Chromosomal Localization and Catalytic Properties of the Recombinant α Subunit of Human Lymphocyte Methionine Adenosyltransferase*

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Human lymphocyte methionine adenosyltransferase (HuLy MAT) consists of heterologous subunits α and β. The cDNA sequence of the α subunit of HuLy MAT from Jurkat leukemic T cells was identical to that of the human kidney α subunit and highly homologous to the sequence of the extrahepatic MAT from other sources. The 3′-untranslated sequence was found to be highly conserved, suggesting that it may be important in regulating the expression of MAT. The extrahepatic α subunit of MAT was found to be expressed also in human liver, and no differences were found in the sequence of the α subunit from normal and malignant T cells. The sequence of two unspliced introns found in the cDNA clones from the Jurkat library enabled us to isolate genomic clones harboring the human extrahepatic α subunit gene and to localize it to the centromere on chromosome arm 2p, an area that corresponds to band 2p11.2.

Expression of the α subunit cDNA in Escherichia coli yielded two peptides with the immunoreactivity and mobilities of authentic α/α′ subunits from HuLy. The Km of the recombinant α subunit was 80 μM, which is 20-fold higher than found for the α/α′ β, holoenzyme purified from leukemic lymphocytes and 4-10-fold higher than found for the rat liver enzyme. The data suggest that the α/α′ subunits mediate the enzyme catalytic activity and that the β subunit may be a regulatory subunit of extrahepatic MAT.

Methionine adenosyltransferase (MAT; 1 S-adenosyl-L-methionine (AdoMet) synthetase, EC 2.5.1.6) catalyzes the formation of AdoMet from ATP and L-methionine (1, 2). In mammals, two major MAT isozymes have been described which differ with respect to tissue distribution and kinetic properties (for review see Ref. 3). Liver contains an isozyme unique to this organ, which has a high Km for L-methionine (Sβ,g of 0.2–0.5 mM) and is relatively insensitive to product inhibition by AdoMet (4, 5). This isozyme has been studied most extensively in the rat, where it has been previously designated the high Km form (6, 7) or the β isozyme (8) but is now called MAT III (4). The rat liver MAT III isozyme has been purified to homogeneity and was found to exist as a homodimer of a single hepatic α subunit (αH) with a predicted mass of 43.7 kDa but which migrates on polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) as a 48-kDa protein (4, 9, 10, 41). The tetrameric form of this same subunit (αH)4, which is known as MAT I, has an “intermediate” Km for L-methionine (about 17–23 μM) (4–6, 11) and can be converted to the dimeric form by treatment with lithium bromide (10). Rat MAT I and MAT III can be separated easily by hydrophobic chromatography on phenyl-Sepharose columns (4). Although human liver expresses the mRNA that encodes the αH subunit (12, 13), which is highly homologous to the rat αH subunit (14), the existence of a MAT I and MAT III equivalent in human liver has yet to be determined. The rat and human liver-specific αH subunits are not expressed in extrahepatic tissues.

Extrahepatic tissues contain MAT isozymes that are very different from that of liver and are characterized by low Km values for L-methionine (3–20 μM) and by their strong feedback inhibition by AdoMet (15, 16). Such isozymes have been partially purified from rat kidney (4, 8), rat lens (17), and human erythrocytes (15, 18) and completely purified from human lymphocytes (19) and bovine brain (20). The human lymphocyte isozyme is strongly inhibited by AdoMet (15, 16, 19). The low Km, isozyme is expressed in all tissues including the liver (4, 7, 21). However, recent studies suggest that different tissues, or even cells from the same tissue at different stages of development, can express different forms of the extrahepatic enzyme (3, 4, 7, 21). In contrast to the rat liver isozymes that represent different polymeric states of the αH subunit (41), the purified enzyme from human leukemic lymphocytes and from bovine brain consists of heterologous subunits, known as α (53 kDa) and β (38 kDa) (19–22). In human T cells the α subunit appears to undergo some type of post-translational modification to yield a 51-kDa protein called α′ (19). MAT from leukemic T cells has a native molecular weight of 185,000 (19) and thus may have a possible subunit structure of α′/β2, but since the molar ratio of

