Cloning, Expression, and Functional Characterization of the 
β Regulatory Subunit of Human Methionine
Adenosyltransferase (MAT II)*

(Received for publication, June 24, 1999, and in revised form, October 18, 1999)

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MAT II, the extrahepatic form of methionine adenosyltransferase (MAT), consists of catalytic αβ/αβ subunits and a noncatalytic β subunit, believed to have a regulatory function. The full-length cDNA that encodes the β subunit of human MAT II was cloned and found to encode for a 334-amino acid protein with a calculated molecular weight of 37,552. Analysis of sequence homology showed similarity with bacterial enzymes that catalyze the reduction of TDP-linked sugars. The β subunit cDNA was cloned into the pQE-30 expression vector, and the recombinant His tagged protein, which was expressed in Escherichia coli, was recognized by antibodies to the human MAT II, to synthetic peptides copying the sequence of native β subunit protein, and to the ρβ protein. There is no cross-reactivity between the MAT II αβ or β subunits. None of the anti-β subunit antibodies reacted with protein extracts of E. coli host cells, suggesting that these bacteria have no β subunit protein. Interestingly, the ρβ subunit associated with E. coli as well as human MAT α subunits. This association changed the kinetic properties of both enzymes and lowered the Km of MAT for L-methionine. Together, the data show that we have cloned and expressed the human MAT II β subunit and confirmed its long suspected regulatory function. This knowledge affords a molecular means by which MAT activity and consequently the levels of AdoMet may be modulated in mammalian cells.

Methionine adenosyltransferase (MAT; S-adenosyl-L-methionine synthetase, EC 2.5.1.6) is an essential enzyme that catalyzes the synthesis of S-adenosylmethionine (AdoMet) from L-methionine (L-Met) and ATP (1, 2). AdoMet is the major methyl group donor, participating in the methylation of proteins, DNA, RNA, phospholipids, and other small molecules (reviewed in Refs. 3–5). In addition, AdoMet is the ultimate source of the propylamine moiety used in polyamine biosynthesis, and it serves as co-factor for other key enzymes in the one-carbon metabolism pathway (3–5). MAT is present in all living species, including thermophilic archaeabacteria, plants, yeast, and mammals (reviewed in Refs. 4 and 6–8). Interestingly, most species have more than one MAT isozyme (6).

In mammals, it is now established that there are at least two MAT isozymes (9–12). MAT I/III is expressed only in liver and has a catalytic subunit designated α1 that is encoded by the MATIA gene (8, 9, 13–16). MAT I and MAT III represent different oligomeric forms of the α1 subunit - MAT III is a dimer, and MAT I is a tetramer of the α1 subunit (9, 17–19). MAT I and MAT III differ considerably in their physical, kinetic, and regulatory properties (8, 9, 20). The MAT II isozyme is expressed in all tissues, including the liver, and has been studied in many tissues including erythrocytes, lymphocytes, brain, kidney, testis, and fetal liver (11, 20–27).

We have been characterizing the human MAT II from human lymphocytes (22, 28–31) and were able to show that the form present in activated lymphocytes consists of distinct subunits (22, 29). The catalytic MAT II αβ subunit, which is encoded by the MAT2A gene, was cloned and characterized and found to be homologous but different from the catalytic α1 subunit of the liver MAT I/III isozyme (13–15, 26, 30). The MAT II αβ subunit, which has a calculated molecular weight of 43,600, migrates on SDS-PAGE gels as a 53-kDa protein and is posttranslationally modified to generate MAT II α2 subunit (22). The catalytic αβ/αβ subunits are found in native MAT II associated with a catalytically inactive subunit designated MAT II β, which migrates on SDS-PAGE as a 38-kDa protein (22, 29, 31). Inasmuch as the MAT II β subunit had no homology to the αβ subunit (22, 33), it was postulated that it is encoded by a different gene, which was putatively designated MAT2B (12).

In this study, we report the complete sequence of cDNA encoding the entire MAT II β subunit, and we show that the protein expressed in Escherichia coli associates with the E. coli as well as the human catalytic α subunits of MAT. The association of β and α subunits changes the kinetic properties of MAT, thereby providing direct evidence for the regulatory role of the MAT II β subunit.

MATERIALS AND METHODS

Cloning of the Human MAT II β Subunit cDNA—Degenerate primers were designed based on partial amino acid sequence of two tryptic peptides (generated by partial digestion of pure MAT II β protein as described previously (29–31). The forward 5’ DEG primer (5’-GTNG-GMNNGARAARGARYTNWSNATHCAYSFTGTGTYNTCC-3’) was based on the sequence of the N-terminal peptide (VGHERELISHVPVPSGSELV), and the reverse 3’ DEG primer (5’-GTYGTYCRTTNCNSWCCART-3’).

