Expression and Functional Interaction of the Catalytic and Regulatory Subunits of Human Methionine Adenosyltransferase in Mammalian Cells*

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Abdel-Baset Halim‡§, Leighton LeGros‡§, Arthur Geller¶, and Malak Kotb‡§**

From the Departments of ‡Surgery, §Microbiology and Immunology, and ¶Biochemistry, University of Tennessee, Memphis, Tennessee 38104 and the §§Veterans Affairs Medical Center, Memphis, Tennessee 38104

Methionine adenosyltransferase (MAT) catalyzes the synthesis of S-adenosylmethionine (AdoMet). The mammalian MAT II isozyme consists of catalytic a2 and regulatory b subunits. The aim of this study was to investigate the interaction and kinetic behavior of the human MAT II subunit proteins in mammalian cells. COS-1 cells were transiently transfected with pTarget vector harboring full-length cDNA that encodes for the MAT II a2 or b subunits. Expression of the His-tagged recombinant a2 (r a2) subunit in COS-1 cells markedly increased MAT II activity and resulted in a shift in the K_m for L-methionine (L-Met) from 15 μM (endogenous MAT II) to 75 μM (r a2), and with the apparent existence of two kinetic forms of MAT in the transfected COS-1 cell extracts. By contrast, expression of the recombinant b (r b) subunit had no effect on the K_m for L-Met of the endogenous MAT II, while it did cause an increase in both the V_max and the specific activity of endogenous MAT. Co-expression of both r a2 and r b subunits resulted in a significant increase of MAT specific activity with the appearance of a single kinetic form of MAT (K_m = 20 μM). The recombinant MAT II a2 and r b subunit associated spontaneously either in cell-free system or in COS-1 cells co-expressing both subunits. Analysis of nickel-agarose-purified His-tagged r a2 subunit from COS-1 cell extracts showed that the β subunit co-purified with the a2 subunit. Furthermore, the a2 and β subunits co-migrated in native polyacrylamide gels. Together, the data provide evidence for a2 and β MAT subunit association. In addition, the β subunit regulated MAT II activity by reducing its K_m for L-Met and by rendering the enzyme more susceptible to feedback inhibition by AdoMet. We believe that the previously described differential expression of MAT II β subunit may be an important mechanism by which MAT activity can be modulated to provide different levels of AdoMet that may be required at different stages of cell growth and differentiation.

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** To whom correspondence should be addressed: University of Tennessee, Memphis, 956 Court Ave. Suite A-202, Memphis, TN 38163. Tel.: 901-448-7247; Fax: 901-448-7208; E-mail: M Kotb@utmem1.utmem.edu.

1 The abbreviations used are: MAT, methionine adenosyltransferase; rMAT, recombinant MAT; r a2, recombinant a2; r b, recombinant β; AdoMet, S-adenosylmethionine; PAGE, polyacrylamide gel electrophoresis.

S-adenosyltransferase (EC 2.5.1.6) catalyzes the biosynthesis of S-adenosylmethionine (AdoMet) from L-methionine (L-Met) and ATP (1). AdoMet is the major methyl donor in transmethylation reactions, including the methylation of DNA, RNA, proteins, and other small molecules. Further, AdoMet is the propylamine donor in the biosynthesis of polyamines (2–5), and it participates as a co-factor in key metabolic pathways (6–8). Inasmuch as AdoMet plays a pivotal role in metabolism, it is not surprising that most species studied to date have more than one MAT isozyme (3).

Mammalian MAT exists in multiple forms that differ in their physical and kinetic properties among distinct species and even among different tissues of the same species. In mammals there are three forms, designated MAT I, II, and III, that differ in their tissue distribution and kinetic properties (7, 9–11). MAT I and III are referred to as the hepatic forms because their expression is confined to the liver. By contrast, MAT II is found in all mammalian tissues that have been examined to date, including erythrocytes, lymphocytes, brain, kidney, testis, and liver (10, 12–18). MAT I is a tetramer and MAT III is a dimer of an identical catalytic subunit, α1, encoded by the MATIA gene (7, 19–22). On the other hand MAT II from leukemic T cells or from activated human lymphocytes is a hetero-oligomer that consists of α2 (53 kDa), α’2 (51 kDa) and β (38 kDa) subunits (13). The α2 and α’2 are the catalytic subunits, whereas β appeared to have a regulatory function (23–25). The α2 and α’2 subunits are immunologically cross-reactive and essentially identical to each other but are quite different from the β subunit. The α2 subunit, which appears to be post-translationally processed to yield α’2 (13), is encoded by the MAT2A gene, which is homologous but different from MATIA gene (11, 19, 24).

Previous studies had shown that physiological activation of human lymphocytes induces down-regulation of the β subunit with coincidental alterations in MAT II kinetic properties (25). We hypothesized that this differential expression of the β subunit may be an important physiological mechanism by which MAT II activity can be modulated. To test this hypothesis, we cloned and expressed both the r a2 (24) and r b subunits** of human MAT II in COS-1 cells, where mammalian post-translational events may affect subunit association and/or enzyme activity. In this study, we provide evidence for the association of MAT II α2 and β subunits with consequent changes in enzyme kinetic and regulatory properties. We believe that this is an important mechanism by which AdoMet levels are controlled during cell growth and differentiation.