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** The abbreviations used are: MAT, methionine adenosyltransferase; PAGE, polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; IPTG, isopropyl-β-D-thiogalactopyranoside; PHA, phytohemagglutinin A; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase(s); ORF, open reading frame; HuLy, human lymphocyte.
each subunit in the holoenzyme is not known, we will refer to a subunit and the liver as acute myelogenous leukemia cells, MAT activity is primarily the subunits of the holoenzyme are not known, we will refer to a specific activity of 1–2 × 10^3 cpm/mg DNA was performed by the method of Sanger et al. (32) with specific oligodeoxyribonucleotide primers and single-stranded templates derived from the holoenzyme, we hypothesized that differences may exist in the sequence of the a subunit of MAT from different tissues and that these differences may affect the association between the a and b subunits of extrahapatic MAT. The present study was undertaken to determine whether differences exist between: (i) MAT a subunit from different human extrahapatic tissues, namely kidney versus lymphocytes; and (ii) MAT a subunit from normal and malignant tissues. We report that the sequence of the a subunit from human kidney and normal or malignant lymphocytes is identical and that the gene for the a subunit of MAT is located on chromosome 2p11.2. We report also the expression of the a subunit in Escherichia coli and demonstrate that, although it is catalytically active in the absence of b subunit, its kinetics are different from the native a, b, form of MAT found in normal and malignant lymphocytes.

EXPERIMENTAL PROCEDURES

Strains and Media—The E. coli strains used were: NM522, which is supE thi Dhsd5 (r-m i lac proAB) [F' proAB lac-ZDM15]; Y1090, which is D(lac)U169 D(lan) araD139 strA supF trpC22: Tn10(pMC9); and strain XL-1 Blue (Stratagene), which is endA1 supE44 hsdR17 (r-m i lac) l recA1 yqA96 lac [F' proAB lac-ZDM15 Tn10 (tet')]. A phase derivatives were plated in top agar consisting of NZCYM (26) containing 0.7% agarose. Plasmid strains were grown on LB or 25 cm; particlesize, 5 mm; poresize, 252 Å; Rainin Instrument Co.), which was equilibrated with 0.1% (w/v) trifluoroacetic acid and 10% HPLC grade acetonitrile (v/v) at a flow rate of 330 Å.

Three subunits. A 260-μm formic acid, a single peak appeared at 42.8 min, which contained all tryptic peptides was achieved by HPLC. A lyophilized sample of purified MAT was dissolved in 70% formic acid and applied to a Vydac C4 column, which was equilibrated with 0.1% (w/v) trifluoroacetic acid and 10% HPLC grade acetonitrile (v/v) at a flow rate of 1% min⁻¹. After 10 min of isocratic perfusion, the acetonitrile concentration was increased at a linear rate of 1% min⁻¹ for the next 14 min and then at a rate of 0.25% min⁻¹ for the next 36 min. The eluate was monitored at 214 and 280 nm, and fractions of interest were lyophilized to dryness and analyzed by gas phase peptide sequencing (28).

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transcripts/μg of RNA. The reaction was incubated for 45 min at 37 °C and terminated by heating at 95 °C for 10 min followed by rapid chilling in ice water for 2 min. The α subunit cDNA was amplified in the presence of Taq polymerase (0.25 unit) using the following primers. For MAT α subunit the forward primer was 5′-GCC-GCT-CCT-CTC-TTA-G-3′, and the reverse primer was 5′-TAA-CTG-CCA-ACA-AGA-TGT-GAC-3′. Together these primers amplified the cDNA product covering the entire ORF of the α subunit cDNA. The amplified PCR product was purified and sequenced either directly by cycle sequencing using the fmol™ sequencing kit (Promega) or cloned into the TA cloning vector (pCR™ II Vector, Invitrogen Corp., San Diego) and subcloned into competent E. coli strain INvα (competent cells for sequencing of positive plasmids by the dideoxy termination method using the Sequenase II kit (Upstate Biotechnology, Inc.). The sequences were compared and aligned to previously derived MAT gene sequences using GenBank.