§§ This work was supported by National Institutes of Health Grant GM-54892–09 and by Merit Review Award Funds from Veterans Affairs. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF182814.

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† The abbreviations used are: MAT, methionine adenosyltransferase; AdoMet, S-adenosylmethionine; PBMC, peripheral blood mononuclear cells; DEG, oligonertate; bp, base pair(s); PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; ORF, open reading frame; ρMAT II β, recombinant MAT II β; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; BSA, bovine serum albumin; contig, group of overlapping clones.

This paper is available on line at http://www.jbc.org

Vol. 275, No. 4, Issue of January 28, pp. 2359–2366, 2000
Printed in U.S.A.
GRAANGTNCCYTTT was based on the sequence of an internal peptide (LDPISIKTFHWHSNGEQT). Total RNA was extracted from the human T cell leukemia lines, Jurkat (ATCC-8163), or MOLT4 (ATCC-15852) using RNazol B (Tel-Test, Inc.). The RNA (2 μg) was reverse transcribed into cDNA using 25 units of avian myeloblastoma virus reverse transcriptase (Promega), oligo(dT)12-18 (Promega), and 1 mm dNTPs (American Pharmacia Biotech). The cDNA was amplified using Taq DNA polymerase and the 5′ and 3′ deg primers. After 30 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 1 min, and extension at 72 °C for 1 min, a 765-bp PCR product was generated. The denaturation at 95 °C for 30 s, annealing at 48 °C for 1 min, and extension at 72 °C for 1 min, a 765-bp PCR product was generated. The sequential methodology was employed to obtain overlapping 5′ sequencing (see Fig. 1). Based on the sequence of Clone 1, nondegenerate primer (5′ reverse transcribed into cDNA as described above, except that a T-subunit-specific primer, 5′-TCTCTCTCTGTTACATGAC (Sigma) was added to a final concentration of 1 mM, and the culture was incubated at 37 °C with 5 units of alkaline phosphatase (Promega) to enhance the efficiency of ligation. SacI- and HindIII-digested Clone 4 was directionally cloned into unique cloning site of the pQE-30 vector. Following ligation of the MAT II β subunit cDNA into the prepared pQE-30 vector, the ligated vector was transformed into E. coli strain ML109. Ten positive clones were fully sequenced using the fmol cycle sequencing system (Promega) to rule out any possible base substitutions, and one representative clone, Clone 4, which represented the full-length β subunit cDNA, was used for further studies. The sequence of the ORF was identical for cDNA representing RNA from Jurkat cells, MOLT4 cells, and normal human PBMC. The MAT II β cDNA was excised from the pGEM-T-easy vector using SacI and HindIII and purified.

Expression vector pQE-30 (Qiagen), which is designed to express proteins containing a His tag at the N-terminal was used. The vector was linearized by digestion with the restriction enzymes SacI and HindIII for 1 h at 37 °C, then purified from an agarose gel, and incubated at 37 °C with 5 units of alkaline phosphatase (Promega) to enhance the efficiency of ligation. SacI- and HindIII-digested Clone 4 was directionally cloned into unique cloning site of the pQE-30 vector. Following ligation of the MAT II β cDNA into the prepared pQE-30 vector, the ligated vector was transformed into E. coli expression strain M15 (Qiagen). Cursory sequencing of 20 positive clones was carried out using the fmol cycle sequencing system (Promega), and the clones were verified for proper frame and orientation of the MAT II β cDNA. A representative clone, Clone 5, was inoculated into LB broth containing 25 μg/ml kanamycin and 100 μg/ml ampicillin. The culture was incubated, with shaking, at 37 °C until an absorbance of 0.5–0.7 was reached, and then isopropyl-β-D-thiogalactoside (Sigma) was added to a final concentration of 1 mM, and the culture was allowed to incubate for an additional 4 h under the same conditions. Bacterial pellets were disrupted using sonication and then analyzed via Western blot and MAT II β-specific polyclonal antibodies that were generated to the two MAT II β peptides (29–31).

**Purification of Recombinant MAT II β**—The MAT II β expression clone, Clone 5, was inoculated into 1 liter of LB broth containing 25 μg/ml kanamycin and 100 μg/ml ampicillin and then incubated with shaking at 37 °C until an absorbance of 0.5–0.7 was reached. Isopropyl-β-D-thiogalactoside (Sigma) was added to a final concentration of 1 mM, and the culture was allowed to incubate for an additional 4 h under the same conditions. Initial attempts to purify His-tagged MAT II β subunit under native conditions via sonication and nickel-nitrioltriacetic acid-agarose resulted in a complex of the E. coli MAT α subunit and the recombinant human β subunit. Therefore, subsequent purification of MAT II β away from the endogenous E. coli MAT α was performed by two methods. The first involved purification of the His-tagged protein under denaturing conditions of 8 M urea, and the second involved separation of SDS-PAGE and elution of the β subunit protein from the gel.

