Purification, Kinetic Properties, and cDNA Cloning of Mammalian Betaine-Homocysteine Methyltransferase*

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Porcine liver betaine-homocysteine methyltransferase (BHMT; EC 2.1.1.5) was purified to homogeneity, and the Michaelis constants for betaine, dimethylacetothetin, and L-homocysteine are 23, 155, and 32 μM, respectively. The maximum rate of catalysis is 47-fold greater using dimethylacetothetin as a methyl donor compared with betaine. Partial amino acid sequence of porcine BHMT was obtained, and inosine-containing redundant oligonucleotide primers were used to amplify an 815-base pair sequence of the porcine cDNA by polymerase chain reaction (PCR). Nondegenerate oligonucleotide primers based on the porcine cDNA were synthesized and used to isolate a 463-base pair fragment of the human cDNA by PCR. The human PCR DNA product was then used to screen a cDNA library by plaque hybridization, and cDNAs encoding human BHMT were isolated. The primary structure of the human cDNA is reported here, and the open reading frame encodes a 406-residue protein of M, 44,969. The deduced amino acid sequence of human BHMT shows limited homology to bacterial vitamin B12-dependent methionine synthases (EC 2.1.1.13). A plasmid containing the human BHMT cDNA fused in frame to the N terminus of β-galactosidase was transformed into *Escherichia coli*, and transformants expressed BHMT activity, an activity that is absent from wild type *E. coli*.

There is considerable interest in the regulation of homocysteine (Hcy) metabolism since even moderate elevations in plasma total homocyst(e)ine (pHcy) have been established as an independent risk factor for the development of arteriosclerotic vascular disease (1). The enzyme betaine-homocysteine methyltransferase (BHMT; EC 2.1.1.5) may have an important role in the modulation of pHcy. BHMT is a cytosolic enzyme that catalyzes the conversion of betaine and Hcy to dimethylglycine and methionine, respectively (2). This reaction also is required for the irreversible oxidation of choline. The only other enzyme known to methylate Hcy in mammalian cells is the folate/vitamin B12-dependent methionine synthase (MS; EC 2.1.1.13).

Large oral doses of betaine have been shown to be an effective treatment for homocystinuria due to deficiencies of cystathionine β-synthase (EC 4.2.1.22) and methylenetetrahydrofolate reductase (EC 1.7.99.5) and inborn errors of cobalamin metabolism (3, 4). The level of pHcy in these individuals decreases significantly with betaine treatment, and the incidence of thromboembolism is significantly reduced. The efficacy of betaine treatment is due, at least in part, to increased methylation of Hcy by the BHMT-catalyzed reaction. Betaine treatment, however, usually does not lower pHcy levels to within the normal range, and the moderately elevated levels that remain are highly correlated with vascular disease. BHMT is therefore a target for the treatment of homocystinuria in that better methyl donors may have more potent pHcy-lowering effects. Furthermore, BHMT activity has been shown to vary with dietary and hormonal treatments in rats (5-7) and dietary treatments in chickens.2 Manipulations of these parameters could improve current therapies for some forms of homocystinuria.

A genetic defect in BHMT could result in hyperhomocyst(e)inemia, and perhaps homocystinuria, since it has been reported that this enzyme is responsible for up to 50% of the Hcy methylation capacity in liver (8). This possibility has been difficult to determine, since fibroblasts and lymphocytes, tissues normally assayed to detect enzyme defects, do not express BHMT activity (9). The lack of a cDNA encoding BHMT has precluded investigations into the molecular mechanisms responsible for dietary and hormonally induced changes in BHMT activity. A human cDNA encoding BHMT would aid in the search for defects in its gene.

This report describes the purification and kinetic constants of porcine liver BHMT as well as the molecular cloning of a partial porcine cDNA and a near full-length human cDNA encoding this enzyme. A fusion construct of the human cDNA with that of *E. coli* MS indicates regions that may be involved in Hcy binding and catalysis of methyl transfer.