Genomic Cloning and Chromosomal Localization of Human MAT α Subunit Gene—PCR primers were chosen to amplify genomic DNA from position 102 of intron B, which was found in the J urkat library, to position 824 of the coding sequence. The sequence of the forward primer was 5′-GGA-GTA-TTG-CTG-AAT-GA-3′, and that of the reverse primer was 5′-ACC-CCA-GCC-AGG-ATA-GT-3′; together these primers amplified a 147-bp product. The primers were used to screen a human P1 genomic library (Genome Systems Inc. St. Louis, MO). The library consisted of 50,000 clones arranged in 26 pools of 2,000 clones each. The positive clones were sequenced to ensure that they harbored the α subunit gene. Highly purified DNA from one of the positive clones was used to probe human chromosomes for the α subunit gene. Purified DNA from P1 clone 2007 was labeled with digoxigenin dUTP by nick translation. Labeled probe was combined with sheared human DNA and hybridized to normal metaphase chromosomes derived from PHA-stimulated peripheral blood lymphocytes in a solution containing 50% formamide, 10% dextran sulfate, and 2× SSC. Specific hybridization signals were detected by incubating the hybridized slides in fluorescein-conjugated antidigoxigenin antibodies. The chromosomes were counterstained with propidium iodide and analyzed.

Other Methods—Bacterial cell extracts were prepared by suspending cell paste in 2–4 volumes of ice-cold 0.1 M Tris-HCl, pH 7.6, and disrupting them by sonication. Cellular debris was removed by centrifugation for 30 min at 20,000 × g, and supernatants were stored at −70 °C. MAT activity was determined as described previously (19) with varying concentrations of L-[3H]methionine. One unit of MAT activity is defined as the amount of enzyme that catalyzes the formation of 1 nmol of AdoMet/h. Protein was determined by the dye-ligand method (36) with bovine serum albumin as a standard. Immunoblots of proteins that had been separated in a 10% SDS-PAGE gel were prepared as described previously (36). The first antibody consisted of a polyclonal rabbit antisera to highly purified human lymphocyte MAT (36). Nonspecific binding of rabbit immunoglobulin to the E. coli proteins was prevented by diluting the antiseraum 1000-fold in a buffer containing 1 mg/ml bovine serum albumin and 0.1% sodium dodecyl sulfonate of NM522. The second antibody was horseradish peroxidase-conjugated, affinity-purified goat antiserum to rabbit immunoglobulin G (Bio-Rad). Color development was accomplished with hydrogen peroxide and 4-chloro-1-naphthol as the chromophore.

RESULTS

Peptide Sequences—Attempts to obtain an amino acid sequence for the intact αα subunit preparation were unsuccessful, indicating that the amino terminus is blocked. Amino acid sequences were obtained for five tryptic peptides containing a total of 46 different residues (Fig. 1).

Comparison of the Extrahapatic α Subunit cDNA Sequences from Different Human Tissues—To determine whether different human tissues express the same α subunit of MAT, we sequenced the α subunit from leukemic T cells. For this purpose, an oligo(dT)-primed J urkat T cell cDNA library in a ZAP vector was generated. To screen this library, we first screened an adult human liver library in λgt11 with a 1.3-kb probe consisting of the entire coding region of rat liver MAT (14) plus 74 bp of 5′-untranslated sequence and 58 bp of 3′-untranslated sequence. Of a total of 2 × 106 plaques screened, only two positive clones were identified, designated XHM4.1 and XHM4.2, with inserts of 1.25 and 1.35 kb, respectively. The sequences of these isolates overlapped and covered a total length of approximately 1.4 kb (see below) and contained an ORF of 118 codons, which was virtually identical to that of human kidney (24). It was therefore appropriate to use these probes for screening the J urkat library. A 0.35-bp EcoRI/HindII fragment that contained almost the entire ORF from isolate XHM4.1 was used to probe the J urkat library. From approximately 5 × 108 plaques, eight clones were isolated and characterized. The overlapping inserts provided a 2,649-bp sequence extending from position 45 to position 2693 relative to what was eventually determined to be the start codon of the ORF of the lymphocyte enzyme (Fig. 1) and the human kidney enzyme (24). Isolate HLYp31.1 was completely sequenced on both strands and extended from position 47 to 1478. Isolate HLYp36.3 was sequenced for 85% of its length on the coding strand (all but positions 1978–2299) and for 82% of the noncoding strand (all but positions 851–1120 and 2621–2620). Other isolates are shown in Fig. 2 and were sequenced for approximately 300 bp on one or both ends to establish their position in the overall sequence. Isolate HLYp29.1 extended from position 45 to 1032 and contained an additional 235 bp of upstream noncoding sequence. The junction between this segment and nucleotide 45 is AG/GG, which is characteristic for the 3′ end of an unspliced intron, which we have designated intron A (Figs. 1 and 2). Further screening of the oligo(dT)-primed J urkat library failed to identify a clone containing sequence upstream to position 45. Therefore, we constructed additional specific- as well as random-primed libraries and probed them with a 36-mer oligodeoxynucleotide (olgoso5, 5′-ATCGGATCTCCACAAATCTTATCGGGTGCG CTT-C′), which consists of a 3′ end of 26 nucleotides complementary to a previously obtained sequence between positions 79 to 104 (Fig. 1), preceded by 10 nucleotides, which are not complementary to the sequence and contain a BamHI site. A single positive clone, designated HLYp49.1, was obtained from the specific-primed library and gave upstream sequence to −82 (Fig. 1).