To purify recombinant MAT II β protein under denaturing conditions, the cell pellet was lysed at room temperature by stirring the pellet in a buffered solution containing 8 M urea, pH 8.0 (denaturing conditions). Once the solution became translucent, the cellular debris from resting peripheral blood mononuclear cells (PBMC) total RNA was amplified by PCR using Pfu DNA polymerase (Promega) and the MAT II β 5′ subunit-specific primer, 5′-TCTCTCTCTGTTACATGAC (Sigma) and the MAT II β 3′ subunit-specific primer, 3′-TCTCTCTCTGTTACATGAC (Sigma) was added to a final concentration of 1 mM, and the culture was incubated at 37 °C with 5 units of alkaline phosphatase (Promega) to enhance the efficiency of ligation. SacI- and HindIII-digested Clone 4 was directionally cloned into unique cloning site of the pQE-30 vector. Following ligation of the MAT II β cDNA into the prepared pQE-30 vector, the ligated vector was transformed into E. coli expression strain M15 (Qiagen). Cursory sequencing of 20 positive clones was carried out using the fmol cycle sequencing system (Promega), and the clones were verified for proper frame and orientation of the MAT II β cDNA. A representative clone, Clone 5, was inoculated into LB broth containing 25 μg/ml kanamycin and 100 μg/ml ampicillin. The culture was incubated, with shaking, at 37 °C until an absorbance of 0.5–0.7 was reached, and then isopropyl-β-D-thiogalactoside (Sigma) was added to a final concentration of 1 mM, and the culture was allowed to incubate for an additional 4 h under the same conditions. Bacterial pellets were disrupted using sonication and then analyzed via Western blot and MAT II β-specific polyclonal antibodies that were generated to the two MAT II β peptides (29–31).

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were removed by centrifugation. The clarified supernatant was loaded onto a nickel-nitrilotriacetic acid-agarose column (Qiagen) to capture the His-tagged protein. The column was washed with several volumes of buffered 8 M urea, pH 6.5, until a A280 of 0.001–0.005 is reached. Elution of the recombinant MAT II β (MAT II β) was carried out using buffered 8 M urea, pH 5.9 and 4.5. The eluted protein was extensively dialyzed against 50 mM Tris-HCl, pH 7.5, and then concentrated and analyzed by Western blots and silver-stained SDS-PAGE for determination of size and purity. The pure recombinant human MAT II β subunit protein was used to immunize rabbits and to generate polyclonal antibodies as detailed below.

In some experiments, the nickel-agarose purified proteins were separated on SDS-PAGE and visualized by impregnation in cold 300 mM NaCl, and the band corresponding to the 38–39-kDa protein was excised, and the protein was electroeluted from the gel into Tris-glycine buffer, pH 8, dialyzed against 10 mM ammonium bicarbonate and then lyophilized. The lyophilized protein was reconstituted in 50 mM Tris-HCl, pH 7.5, and analyzed by Western blotting and silver staining for size and purity. The purified β subunit protein was also analyzed for functional activity in MAT assays containing E. coli MAT II α or recombinant human MAT II α protein. The nickel-agarose and SDS-PAGE purified β subunit protein retained functional activity.

Production of Polyclonal Antibodies to MAT II β Subunit—Polyclonal antibodies were generated to the β subunit synthetic peptides (VNLVDEGDGKGQ EGFKDENGAF and VNLVDEGDGKGQ EGFKDENGAF) and the recombinant human MAT II β subunit protein. Both antibodies were generated in male New Zealand White rabbits (Myrtle’s Rabbitry, Huntington Station, NY), and the intensity of the desired band was integrated and expressed in arbitrary units.

**RESULTS**

cDNA and Predicted Protein Sequence of the Human MAT II β Subunit—The cloning strategy was based on the design of degenerate primers representing partial amino acid sequence of an N-terminal (19-mer) and an internal (17-mer) peptides of the trypsin digested β subunit protein, purified from human lymphocytes (29, 30). Complementary DNA prepared from Jurkat cells mRNA was amplified with the degenerate primers, 5’ DEG and 3’ DEG, that would encode partial sequences of the two β subunit peptides. The cDNA from positive clones were isolated at the preparative level and entirely sequenced in both directions. The cDNA clones that contained sequences corresponding to both peptides were selected for further sequencing.
and Clone 1 (765 base pairs), which represented the consensus sequence, was fully characterized for the design of nondegenerate primers. Clone 1, which represents a partial sequence of the $b$ subunit cDNA, would be expected to encode for 255 amino acids. However, because the $b$ subunit migrates on SDS-PAGE as a 38-kDa protein, we estimated that at least 60–80 amino acids or 180–240 base pairs were still unaccounted for. Accordingly, sequence-specific primers based on the sequence of Clone 1 were synthesized and used in 3’ and 5’ RACE to obtain the complete ORF of the $b$ subunit cDNA (Fig. 2).