EXPERIMENTAL PROCEDURES

Materials—[methyl-14C]Choline chloride (55 mCi/mmol; 200 μCi/ml), [α-32P]dCTP (3000 Ci/mmol; 10 μCi/ml), and random primer DNA labeling kit were obtained from Amersham Corp. Betaine hydrochlo-

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1 The abbreviations used are: Hcy, homocysteine; BHMT, betaine-homocysteine methyltransferase; DMAT, dimethylacetothetin; MS, methionine synthase (vitamin B12-dependent); pHcy, plasma total homocyst(e)ine, denoting oxidized and reduced forms; PCR, polymerase chain reaction; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl)methyl)glycine.

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ride, choline chloride, choline oxidase, Amberlite CG-50, and Dowex 1 were purchased from Sigma. Hydroxyapatite (Bio-Rad) and DEAE cellulose were purchased from Bio-Rad and Whatman, respectively. All other reagents were of the highest purity commercially available. Porcine livers were generously supplied by the Meat Science Laboratory at the University of Illinois, Urbana.

*Bacteria, Bacteriophage, and Plasmids—*E. coli* strains XL-1-Blue MRF' and SOLR were obtained from Stratagene, and strain C600 was purchased from Sintech. Porcine liver cDNAs were purchased from Clontech (ρ101) and Stratagene (λ ZAP XR), respectively. Libraries were titered according to manufacturers' protocols. PCR fragments were ligated into plasmid pCR II and transformed into *E. coli* strain INVdF' (Invitrogen). Human cDNAs were isolated in plBluescript SK+ using the automatic subcloning feature of λzAP. DNA sequencing of 3' end was accomplished by converting 3'-hydroxyl group to 3'-methyl group. Isolation of cDNAs—BHMT assay—BHMT activity was measured as described by Finkelstein and Mudd (13) with several modifications. DL-Hcy was prepared by Method of Bradlow (16) using bovine serum albumin as standard. BHMT purity was estimated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate using a 5% stacking gel and a 12% separation gel. The discontinuous buffer system of Laemmli (17) was used in a miniprotein II slab gel apparatus (Bio-Rad). Protein was visualized by a Coomassie staining procedure.

**Purification of Porcine Liver BHMT and Derived Peptides—**BHMT (4 μg) was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis as described above. The protein was blotted onto a polyvinylidene difluoride membrane using a Tris-glycine transfer buffer in a mini-trans-blot cell (Bio-Rad) following the manufacturer's suggested protocol. The protein was visualized by Coomassie staining, and the band was excised and stored at −20°C until sequenced. Amino acid sequence was determined using an Applied Biosystems 477A protein sequencer at the Biotechnology Center of University of Illinois, Urbana.

The following procedure was used to generate peptide fragments of porcine BHMT. An aliquot of protein (500 μg) was dialyzed against 5 mM ammonium bicarbonate (1 liter) and lyophilized to dryness. The sample was resuspended in 200 μl of 10% acetic acid and heated at 50°C for 72 h. The sample was neutralized with triethylammonium acetate and lyophilized. A portion of the sample (25 μg) was dissolved in sample buffer, and peptides were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis using a 4% stacking gel and a 15% separation gel. The Tris-Tricine buffer system of Schagger and Von Jagow was used (18). The peptides were transferred onto a polyvinylidene difluoride membrane and visualized by Coomassie staining. The bands were excised and stored at −20°C until sequenced as described above.