Ten clones were obtained from the random-primed library, and one of these, designated HLYp45.1, was characterized in detail. It contained the sequence between positions −94 and +847 but was also found to have an additional 191 bp between positions +768 and +769. The sequences at the 5′ and 3′ junctions of this segment, CAG/ATG and AG/GG, are characteristic for an unspliced intron, which we have designated intron B (Figs. 1 and 2). The single EcoRI site in this putative intron is unlikely to be a cloning artifact because it does contain the adjacent Nol site present in the adapter used for cloning. The final cDNA sequence of 2,787 bp contains a 395-codon ORF encoding a 43.6-kDa polypeptide in which all five of our chemically determined tryptic peptide sequences are present (Fig. 1). The putative start codon at position +1 is found within the sequence CAACATGA, which is identical to the −4 to +4 consensus initiator sequence C(A/G)CCATGG (37) at 6 of 8 positions. The Met-Asn sequence that begins this ORF would be expected to be acetylated in eukaryotes to give N-acetyl-Met-Asn (38), which would explain our finding of a blocked amino terminus for the purified αα peptide. The sequence of the HuLy MAT was identical to that of human kidney MAT (24). However, the J urkat library provided additional 3′-untranslated sequence totaling 1,505 bp, not including the poly(A) tail and including the polyadenylation signal sequence AAATAAA (39, 40) beginning 23 bp upstream of the poly(A) region.

No sequence differences were noted in the various isolates obtained from the three J urkat T cell libraries. The two clones obtained from the adult human liver library corresponded to positions 830–2068 (XHM4.1) and positions 854–2236 (XHM4.2) and were sequenced for about 500 bp from both ends. The sequence of the XHM4.1 and the XHM4.2 fragments was iden-
FIG. 1. Nucleotide sequences of cloned DNA. Panel A shows the cDNA for the α9α9 subunit of human lymphocyte MAT. The sequence was determined from clones isolated from three different Jurkat T cell libraries. The deduced amino acid sequence is shown for the ORF. Nucleotides are numbered beginning at the first position of the ORF. Amino acid positions are shown in parentheses. The positions of putative introns A and B are shown as well as the HindIII site and a polyadenylation signal. Shaded amino acid residues correspond to tryptic peptide sequences that were chemically determined from purified α9α9 subunit. Panel B shows the sequence of partial intron A, which consists of 245 bp upstream of position 45 in isolate HL y29.1, and the sequence of the 191-bp intron B, which is complete and was found between positions 786 and 769 in isolate HL y45.1.
tical to the α subunit cDNA sequence from Jurkat cells. An A and a T located at positions 837 and 846 of human lymphocyte and kidney cDNA were replaced by a G and a C in the XHM4.1 and XHM4.2 fragments from human liver. This is most likely a cloning artifact related to a 5-methyl cytosine spontaneous mutation in E. coli strains that are able to perform the methylation reaction. In these strains the mutation creates a GC to AT transition. However, these base substitutions, which occurred in the third positions of codons 278 and 281, gave synonymous codons for glycine and phenylalanine, respectively. XHM4.1 contained a C at position 2222 in the 3' untranslated region in place of the T found in human lymphocyte cDNA. These data show that the extrahepatic α subunit of MAT is also expressed in human liver.