A minimum of 10 positive clones from 3’ or 5’ RACE were sequenced in both directions, and Clone 2 represented the consensus of the 5’ RACE clones, whereas Clone 3 represented that of the 3’ RACE clones. The sequence of Clone 2, which was generated by 5’ RACE, showed that Clone 1 was only missing the ATG start codon at its 5’ end. The authenticity of this ATG codon as the initiator codon was confirmed in several ways. First, this ATG is in frame with sequences that encode for amino acids found in the N-terminal $b$ subunit peptide (Fig. 2). Second, the sequence of bases flanking this ATG codon are in accordance with sequences identified by Kozak (34) as consensus sequences preceding or following an authentic initiator codon. For example, the sequence $(\ldots)GnnATGG(\ldots)$ showed the second highest incidence of functional initiator codons (130 of 699 tested) in vertebrate mRNAs analyzed (34). In addition, the presence of a C at position −1 and the presence of a G at

![Fig. 3. Amino acid sequence homology between the MAT II $b$ subunit and bacterial enzymes that catalyze the reduction of TDP-linked sugars.](image)

The amino acid sequence of the MAT II $b$ subunit was aligned with dTDP-6-deoxy-l-mannose-dehydrogenase (dTDP-DMD; accession number AAC40275; A) and UDP-glucose 4-epimerase (UDP-GE; accession number Q56623; B) using the CLUSTALW program available on the Network Protein Sequence Analysis site of the Pole Bio-informatique Lyonnais in Lyon, France. Alignment with dTDP-DMD revealed 26.2% identity, with 18.24% strong homology and 10% weak homology. Alignment with UDP-GE revealed 22.75% identity, with 20.22% strong homology and 11.52% weak homology.
sequences of 5′- and 3′-flanking regions and an open reading frame of 1002 base pairs. The cDNA encodes for 334 amino acids with a calculated molecular weight of 37,551.81 and has a pi of 6.90. Thus, unlike the MAT II α2 and α5 subunits, which migrate at higher than expected molecular weight on SDS-PAGE (22, 29, 30), the MAT II β subunit migrates at its expected molecular mass of 38 kDa.

Analysis of the DNA and the protein sequences on data bases from GenBank™ and the Biological Information Resource site using, among others, the Advanced Blast and SwissPort data bases, revealed up to 28% homology between the MAT II β subunit and a family of bacterial enzymes that catalyze the reduction of TDP-linked sugars such as dTDP-4-dehydrorhamnose reductase, several nucleoside-diphosphate-sugar epimerases (37), and other proteins involved in the synthesis of polysaccharides (38). The alignment with two representative proteins, dTDP-6-deoxy-L-mannose-dehydrogenase and UDP-glucose 4-epimerase, is shown in Fig. 3. Analysis for DNA sequence homology revealed identity between several segments of the MAT II β cDNA and several human, mouse, and rat DNA sequences, which were part of contigs that were sequenced in both directions, and the consensus sequence, represented by Clone 4, is shown in Fig. 2.

Sequence Comparisons—The cloned full-length cDNA contained standard 5′- and 3′-flanking regions and an open reading frame of 1002 base pairs. The cDNA encodes for 334 amino acids with a calculated molecular weight of 37,551.81 and has a pi of 6.90. Thus, unlike the MAT II α2 and α5 subunits, which migrate at higher than expected molecular weight on SDS-PAGE (22, 29, 30), the MAT II β subunit migrates at its expected molecular mass of 38 kDa.