**Isolation of cDNAs—**The following redundant inosine-containing oligonucleotide primers were designed from the N-terminal and internal amino acid sequence of porcine liver BHMT, respectively: 5'-GCCICGTTGGIGGAG/TC/A/AA/GAA/A/G/GC-3' (sense) and 5'-GG(TC/TC/T/ C/A/G/A/I/C/CA/GA/A/G/G/GC-3' (antisense). These primers were isole to a portion of the BHMT-encoding porcine cDNA by PCR. The final concentrations of the PCR reaction mixture were as follows: 10 mM Tris-HCl (pH 8.3), 50 mM potassium chloride, 0.5 μM each primer, 200 μM each dNTP, 2.5 mM magnesium chloride, and 12.5 × 10−6 Agt10 bacteriophage containing porcine liver cDNA. Three units of Taq polymerase (Perkin-Elmer) was used in a final volume of 100 μl. The first cycle used a 5-min denaturation step (97°C), a 1-min annealing step (50°C), and a 2-min elongation step (72°C). This sequence was followed by 28 cycles of 94°C, 50°C, and 72°C, for the denaturation, annealing, and elongation steps (1 min each, respectively). A final cycle used an elongation step of 10 min to complete any unfinished products. A single PCR product of approximately 800 base pairs was observed on 1% agarose gel and ligated into pCR II (Invitrogen). Both strands of the insert were sequenced.

The following nonredundant oligonucleotide primers were synthesized based on nucleotides 205-227 and 646–667 of the pig cDNA (see Fig. 2), respectively: 5'-GTCAGCAGGACCTCTCACC/TA-TCTCTTA-3' (sense) and 5'-TAATTGTTGGGTCAAAAATGCGA-3' (antisense). These primers were used to isolate a portion of the BHMT-encoding human cDNA by PCR. The template for PCR was a human cDNA library in AZAP XR (7 × 106 clones). PCR reactions were carried out as described above except that the annealing temperature was 55°C and there were a total of 35 cycles performed. One PCR product of approximately 500 base
pairs was observed on a 1.2% agarose gel. This fragment was ligated into pCRII, and both strands of the insert were sequenced.

The human liver cDNA library (AZAP XR) was then plated, and the bacteriophage plaques were lifted onto nylon membranes (DuPont, Hybond-N). The membranes were screened as described by the manufacturer's protocol provided with the library. The hybridization probe was made from the human PCR fragment (described above) that was labeled with [α-32P]dCTP by a random primer extension procedure. A total of 2,200 plaques were screened.

Primers were synthesized at the Biotechnology Center of the University of Illinois, Urbana. DNA sequencing was performed using an Applied Biosystems 373A automated DNA sequencer at the same facility.

RESULTS

Purification and Kinetic Constants of Porcine Liver BHMT—BHMT was purified by heat-treating a crude extract followed by chromatography on hydroxyapatite and DEAE-cellulose (Table I). This procedure results in a preparation that displays a single band of Mr 45,000 on a sodium dodecyl sulfate-containing polyacrylamide gel (Fig. 1). The Vmax of porcine liver BHMT using the standard assay is very low (4 μmol h⁻¹ mg⁻¹).

The kinetic constants of betaine, DMAT, and l-Hcy for the porcine liver enzyme were determined from initial rate data. The Michaelis constants were 23, 155, and 32 μM for betaine, DMAT, and l-Hcy, respectively. BHMT displayed a 47-fold increase in Vmax and a 7-fold increase in Vmax/Km when DMAT was the methyl donor relative to when betaine was used (Table II). The Vmax of BHMT using the standard assay (4160 units/mg) was about 2-fold higher than that obtained using the kinetic assay, whether l-Hcy (2036 units/mg) or betaine (1997 units/mg) was the varied substrate. The highest concentration of variable substrate used in all kinetic analyses ended up being slightly over 5 times the final Km, determination. As with the rat enzyme (19), no indication of substrate inhibition was observed, and reducing agents had no effect on Vmax.

The possibility of substrate activation at levels higher than 5 times the Km value reported here needs to be investigated.

Preliminary kinetic analysis indicated that the Km for D.-Hcy was exactly twice that of L-Hcy, suggesting that D-Hcy is not a substrate and has very low affinity for BHMT. Activity was then measured using 100 μM D-Hcy and/or 100 μM L-Hcy. The level of betaine was saturating (2 mM), and the ionic strength was held constant to the standard assay. The results indicated that porcine BHMT can methylate D-Hcy at about 15% the rate observed for L-Hcy. When BHMT was assayed with 100 μM of both D-Hcy and l-Hcy, the rate of methionine production was 95% of that observed with l-Hcy alone. These data indicate that D-Hcy has very low affinity for the enzyme and that L-Hcy is preferentially methylated when a mixture of the enantiomers are used (not shown).

