosolic and rabbit cytosolic enzymes; $H_4PteGlu_n$, tetrahydropteroylglutamate with n the number of γ-linked glutamyl residues; 5,10-CH^+-$H_4PteGlu_n$, 5,10-methenyl tetrahydropteroylglutamate; wt, wild-type SHMT; BES, *N*,*N*-bis(2-hydroxyethyl)-2-amino-ethanesulfonic acid.

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role in the biosynthesis of DNA, protein, and many other important cellular reactions, such as methylation of nucleic acids [2]. In mammals, SHMT is present in both the mitochondria (mSHMT) and cytoplasm (cSHMT), and the enzymes are encoded by separate genes [8,9]. Studies suggest that mSHMT is responsible for generating more than 90% of the one-carbon groups used in purine and thymidylate biosynthesis, whereas cSHMT functions mainly in the direction of serine synthesis [10–12]. It is believed that cSHMT is the key enzyme responsible for regulating and maintaining the homeostasis of the intracellular one-carbon pool [9]. A second reaction catalyzed by SHMT is the irreversible conversion of 5,10-CH^{+}-H_4PteGlu$_n$ to 5-CHO-H_4PteGlu$_n$ [13,14]. Studies strongly suggest that 5-CHO-H_4PteGlu$_n$ plays a regulatory role in folate homeostasis [15–17].

The 3-D structures of SHMT from many species, including *Escherichia coli*, human, rabbit, mouse, and *Bacillus stearothermophilus*, have been solved [18–22]. The enzyme exists as homodimers in prokaryotes and homotetramers in eukaryotes. To be a fully active enzyme, SHMT requires pyridoxal phosphate (PLP), which is tightly bound as an internal aldimine linkage to the ε-amino group of a lysyl residue in the active site of the enzyme [23,24]. Most of the SHMTs studied to date have the PLP binding stoichiometry of one per subunit, except an isoform found in *Mycobacterium tuberculosis*, which contains only one molecule of PLP per enzyme dimer [25]. *E. coli* SHMT forms a tight homodimer with the active site at the interface of two monomers. The tetrameric cSHMT has four active sites and is best described as a dimer of obligate dimers [20,21]. Each monomeric subunit within the obligate dimer contributes catalytically important amino acid residues to the active site [21,26].

Currently, two variations have been found by single strand conformational polymorphism in the coding region of the gene coding for human cSHMT [27]. The first one, the G1181A transition is located in exon 12 and leads to a serine to asparagine substitution at position 394 (S394N). There is only one report regarding the single nucleotide polymorphism at this position, which was heterozygously detected in one neural tube defect patient. The second variation, C1420T, is located in exon 13 and results in an amino acid substitution of leucine to phenylalanine at position 474 of the protein (L474F). The variant T allele of the 1420C > T polymorphism in hcSHMT was reported to associate with a protective effect in neural tube defect mothers and malignant lymphoma patients [28–31]. Individuals with the 1420CC genotype showed significantly lowered plasma folate and higher hCys levels than the 1420CT/TT genotype in mothers of children with neural tube defects [27]. However, none of these studies addressed the question of how the variant genotype affects the properties of hcSHMT structurally and functionally, although the need for additional studies on the properties of these mutant enzymes was suggested [27]. The lack of functional analysis on the hcSHMT polymorphic enzymes could be the result of the difficulty of being able to directly measure hcSHMT activity present in clinical samples, due to the low intracellular concentration of hcSHMT in white cells, and in separating the activity of the cytosolic isoform from the mitochondrial isoform. It is obvious that additional studies are required before any conclusion can be made for the possible role played by hcSHMT in controlling hCys metabolism and in the etiology of other diseases. There is a critical need to establish whether a polymorphism in hcSHMT results in any functional change in vitro before initiating clinical studies for epidemiological screening for the polymorphic genotypes or pharmacologic treatment, since both studies are laborious and costly. It is difficult to predict whether a mutation will alter function without biochemical analysis of the mutant protein.

In the present paper, we report the biochemical properties of these two polymorphic mutations in rabbit and human cSHMTs. Both hcSHMT and rcSHMT[2] C1420T and G1181A mutants were constructed by site-directed mutagenesis methods and the mutant enzymes purified and analyzed for effects on catalytic activity, stability, and substrate and coenzyme affinity. We found that the human L474F mutant has slightly increased affinity for glycine, but reduced affinity for 5-CHO-H_4PteGlu$_5$. Surprisingly, the substitution of Ser394 with Asn significantly decreases the binding affinity of glycine and folates without altering the activity and thermostability of both hcSHMT and rcSHMT. However, both mutants have significantly slower rates for binding PLP in converting the apoenzyme to the active holoenzyme. Structures of the wt enzymes are analyzed for how the two mutations might cause the observed differences in properties.

Materials and methods

Materials

All coenzymes, buffers, amino acids, column chromatography supports, reagents for bacterial growth, and ultra pure urea were purchased from either Sigma–Aldrich or Fisher. (6S) H_4PteGlu was a generous gift from Eprova AG (Schaffhausen, Switzerland). 6(RS)

[2] The numbering system from the DNA sequence for rabbit cSHMT shows that S394 is the same as human cSHMT, but because the rabbit enzyme has been shown by amino acid sequence analysis to be missing the first methionine the numbering according to a previously published account would be Ser393 [59]. A one amino acid insert in the rabbit enzyme results in Leu474 in hcSHMT being at position 475 in rcSHMT. However, for sake of clarity we will refer to both the human and rabbit mutations as L474F.