Comparison of the α Subunit cDNA Sequence in Normal and Malignant Human Lymphocytes—To determine whether sequence differences exist between MAT α subunit in normal and malignant tissues, the α subunit cDNA from normal human T cells was also sequenced. Primers that bracket different regions of the ORF and including positions 244 to 1122 were used to amplify cDNA from normal T cells that have been activated for 48 h with PHA. In resting T lymphocytes MAT activity is predominately associated with α (p68), which is replaced by the α/α9 subunit when T cells are activated. Activation with PHA was, therefore, necessary to induce α subunit mRNA expression, which is normally expressed at very low level in resting T cells.2 The amplified PCR product was either directly sequenced or subcloned into a TA cloning vector and then sequenced. Sequencing covered positions 44 to 1229 were used to amplify cDNA from normal T cells that have been activated for 48 h with PHA. 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Genomic Cloning and Chromosomal Localization of the Human Extrahepatic α Subunit of MAT—Purified DNA from P1 clone 2007 was labeled with digoxigenin dUTP and hybridized to normal metaphase chromosomes derived from PHA-stimulated peripheral blood lymphocytes. Specific hybridization signals were detected using fluorescein-conjugated antidigoxigenin antibodies and counterstaining with propidium iodide. Hybridization with clone 2007 that harbors the α subunit gene. The gene was localized to chromosome arm 2p, an area that corresponds to band 2p11.2. Chromosome 2 centromere-specific probe (D2Z1) was labeled with biotin dUTP and cohybridized with the P1 clone labeled with digoxigenin dUTP. Specific hybridization signals were detected using fluorescein-conjugated antidigoxigenin and Texas red avidin followed by counterstaining with 4,6-diamidino-2-phenylindole.
that clone 2007 is located immediately distal to the centromere on chromosome arm 2p, an area that corresponds to band 2p11.2.

Expression of \( \alpha' \) Subunit cDNA in E. coli—Digestion of pDR540 with HindIII and BamHI gave a 91-bp fragment containing the tac promoter and a Shine-Dalgarno sequence (42), which was situated just upstream of the BamHI site. pKMN1 was constructed by ligation of this fragment into the polylinker region of pT7T3 19U. A 1.34-kb BamHI/EcoRI fragment, which contained all but the amino-terminal 143 bp of the human lymphocyte MAT \( \alpha' \) subunit coding region (Fig. 4), was then purified from HLY31.1 and ligated into pKMN1 to give pMAT351. A fragment containing the missing amino-terminal portion of the coding region was synthesized by PCR with template DNA from HLY45.1 and an upstream primer, \( 5'-\text{GGAATTCGATCCTATGAACGGACAGCTCAACGG}-3' \), which consists of 13 nucleotides at the 5' end containing a BamHI site followed by a T and then 20 nucleotides corresponding to the noncoding strand at positions 1–20. The downstream primer, \( \text{oligo} \text{\textunderscore}g_{\text{ser}} \), was identical with the coding strand from position 152–127. Following digestion of the PCR product with BamHI, the 148-bp fragment was purified by PAGE and inserted into the BamHI site of pT7T3 18U. Several isolates were sequenced to verify the absence of PCR-generated mistakes. The BamHI fragment from one such isolate was then inserted into pMAT351, following transformation into NM522, a recombinant plasmid containing a complete ORF oriented downstream of the tac promoter was identified by DNA sequencing and designated pMAT355 (Fig. 4). The same methods were used to construct pMAT356, which is identical to pMAT355 except that the starting vector was pT7T3 18U.

The lac operon strain NM522, containing either pMAT355 or pMAT356, was grown on 2 × YT medium to a density of about 4 × 10^8 cells/ml and IPTG was added at 0.5 mM. Control cultures were treated in the same way but did not receive IPTG. After an additional 2.5 h of growth, cells were harvested, and the expression of the \( \alpha' \) subunit was examined. Immunoblots of an SDS-PAGE gel probed with a polyclonal antibody to highly purified human lymphocyte MAT holoenzyme (23) showed two bands in extracts from IPTG-induced NM522 (pMAT355), which migrated almost identically with the 53-kDa \( \alpha \) band and the 51-kDa \( \alpha' \) band of the human lymphocyte MAT holoenzyme (Fig. 5). These two bands were not present in the extract from uninduced NM522 (pMAT355). By comparison with standards and by assuming equivalent immunoreactivity, we estimated that the \( \alpha' \) subunit protein accounted for approximately 6% of the total protein in induced NM522 (pMAT355).