Porcine liver BHMT is inhibited by dimethylglycine and the demethylated product of DMAT, methylthioacetate, but not sarcosine (Table IV).

Amino Acid Sequencing of Porcine Liver BHMT—The N-terminal amino acid sequence of porcine BHMT was determined to be APVGDKKAKKGLERLNGEVT. Treatment with dilute acid (partially) cleaved the protein into five major and several minor peptides that could be separated on a polyacrylamide gel containing sodium dodecyl sulfate (not shown). This procedure has been reported to cleave at Asp-Pro sequences. Two peptides were sequenced and were subsequently discovered to be partially overlapping: XGKQGFIDLP and LPEFPPGLEPRVATR. These porcine BHMT peptide sequences were found in the deduced amino acid sequence of the porcine cDNA (Fig. 2), and nearly identical sequences are present in the human enzyme (residues 22–22 and 257–273; Fig. 3). The first internal peptide sequence was preceded by Pro-Asp in the deduced amino acid sequences of both porcine and human cDNAs. The second internal peptide was preceded by an Asp residue that was part of an Asp-Leu-Pro sequence, also in the deduced sequences of both porcine and human cDNAs.

Nucleotide and Deduced Amino Acid Sequence of BHMT cDNAs—Redundant inosine-containing oligonucleotide primers, based on porcine amino acid sequence, were used to amplify an 815-base pair cDNA fragment encoding a portion of porcine BHMT by PCR (Fig. 3). The deduced amino acid sequence of this cDNA fragment contained all the amino acid residues that were identified from peptide sequencing. The

TABLE I

| Fraction       | Volume (ml) | Activity (units/mL) | Protein (mg/mL) | Specific Activity (units/mg) | Purification | Yield (%) |
|----------------|-------------|---------------------|-----------------|-----------------------------|--------------|-----------|
| 1. Crude       | 25          | 2900                | 33              | 88                          | 1            | 100       |
| 2. Heat-treated| 17          | 3100                | 4.5             | 689                         | 8            | 73        |
| 3. Hydroxyapatite| 52      | 688                 | 0.34            | 2020                        | 23           | 49        |
| 4. DEAE-cellulose| 15          | 790                 | 0.19            | 4160                        | 47           | 16        |

* Units are nmol of methionine formed per hour.

FIG. 1. Sodium dodecyl sulfate gel electrophoresis of porcine liver BHMT after chromatography on DEAE-cellulose. Experimental details are described under “Experimental Procedures.” Protein was visualized by a Coomassie staining procedure. The electrophoretic mobilities of standards can be seen in lane 1. The standards are as follows: bovine serum albumin (66 kDa), egg albumin (45 kDa), glycer-aldehyde-3-phosphate dehydrogenase (36 kDa), bovine carbonic anhydrase (29 kDa), bovine pancreas trypsinogen (24 kDa), and soybean trypsin inhibitor (20 kDa). Lane 2 shows DEAE-cellulose-purified porcine liver BHMT (2 μg).

TABLE II

| Substrate | Km (μM) | Vmax (rel) | Vmax/Km (rel) |
|-----------|---------|------------|---------------|
| Betaine   | 23      | 1          | 1             |
| DMAT      | 155     | 47         | 7             |

* Values are relative to those obtained with betaine as substrate. For Vmax (rel), 1 equals 2 μmol h⁻¹ mg⁻¹ (kcat = 0.025 s⁻¹). For Vmax/Km (rel), 1 equals 1090 s⁻¹ M⁻¹.
The 5'-untranslated region and open reading frame of pTG9 was in frame with the N-terminal portion of the β-galactosidase sequence present in the pBluescript (SK−) vector. Crude extracts of E. coli strain SOLR containing pTG9 expressed BHMT activity, whereas cells transformed with vector alone had no activity (Table III). These data confirm that the cDNA insert in pTG9 encodes human BHMT.