Expression of the Recombinant β Subunit Protein in E. coli—Clone 4, which represented the full-length cDNA encoding the complete ORF (1002 base pairs) for the β subunit, was directionally cloned into the pQE-30 expression vector, which was designed to express the β subunit protein with a poly-His tag at the N-terminal end of the molecule. Clone 5, which represented 20 sequenced clones, provided expression of the β subunit protein in E. coli. A is Coomassie Blue-stained SDS-PAGE of crude protein extracts from E. coli cells (lane 1), E. coli cells that were transfected with the pQE-30 vector harboring the MAT II α2 subunit cDNA and expressing the recombinant α2 subunit protein (lane 2), and E. coli cells that were transfected with the pQE-30 vector harboring the MAT II β subunit cDNA (lane 3). B is a Western blot of the same crude material analyzed in A. The blot was probed with antibodies to the β subunit protein. C is a silver-stained SDS-PAGE of His-tagged recombinant β subunit protein purified from E. coli cells that were transfected with the pQE-30 vector harboring the MAT II β subunit cDNA, using Ni-agarose column purification (lane 1) and His-tagged recombinant β subunit protein purified on nickel-nitrilotriacetic acid-agarose column and further purified and eluted from a preparative SDS-PAGE (lane 2) as detailed under "Materials and Methods" and "Results." D is a Western blot of the same material analyzed in C and probed with antibodies to both the MAT α2 and β subunits. C, lane 1 is the His-tagged recombinant β subunit protein eluted from the nickel-agarose column, and lane 2 is the eluted material subjected to further purification and electrophoresis from a preparative SDS-PAGE. The antibodies to the MAT II α2 subunit recognized the cross-reactive E. coli MAT α2 subunit, which co-purified with the recombinant His-tagged β subunit (lane 1), but there was no contaminating α subunit in the SDS-PAGE eluted material (lane 2). E and F represent the same blot shown in D, stripped and reprobed with antibodies to the MAT II α2 subunit or with antibodies to the MAT II β subunit, respectively.

Our previous studies (33), there was no immunological cross-reactivity between the MAT II α and β subunits. Furthermore, no reactivity was detected between the anti-β peptides and protein extracts of E. coli host cells that were either untransfected or that were transfected with the same vector and expressing the human recombinant MAT II α2 subunit cDNA (Fig. 4D) (30).

When the His6-tagged recombinant MAT II β subunit was purified on nickel-nitrilotriacetic acid-agarose column and eluted with 300 mM imidazole, analysis of the dialyzed protein on silver-stained SDS-PAGE showed that several protein bands co-purified with the recombinant protein (Fig. 4C, lane 1). Further analysis by Western blot, which was probed with
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E. coli MAT

\[ y = 2.1996x - 1.7126x \]

\[ R^2 = 0.996 \]

\[ Km = 84 \mu M \]

\[ L-Met 20-250 \mu M \]

E. coli MAT

\[ y = 3.5105x + 3.4777x \]

\[ R^2 = 0.996 \]

\[ Km = 65 \mu M \]

\[ L-Met 5-20 \mu M \]

coli MAT + rMAT II b

\[ y = 1.9042x + 0.18457x \]

\[ R^2 = 0.996 \]

\[ Km = 87 \mu M \]

\[ L-Met 40-200 \mu M \]

coli MAT + rMAT II b

\[ y = 2.0006x - 0.10252x \]

\[ R^2 = 0.999 \]

\[ Km = 36 \mu M \]

\[ L-Met 2.5-30 \mu M \]

FIG. 5. Lineweaver-Burk plots showing the effect of recombinant β subunit protein on E. coli MAT kinetic properties. Protein extracts from untransformed E. coli cells (A and B) or from E. coli expressing recombinant MAT II β subunit protein (C and D) were assayed for MAT activity as described under “Materials and Methods” at different concentrations of L-Met. A and C represent the data at the high L-Met concentration range; B and D represent the data at the low L-Met concentration range. The velocity was calculated in units/ml, where 1 unit of MAT activity is defined as the amount of enzyme that catalyzes the formation of 1 nmol of AdoMet in 1 h.

DISCUSSION

The existence of multiple isozymes of MAT in mammalian tissues is well established (reviewed in Refs. 4 and 6). Whereas, the liver-specific enzyme appears to be a homodimer or tetramer of a single α subunit, the extrahepatic MAT II enzyme, which is expressed in all tissues, appears to consist of nonidentical subunits, α2 and β. In 1985, Koth and Kredich (22) reported that native MAT II from human leukemic cells has a molecular weight of 185,000 and consists of two related subunits α2 and β, which migrated on SDS-PAGE as 53- and 51-kDa proteins and had an identical V8-protease peptide antibodies to either the α or the β subunits or both, showed that the endogenous E. coli MAT α protein was co-purifying with the recombinant human MAT II β subunit protein (Fig. 4, D and E, lanes 1). To purify the recombinant MAT II β subunit protein away from the endogenous E. coli MAT α protein, the nickel-agarose purified proteins were separated on SDS-PAGE, the protein bands were visualized by impregnation in cold 300 mM KCl, and the band corresponding to the 38–39-kDa protein was excised. The protein was electroeluted from the gel slice into Tris-glycine buffer, pH 8, dialyzed, lyophilized, reconstituted in 50 mM Tris-Cl buffer, pH 7.5, and reanalyzed. Analysis of this purified protein on silver-stained SDS-PAGE showed the migration of a single 38–39-kDa protein (Fig. 4, C, lane 2), and the Western blot revealed that only the recombinant human MAT II β subunit protein was present in this fraction (Fig. 4, D and F, lanes 2). As will be discussed below, the gel-purified β subunit protein retained functional activity.