**DISCUSSION**

The existence of multiple isozymes of MAT in mammalian tissues is well established (for review see Refs. 3 and 43).
Whereas the liver-specific enzyme appears to be a homodimer or tetramer of a single α subunit (41), the extrahepatic enzyme that is expressed in all tissues appears to consist of nonidentical α and β subunits. Several complementary pieces of evidence indicate that the cDNA we have characterized from Jurkat T cell libraries is that of the α subunit of human lymphocyte MAT. First, the deduced amino acid sequence is identical to that of the α subunit from human kidney (24) and highly homologous to that of other MATs (see below). Second, the sequence contains the five tryptic peptide sequences that were chemically determined from purified, native α/α9 subunit. Third, expression of this cDNA in E. coli gave two peptide bands that comigrated with authentic α and α9 subunits in SDS-PAGE and reacted with antiserum to human lymphocyte MAT. The fact that the α subunits of MAT in human lymphocytes, kidney, and liver are identical suggests that the sequence of this subunit is the same in all tissues.

Several studies have reported that MAT activity is elevated in malignant cells (6, 22, 44–46). Furthermore, Liau et al. (6, 7, 47, 48) demonstrated the presence of an altered form of MAT in tumors. In this study we show that the sequence of the α subunit is the same in normal and malignant lymphocytes. These findings do not rule out the possibility that the expressed protein may be altered post-translationally in malignant lymphocytes. However, immunoblot analysis of cell extracts shows a very similar pattern for leukemic and normal activated T lymphocytes (21, 22). Additional studies are needed to determine whether differences exist in the regulation and expression of MAT in normal and malignant tissues. These studies may facilitate the development of specific inhibitors that specifically block MAT in malignant tissues (44, 49–51).

Comparisons of the deduced amino acid sequence for the human lymphocyte or kidney α subunit with those of non-mammalian MAT (not shown) revealed identities of 49% with the E. coli enzyme (52), 68% with the Arabidopsis thaliana enzyme (53), and 69% with the two yeast enzymes (54, 55). Identities were found at 84% of positions with the liver αh subunit from human, rat, and mouse (13, 14, 56), and at 98.7% of positions with the rat kidney enzyme (57). This homology at the molecular level confirms previous reports showing that the
α subunit of human lymphocytes is immunocross-reactive with MAT from E. coli, yeast, and rat liver (23). Therefore, earlier reports by Abe et al. (9), which failed to detect cross-reactivity between the hepatic and the kidney enzymes in rat, may represent differences in the amount of proteins or the antibodies used in the immunologic studies.

Earlier studies by Horikawa et al. (57) suggested that the rat kidney α subunit is not expressed in the liver, but later studies showed that the human kidney α subunit is expressed at very low levels in adult liver (12). However, the data presented here clearly show that the α subunit is expressed in human liver because the sequence of the XHM4.1 and XHM4.2 cDNA fragments, which were isolated from an adult human liver library, was identical to that of the extrahepatic α subunit. The finding that the extrahepatic α subunit is expressed in liver confirms earlier findings by Liu et al. (7) of a human liver isozyme with a low Km for l-Met, and a more recent report by De La Rosa et al. (21) in which immunoblots of human liver extracts revealed that adult human liver also expresses the extrahepatic form of MAT.

The deduced amino acid sequences of the human and rat extrahepatic α subunit differ at only 5 of 395 positions (Fig. 6). The cDNA sequences for the human lymphocyte α subunit and rat kidney enzyme were 93.5% identical. This high degree of identity was even more striking at the 3′-untranslated region, where only a single difference exists (position 39) in the 73 bp downstream of the TGA terminator codons (to position 1260). The 3′-untranslated region was also conserved for the extrahepatic α subunit cDNA expressed in adult liver because fragment XHM4.1 contained an identical sequence in this region differing by only 1 base where a C at position 2222 in the 3′-untranslated region was found in place of the T found in human lymphocyte cDNA. Although the sequences of the 3′-untranslated regions of the hepatic α subunit and the extrahepatic α subunit are quite different, the 3′-untranslated region is highly conserved across different species, suggesting that this region may exert an important regulatory function.