**Homology of Human BHMT with Bacterial Vitamin B12-dependent MS**—A comparison of the deduced amino acid sequence of human BHMT with other sequences using BLASTP revealed regions of homology with several bacterial vitamin B12-dependent MS proteins. Six amino acid alignments between human BHMT and E. coli MS can be seen in Fig. 4. The E. coli enzyme has been the most extensively characterized and is therefore the only one shown for clarity. MS is a large protein (approximately 1200 residues) composed of two distinct domains identified by trypsin sensitivity (20).

Figments B and D align to the C-terminal domain of MS (38 kDa). Fragment B aligns to the N-terminal portion of this domain, an area that also resembles a methyltransferase from Clostridium thermoaceticum (21) that catalyzes a methyl transfer from 5-methyltetrahydrofolate to the cobalt center of a corrinoid/iron-sulfur protein (alignment not shown). Fragment D aligns to the C-terminal portion of this domain, an area that is responsible for binding S-adenosylmethionine (20), a cofactor required for the reductive activation of MS. This alignment is significant, since both S-adenosylmethionine and S-adenosylhomocysteine have been reported to inhibit BHMT activity (22, 23).

Figments A, C, E, and F align to the N-terminal domain of E. coli MS. The N-terminal domain (98 kDa) of E. coli MS is not as well characterized as the C-terminal domain. However, this domain must maintain the determinants for Hcy-binding and also the residues required for catalysis of methyl transfer, since it retains 70% of the activity of the holoenzyme (20). The N-terminal domain, however, can no longer be reductively activated by S-adenosylmethionine once the cobalamin is oxidized to the cob(II)alamin state. Although BHMT fragments C and E display some homology to E. coli MS, these alignments do not correspond to residues that are conserved among other MS proteins (not shown). Alignments A and F match the highly conserved regions of PXXXHHXXXGADGXXEYTF and GGCCTGTPXHI in MS proteins and therefore could be significant for Hcy binding and catalysis of methyl transfer.

**DISCUSSION**

BHMT is found primarily in the liver and kidney of mammals (2, 5, 24, 25), and it has been purified to homogeneity from rat (26, 27), horse (28), and human (29) liver. A preparation of the enzyme from porcine liver has been reported that was estimated to be 90% pure (30). These reports consistently show that liver expresses very high levels of this enzyme, ranging from 0.6 to 1.6% of the total soluble protein in crude extracts, and that the enzyme is a hexamer of identical subunits of Mr 45,000.

The porcine liver BHMT purification reported here enriched activity 47-fold, with a final yield of 16%. The preparation was judged to be homogenous after sodium dodecyl sulfate polyacrylamide gel electrophoresis yielding a single band of Mr 45,000 (Fig. 1). The enrichment required to obtain homogenous enzyme varied from 47- to 98-fold. The low specific activity of the purified enzyme is similar to that reported for rat (27) and human (29) liver BHMT. The specific activity of purified BHMT is also similar to that of E. coli vitamin B12-dependent methionine synthase (EC 2.1.1), another transferase that translocates a nitrogen-bound methyl group to Hcy (31).
The $K_m$ estimates for betaine and l-Hcy are similar in magnitude to previous reports on the rat and human enzymes (19, 27, 29). The $K_m$ estimate for l-Hcy should be considered a maximum estimate. Although dithiothreitol was used to slow the oxidation of Hcy, no effort was made to correct for loss of substrate due to disulfide formation. Furthermore, the hydroxide-dependent conversion of Hcy thiolactone to Hcy is not quantitative (14).