The identity of the immunoreactive band with the MAT II β subunit protein was verified by the fact it was recognized by antibodies generated to either the β subunit partial peptides as well as by antibodies to the whole recombinant human MAT II β subunit protein (data not shown). Again, neither antibody to the β subunit showed any reactivity with any E. coli protein (Fig. 4).

Functional Analysis of the Recombinant Human MAT II β Subunit Protein—The recombinant human MAT II β subunit protein was found to have no MAT catalytic activity; however, it modulated the kinetic properties of the MAT II α2 catalytic subunit. The nickel-agarose and SDS-PAGE-purified recombinant MAT II β subunit protein associated spontaneously with E. coli MAT α subunit as well as with the recombinant human MAT II α2 subunit. The effect of the MAT II β subunit protein on the kinetic activity of the E. coli MAT or the recombinant human MAT II α2 was analyzed. In the absence of the MAT II β subunit, both the E. coli MAT and the recombinant human MAT II α2 exhibited normal Michaelis and Menten kinetics with the apparent presence of a single catalytic form (Figs. 5 and 6).

The \( K_m \) for L-Met of the E. coli MAT α subunit was 80–90 \( \mu M \) at high L-Met concentrations (Fig. 5A) and 65–80 at low L-Met concentrations (Fig. 5B). In the presence of the MAT II β subunit, however, two kinetic forms appeared one with a \( K_m \) for L-Met of 80–90 \( \mu M \) (Fig. 5C) and another with a \( K_m \) of 30–38 \( \mu M \) (Fig. 5D). Similarly, the \( K_m \) for L-Met of the human MAT II α2 catalytic subunit alone was 60–100 \( \mu M \) (Fig. 6), and in the presence of the rβ subunit two kinetic forms were evident with \( K_m \) values of 76 \( \mu M \) (Fig. 6C) and 22 \( \mu M \) (Fig. 6D). Together, these observations confirm previous suggestions (31) that one of the functions of the β subunit is to lower the \( K_m \) of MAT for L-Met.
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The kinetics of recombinant human MAT II α2 subunit in the absence (A and B) or presence (C and D) of the recombinant MAT II β subunit protein was performed as described under “Materials and Methods” at different concentrations of l-Met. A and C represent the data at the high l-Met concentration range; B and D represent the data at the low l-Met concentration range. The velocity was calculated in units/ml, where 1 unit of MAT activity is defined as the amount of enzyme that catalyzes the formation of 1 nmol of AdoMet in 1 h.

banding pattern. By contrast, the β subunit migrated on SDS-PAGE as a 38-kDa protein and had a peptide banding pattern that was quite distinct from that of the two α2 subunits, suggesting that β is distinct from the α2 subunit. Antibodies to the pure MAT II enzyme from human leukemic cells, whose purity was verified by analytical ultracentrifugational analysis, recognized both α2 and β subunits in human lymphocytes and showed cross-reactivity with E. coli and yeast MAT α subunits, which is not surprising given the now known high degree of primary sequence homology of MAT catalytic subunits in the many species analyzed; however, there was no indication that the anti-human MAT II antibodies were recognizing a protein similar to the β subunit in either E. coli or yeast cell extracts (33). Moreover, polyclonal antibodies to E. coli or yeast MAT reacted with the human MAT I, II, and III α subunits but failed to recognize the MAT II β subunit protein. Together, these findings strongly suggested that the β subunit of MAT II is distinct from the MAT α2/α2 subunits, and we proposed that it is probably encoded by a distinct gene, which we putatively designated MAT2B (12).

Several complementary forms of evidence indicate that the cDNA we have characterized from Jurkat T-cells, MOLT-4 cells, and normal peripheral blood mononuclear cells is that of the human MAT II β subunit. First, the deduced amino acid sequence contains sequences that are identical to those of the two tryptic human lymphocyte β subunit peptide sequences (29). Second, expression of this cDNA in E. coli gave a protein band that migrated at the expected size of the authentic β subunit in SDS-PAGE (Fig. 5) and reacted with antiserum to the human lymphocyte MAT II, to the tryptic peptides, and to the recombinant MAT II β subunit protein. More important, the fact that the cloned protein lowered the Km for L-Met in human lymphocytes and resulted in down-regulation of its products (31). These studies led us to propose that the MAT II β subunit may have regulatory properties. This notion was further emphasized by the studies of LeGros et al. (31), who in 1997 demonstrated that physiological stimulation of human lymphocytes with supernatants resulted in down-regulation of β subunit expression. The disappearance of the β subunit was accompanied by a change in MAT kinetic properties with the MAT II α2/α2 enzyme form exhibiting a 3-fold higher Km for l-Met and, more importantly, showing resistance to feedback product inhibition by AdoMet and resulting in a 5–6-fold increase in intracellular AdoMet levels (31). These studies led us to propose that the β subunit regulates MAT II activity by lowering the Km for l-Met and rendering the enzyme more resistant to feedback inhibition by its products (31). The data presented here, as well as those presented in Halim et al., provide direct evidence that these hypotheses are correct and confirm the regulatory role of the β subunit of MAT II.