The finding of the two introns in the clones derived from the J urkat library was useful in that it allowed us to select genomic clones that harbor the extrahepatic α subunit gene. DNA from these clones enabled us to determine that the gene for the human extrahepatic α subunit of MAT is located on chromosome arm 2p, an area that corresponds to band 2p11.2. Recently, the gene for the mouse liver enzyme was localized to chromosome 7 (56). To our knowledge there are no reports of the chromosomal localization of the human liver α subunit. Studies that investigate the genomic organization and regulation of MAT isozymes will undoubtedly reveal molecular mechanisms controlling the expression of these enzymes in different mammalian tissues as well as in normal and malignant tissues.

Expression of the α subunit in E. coli produced two immunoreactive peptides that are probably equivalent to the α and α′ peptides of HuLy MAT (19, 23). Total identity would not be expected because of differences in amino-terminal processing, which would give NH2-Met-Asn or perhaps NH2-Asn in E. coli (58, 59) and N-acetyl-Met-Asn in lymphocytes (38). Such differences may account for the slightly faster migration of the E. coli-expressed products in SDS-PAGE (Fig. 5). It seems likely, however, that the E. coli products with apparent masses of 53 and 51 kDa are related to each other in the same way that the lymphocyte α and α′ subunits are related. Since mRNA processing and specific post-translational modifications are generally very different between prokaryotes and eukaryotes, we favor a relatively non-specific process such as proteolysis or deamidation to account for differences between the α and α′ subunits of the lymphocyte enzyme and between their counter-parts produced by the E. coli expression strain. Amino acid sequences of the lymphocyte αα′ subunit that are not present in the rat liver enzyme are possible targets for such a process since the latter gives only a single band in SDS-PAGE. It is of interest in this regard that the deduced αα′ sequence contains two Asn-Gly dipeptides (at positions 6–7 and 170–171) that are not present in the rat liver enzyme (Fig. 6). The asparaginyl residue in this sequence is known to be particularly susceptible to nonenzymatic succinimide formation with subsequent hydrolysis to an isoaspartyl residue (60, 61). If the difference between the α and α′ subunits is due to such a modification, one would expect the rat kidney enzyme also to show two bands in SDS-PAGE because its deduced sequence contains the same two Asn-Gly dipeptides. We are unaware of any report on the subunit structure of the rat kidney enzyme.

The 43.6-kDa polypeptide predicted from the ORF of the cDNA is considerably smaller than the values of 53 and 51 kDa estimated by SDS-PAGE for the α and α′ subunits of human lymphocyte MAT (19, 23). This discrepancy also exists for the HuLy α subunit expressed in E. coli and for the rat liver αα′ subunit, which has a predicted mass of 43.7 kDa (14) but an estimated mass of about 48 kDa by SDS-PAGE analyses (4, 9, 10, 62). Anomalously slow migration in SDS-PAGE has been reported for other proteins and in some instances has been attributed to excess charge density (63) or more specifically, to an unusually high content of acidic amino acids (64, 65). Although the entire deduced αα′ peptide has a net charge of only −7, many of the basic residues are located in the carboxy-terminal portion, and the first 240 amino acids have a net charge of −19. A similar asymmetric charge distribution is found in the rat liver enzyme.

The extrahepatic form of MAT has been studied extensively in human lymphocytes (16, 19, 21–23, 66, 67), human erythrocytes (15, 18), rat lens (17, 68), rat kidney (4), and bovine brain (20). The presence of the α and β subunits has been demonstrated in human lymphocytes (19, 21–23), human mature erythrocytes (18), and bovine brain (20) and has been reported to exist in Erlich ascites tumor and calf thymus (20); there have been no reports of the existence of the β subunit in kidney tissues. There is strong evidence to suggest that the α subunit is the catalytic subunit, whereas β may have a regulatory function. Extracts from the E. coli expression strain contained levels of MAT activity which were 20-fold higher than those from E. coli itself. However, a preliminary kinetic analysis of unpurified enzyme indicates that it has a Km of 80 μM, which is 20-fold higher than the 4 μM value of MAT from leukemic T cells and 5-fold higher than the 16 μM value for enzyme in crude resting human lymphocytes. Perhaps these findings only reflect differences between lymphocytes and E. coli in amino-terminal processing or some other post-translational modification, but it is intriguing to speculate that the function of the β subunit may be to impart the kinetic properties that distinguish the lymphocyte enzyme from the hepatic enzyme, i.e., a low Km for L-methionine and sensitivity to product inhibition by AdoMet (15, 19).

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