The level of Hcy in rat and mouse liver is approximately 4 mM (32); therefore, it is likely that liver Hcy levels are lower than the $K_m$ of Hcy for BHMT. As with Hcy, the intracellular concentration of betaine has been determined only for the rodent

**Fig. 3.** Nucleotide sequence of a human liver BHMT cDNA and derived amino acid sequence. Region amplified by PCR using nonredundant primers based on porcine sequence are doubly underlined.
The concentration has been shown to be dependent upon dietary choline and betaine and generally ranges from 1 to 10 \( \mu \text{mol/g} \) of liver. These levels are significantly higher than the \( K_a \) of betaine for porcine BHMT (23 \( \mu \text{M} \)) and the \( K_a \) of betaine reported for the rat and human enzymes (48 and 100 \( \mu \text{M} \), respectively). These data indicate that rat liver BHMT is saturated with betaine, although this may not be true for all species. For example, choline oxidase, the first committed reaction for choline oxidation, is reportedly 17–60-fold higher in rat liver than human liver (37, 38); therefore, humans probably have significantly lower levels of hepatic betaine. The fact that choline or betaine treatment for homocystinuria lowers pHcy supports the idea that BHMT is not normally saturated with betaine in human liver.

The higher \( V_{\text{max}} \) obtained for the methylation of Hcy using DMAT relative to betaine is consistent with previous reports using the purified horse (28) and porcine (39) liver enzymes. These studies used saturating concentrations of both Hcy and methyl donor and measured methionine production by either colorimetric or microbiological assays. It was found that the methylation rate of Hcy using DMAT was 1–2 orders of magnitude greater than that observed using betaine. Comparison of the two substrates using crude rat liver preparations indicated that DMAT was 20 times more effective than betaine at stimulating the production of methionine (40–42).

Dimethylglycine has been reported to be a potent inhibitor of porcine (39), rat (19), and human BHMT (29, 43), although a \( K_a \) has never been reported. This report compared the relative affinities of dimethylglycine, methylthioacetate, and sarcosine for porcine BHMT (Table IV). Methylthioacetate, the demethylated product of DMAT, has a lower affinity for BHMT than dimethylglycine. Sarcosine, an isostere of methylthioacetate, displays no affinity for the enzyme under the assay conditions used. The lower affinity methylthioacetate has for BHMT partly explains the higher \( V_{\text{max}} \) of BHMT when DMAT is used instead of betaine as methyl donor, since the maximum velocity of the reaction is in part a function of the off rates of the products.

Betaine is presently used as an effective treatment for vitamin-nonresponsive homocystinuria. This treatment significantly reduces pHcy and improves clinical prognosis. Two different mechanisms likely contribute to the pHcy-lowering effect of betaine. The first is the direct methylation of Hcy by the BHMT-catalyzed reaction. A second effect could be mediated by the subsequent oxidation of dimethylglycine to glycine, catalyzed by dimethylglycine dehydrogenase (EC 1.5.99.2) and sarcosine dehydrogenase (EC 1.5.99.1), respectively. These reactions introduce one carbon unit into the folate pool as \( N^5,N^10 \)-methyleneetetrahydrofolate. This anaplerotic effect could enhance the folate-dependent pathway of Hcy methylation in some forms of homocystinuria. Although the use of betaine in the treatment of homocystinuria elicits a pHcy-lowering response, Allen et al. (43) have pointed out that this treatment usually does not lower pHcy to within the normal range (10–15 \( \text{mm} \)). The hyperhomocyst(e)inemia that persists, generally 30–80 \( \mu \text{mol} \), is significantly correlated with vascular disease.

The inability of betaine treatment to normalize pHcy in homocystinurics is likely related to the kinetic properties of the BHMT reaction. It has been shown that concomitant with a decrease in pHcy during betaine treatment, there is also a dramatic increase in plasma (43) and urinary (44) betaine and dimethylglycine. Up to 37-fold increases in plasma dimethylglycine (250 \( \mu \text{M} \)) were observed in individuals receiving betaine for homocystinuria compared with a control population. These data indicate that the subsequent oxidation of dimethylglycine is insufficient and therefore accumulates in tissues. Kinetic studies using the purified porcine enzyme indicate that dimethylglycine inhibits BHMT activity uncompetitively when Hcy is varied at either subsaturating (25 \( \mu \text{M} \)) or saturating (250 \( \mu \text{M} \)) levels of betaine. It is likely that liver BHMT is inhibited by dimethylglycine in vivo.