The sequence of the β subunit cDNA was identical for Jurkat T cells, MOLT-4 cells, and PBMC from different individuals.

FIG. 6. Lineweaver-Burk plots showing the effect of recombinant β subunit protein on recombinant MAT II α2 subunit kinetic properties. The kinetics of recombinant human MAT II α2 subunit in the absence (A and B) or presence (C and D) of the recombinant MAT II β subunit protein was performed as described under “Materials and Methods” at different concentrations of l-Met. A and C represent the data at the high l-Met concentration range; B and D represent the data at the low l-Met concentration range. The velocity was calculated in units/ml, where 1 unit of MAT activity is defined as the amount of enzyme that catalyzes the formation of 1 nmol of AdoMet in 1 h.
The ORF for the MAT II β subunit, which begins with the Met-Val sequence, encodes for a 334-amino acid protein and in concordance with the calculated molecular weight, and the recombinant protein migrates on SDS-PAGE as 38-kDa protein. Thus, the MAT II β subunit, unlike the αβ subunit, migrates at its expected molecular size. The MAT II holoenzyme is known to be very hydrophobic inasmuch as it binds very strongly to phenyl- Sepharose columns and can only be eluted with 40% Me₂SO (22). The Kyte-Doolittle hydrophobicity plots of human MAT II β subunit protein (data not shown) revealed two prominent hydrophobic segments, whereas the αβ subunit has three minor and one major hydrophobic segment. Further studies are required to determine whether the oligomerization of the MAT II αβ and β subunits is responsible for the strong hydrophobic property of the enzyme.

The presence of the β subunit protein was previously detected in human erythrocytes, lymphocytes, bovine brain, Ehrlich’s ascites tumor, and calf thymus (22, 25). Inasmuch as antibodies to the β subunit did not react with any E. coli protein, it appears that these bacteria lack this subunit. Similarly, we were unable to detect the β subunit in yeast cell extracts (data not shown). Although the presence of the β subunit in other species needs to be further investigated, it is possible that this protein is only present in mammalian tissue. In fact, we have evidence that the β subunit is expressed in mouse lymphoid tissues, and the presence of DNA homology between segments of the MAT II β cDNA and sequences found in randomly sequenced DNA contigs of cDNA libraries from mouse and rat tumors confirm the presence of this protein in other mammals. However, future studies will determine the species and tissue distribution of this protein.

The homology of the MAT II β subunit with bacterial enzymes that catalyze the reduction of TDP-linked sugars (38) is remarkable and may provide clues as to the mechanism by which the β subunit alters the kinetics of the α subunit or may reveal additional functions for this protein. Despite the stretches of identity and strong sequence similarity between these proteins, the anti-MAT II β antibodies do not react with either E. coli or yeast extracts. Further, there is no evidence that other proteins present in E. coli extract interact with the MAT α subunit or with His-tagged recombinant human MAT II αβ subunit expressed in E. coli.

In summary, we have cloned and expressed the β subunit of human MAT II and confirmed its regulatory function. The mode of interaction between the MAT II αβ and β subunits remains to be determined; however, our recent studies suggest that the two subunits spontaneously associate. The association between the human MAT II β subunit and the E. coli MAT α subunit is quite interesting because it may provide important clues as to the structural requirements for the association between the α and β subunits of MAT. The association between the MAT II subunits has a strong effect on the MAT kinetic properties, and we believe that this may be an important means by which the enzyme activity is regulated in vivo and a way to alter AdoMet levels to meet cellular requirements and regulate cell function.

REFERENCES

1. Cantoni, G. L. (1953) J. Biol. Chem. 204, 403–416
2. Mudd, S. H. (1973) The Adenosyltransferases: The Enzymes: Group Transfer (Bayer, P. D., ed) 3rd Ed., Part A, pp. 121–154, Academic Press, New York
3. Finkelstein, J. D., Kyle, W. E., Matin, J. L., and Pick, A. M. (1975) Biochem. Biophys. Res. Commun. 66, 81–87
4. Tabor, C. W., and Tabor, H. (1984) Adv. Enzymol. Relat. Areas Mol. Biol. 56, 251–285
5. Mudd, S. H., Levy, H. L., and Skovby, F. (1995) Disorders of Transsulfuration: The Molecular and Metabolic Basis of Inherited Diseases (Scriber, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) 7th Ed., 1279–1327, McGraw-Hill, Inc., New York
6. Koth, M., and Geller, A. M. (1993) Pharmacol. Ther. 59, 125–143
7. Chiang, P. K., Gordon, R. K., Tal, J., Zeng, G. C., Doctor, B. P., Pardhasaradhi, K., and McCann, P. P. (1996) FASEB J. 10, 471–480
8. Mato, J. M., Alvarez, L., Ortiz, P., and Pajares, M. A. (1997) Pharmacol. Ther. 73, 265–280
9. Hoffman, J. L. (1983) Methods Enzymol. 94, 223–229
10. Mato, J. M., Alvarez, L., Ortiz, P., Mingorance, J., Duran, C., and Pajares, M. A. (1984) Adv. Exp. Med. Biol. 160, 113–117
11. Okada, G., Teraoka, H., and Tsukada, K. (1981) Biochemistry 20, 934–940
12. Koth, M., Mudd, S. H., Mato, J. M., Geller, A. M., Kredich, N. M., Chou, J. Y., and Cantoni, G. L. (1997) Trends Genet. 13, 51–56
13. Horikawa, S., and Tsukada, K. (1991) Biochem. Int. 25, 81–90
14. Alvarez, L., Corrales, F., Martin-Duce, A., and Mato, J. M. (1993) Biochem. J. 283, 481–486
15. Sakata, S. F., Shelly, L. L., Ruppert, S., Schultz, G., and Chou, J. Y. (1993) J. Biol. Chem. 268, 13978–13986
16. Ubagazi, T., Lei, K. J., Huang, S., Mudd, S. H., Levy, H. L., and Chou, J. Y. (1995) J. Clin. Invest. 96, 1943–1947
17. Suma, Y., Shimizu, K., and Tsukada, K. (1986) J. Biochem. (Tokyo) 100, 67–75
18. Pajares, M. A., Corrales, F., Duran, C., and Alvarez, L. (1992) FEMS Lett. 99, 1–4
19. Mingorance, J., Alvarez, L., Pajares, M. A., and Mato, J. M. (1997) Int. J. Biochem. Cell Biol. 29, 485–491
20. Sullivan, D. M., and Hoffman, J. L. (1983) Biochemistry 22, 1636–1641
21. Oxen, K., and Clarke, S. (1983) Biochemistry 22, 2987–2986
22. Crooks, M. A., and Kredich, N. M. (1988) J. Biol. Chem. 263, 3923–3930
23. Langkamp-Henken, B., Geller, A. M., LeGro, H. L., Jr., Price, J. O., De La Rosa, J., and Koth, M. (1994) Biochim. Biophys. Acta 1201, 397–404
24. Liu, M. C., Chang, C. F., Belanger, L., and Grenier, A. (1979) Cancer Res. 39, 162–169
25. Mitsu, K., Teraoka, H., and Tsukada, K. (1988) J. Biol. Chem. 263, 11211–11216
26. Horikawa, S., Sasuga, J., Shimizu, K., Otasa, H., and Tsukada, K. (1990) J. Biol. Chem. 265, 13683–13686
27. Gil, B., Casado, M., Pajares, M. A., Bosca, L., Mato, J. M., Martin-Sanaz, P., and Alvarez, L. (1996) Hepatology 24, 876–81
28. Koth, M., and Kredich, N. M. (1990) Biochim. Biophys. Acta 1039(2), 253–60
29. De La Rosa, J., LeGro, H. L., Jr., Geller, A. M., and Koth, M. (1992) J. Biol. Chem. 267, 10699–704
30. De La Rosa, J., Ostrowski, J., Hryniewicz, M. M., Kredich, N. M., Koth, M., LeGro, H. L., LeGro, J., Valentine, M., and Geller, A. M. (1995) J. Biol. Chem. 270, 21860–8
31. LeGro, H. L., Jr., Geller, A. M., and Koth, M. (1997) J. Biol. Chem. 272, 16040–7
32. Klapper, M. (1977) Biochim. Biophys. Acta 78, 1018–1024
33. Koth, M., Geller, A. M., Markham, G. D., Kredich, N. M., De La Rosa, J., and Beachen, E. H. (1990) Biochim. Biophys. Acta 1040, 137–144
34. Kozak, M. (1989) J. Cell Biol. 109, 229–231
35. Fitzgerald, M., and Shenk, T. (1981) Cell 24, 251–260
36. Proudfoot, N. J., and Brownlee, G. G. (1976) Nature 263, 211–214
37. Maccresi, P., Daude, N., Dalapiccola, B., Novelli, G., Allen, R., Okano, Y., and Reichardt, J. (1998) Mol. Genet. Metab. 63, 26–30
38. Pisowiczki, K., Mansouri, K., and Piepersberg, W. (1991) Mol. Gen. Genet. 231, 113–123