This report indicates that DMAT is a more specific substrate for BHMT than betaine and that the demethylated product of DMAT, methylthioacetate, has lower affinity for BHMT than dimethylglycine. DMAT may be useful for the treatment of homocystinuria. Early work initiated by Maw and Du Vigneaud (45) indicated that DMAT can replace choline (or betaine) in rodent diets devoid of methionine but containing homocysteine. DMAT supported growth and prevented fatty liver and renal hemorrhage. Methyl donor-deficient diets have been shown repeatedly to result in growth depression, fatty liver, and, when given to weaning animals, renal hemorrhage.
The subsequent metabolic fate of methylhydroxacetate is not completely understood. However, when rats were given 60 mg of DMAT by either diet or subcutaneous injection, over 50% of the sulfur from this compound was found as sulfate in the urine when measured after 24 h (46). There have been no reports on DMAT transport or renal clearance. Although previous studies were relatively short term (3 weeks), it seems that DMAT is nontoxic to animals at levels that might be efficacious in the treatment of homocystinuria. It should be pointed out that a homologue of DMAT, dimethylpropiothetin, is also a substrate for BHMT (28, 39–41). The rate of methionine formation using dimethylpropiothetin was consistently shown to be between those of betaine and DMAT when assayed at saturating concentrations of substrates. Dimethylpropiothetin is a naturally occurring compound (47), and its demethylated product, methylthiopropionate, is an intermediate in the transamination pathway of methionine catabolism (48). Dimethylpropiothetin can also replace choline (or betaine) in rodent diets devoid of methionine but containing homocysteine (45). Further studies on the metabolism and toxicity of DMAT and dimethylpropiothetin are warranted, since these compounds may improve the nutritional management of homocystinuria. The recent generation of transgenic mice deficient in cystathionine β-synthase will be useful for the comparison of betaine versus thetin as pHcy-lowering treatments for homocystinuria (49).

An important objective of this research was to isolate a cDNA encoding a mammalian BHMT. Toward this end, porcine BHMT was sequenced, and oligonucleotide primers were designed and subsequently used to isolate a partial cDNA encoding porcine BHMT by PCR. This partial cDNA encoded 272 amino acids of porcine BHMT (Fig. 2). Nonredundant primers based on the porcine cDNA were then used to isolate a partial cDNA encoding human BHMT. This partial cDNA encoded 154 amino acids of human BHMT (Fig. 3). The human PCR fragment was used to screen a cDNA library by plaque hybridization, and 12 positive clones were isolated that had similar restriction maps. The BHMT cDNAs represented about 0.5% of the total number of clones in the library, consistent with the high expression of this protein in liver. The vector containing the longest cDNA insert (pTG9) was sequenced (Fig. 3), and the open reading frame encoded a 406-residue protein of M, 44,969. An alignment of the porcine and human deduced sequences displayed 94% amino acid identity over 272 amino acids (Fig. 2). The deduced human protein is similar in size to the porcine liver enzyme (Fig. 1) and the enzymes isolated from human (29) and rat (27) liver. The N terminus of the intact porcine protein begins with an Ala, and this residue aligns with the second amino acid of the deduced human sequence. Since BHMT is a cytosolic protein, it is unlikely that a large signaling peptide is posttranslationally removed, and therefore, the 2.4-kilobase posttranslationally removed, and therefore, the 2.4-kilobase

The possession of DNA sequence encoding BHMT provides the opportunity to screen for mutations in the BHMT gene. The effects of missense mutations on enzyme catalysis can easily be evaluated, since the enzyme can be expressed in E. coli. Furthermore, the specificity of the human enzyme toward their substrates can be evaluated, and more detailed studies on the mechanism of the BHMT reaction can proceed. The human cDNA will also allow studies concerning the regulation of expression of this enzyme by diet and hormones.

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Betaine-Homocysteine Methyltransferase

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