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**Materials and Methods**

Cell Culture

The COS-1 cells (African green monkey kidney fibroblasts, ATCC CRL 1650) were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (Atlanta Biologicals) and 10 mM L-glutamine (Cellgro).

Cloning of MAT II α, and β Subunits cDNA into Mammalian Expression Vectors

The full-length cDNA encoding human lymphocyte (HuLy) MAT II α2 protein (24) was cloned into the pQE30 vector (Qiagen) designed to express the protein with a N-terminal six-histidine (His) tag, and we introduced additional bases that encode for an enterokinase site to allow removal of the tag. The HuLy MAT II β cDNA was cloned into the same expression vector without the polyhistidine or the enterokinase site. The recombinant cDNAs encoding either the α2 or the β subunits were transferred from the pQE30 vector to the mammalian expression pTargeT vector (Promega) to generate pTarget/T/MAT2A and pTarget/T/MAT2B, respectively. Briefly, primers were designed to amplify the full-length cDNA encoding either the α or β subunits from the pQE30 vector. Amplification was done using Taq polymerase (Promega) and pfu (Stratagene) in the ratio of 5:1, and the amplified product containing an A-overhang was separated on 1% agarose gel in 0.5 × TBE, purified, ligated into the mammalian expression vector, pTargetE, and used to transform *Escherichia coli* strain JM109 competent cells by heat shock. Positive colonies were selected and subcultured, and the plasmid DNA was purified (Qiagen). The purified plasmid DNA was tested for the presence of the cloned inserts of the correct size and orientation by both PCR and EcoRI restriction site analysis. DNA from six colonies containing the proper insert in the right direction were subjected to manual sequencing of the entire cDNA in both directions using DNA cycle sequencing reagents (Promega), and the sequence was compared with the previously confirmed cDNA sequence for the α2 (24) or for the β subunit sequence to ensure that no mutations were introduced during amplification. One representative clone for either the α2 or the β subunit insert was grown up for large scale preparation of vector (Qiagen).

Transfection of COS-1 Cells

Transfection of COS-1 cells with pTarget/T/MAT2A or pTarget/T/MAT2B was done using the cationic lipid reagent, Transfast (Promega). Preliminary experiments were carried out to optimize the transfection conditions. Typically, 1.5 × 10⁶ cells were plated in a 100-mm dish 1 day prior to transfection. For each plate, 15 μg of vector DNA was mixed with Transfast at a ratio of 1:1 and incubated in a protein-free medium for 2 h. For cells co-transfected with both α2 and β vectors, 12 μg of DNA from each was used. For each experiment, one untransfected plate served as a normal control, and a second plate was transfected with the pTargetE vector only (mock-transfected cells). After 48 h, cells were harvested by trypsinization with a solution containing 0.5% trypsin, and 0.53 mM EDTA in Hanks' balanced salt solution (from Cellgro), and the trypsinization was stopped by the addition of media supplemented with 10% fetal calf serum. The harvested cells were washed three times in 1 ml of Hanks' balanced salt solution, counted, and then resuspended in 1× extraction buffer containing protease inhibitors (50 mM Tris, pH 7.4, 100 mM NaCl, 5 mM MgCl₂, 4 mM dithiothreitol, 0.1 μM aprotinin, 0.5 μM phenylmethylsulfonyl fluoride, and 30 μg/ml soybean trypsin inhibitor). Cells were lysed by three cycles of quick freezing and thawing, and the lysate was centrifuged at 15,000 × g for 10 min at 4 °C. If not immediately used, the cell extracts were stored at -80 °C. Protein assays were performed using the bichromonic acid reagent (Sigma) following the manufacturer's instructions.

Analysis of Expressed MAT II α2 and β Subunits

Western Blot—Extracts from normal, mock-transfected, and α2- or β-transfected COS-1 cells were prepared as described above, and 40 μg of protein from each cell extract were loaded onto 7.5% SDS-polyacrylamide gel (SDS-PAGE) after 1:1 dilution in 2× sample loading buffer (60 mM Tris, pH 6.8, 4% SDS, 5% 2-mercaptoethanol, and 5% glycerol) and boiling in a water bath for 5 min. For 17 × 17-cm gel size, which was 10 min at 100 V, and after the tracking dye left the stacking gel the current was kept constant at 30 mA for the remainder of the run. The gel was electroblotted onto a nitrocellulose membrane (Bio-Rad) for 1.5 h at 400 mA. After blocking overnight in 6% nonfat dry milk in Tris-buffered saline, the blot was sequentially incubated with primary rabbit anti-α2 or anti-β antibodies prepared as described previously (27, 28) followed by a secondary goat anti-rabbit antibody conjugated to horseradish peroxidase (Southern Biotechnology Associates, Inc.). The signal was initiated by the chemiluminescence reagents (ECL, Amersham Pharmacia Biotech) and detected using X-Omat film from Kodak. Molecular weight markers and recombinant MAT II (rMAT II) subunit proteins purified for *E. coli* extracts were used to determine the migration of α2 and β subunits.

Kinetic Properties of Expressed MAT II Subunits—MAT activity in cell extracts was assayed as described previously (13). The assay mixture contained 5 mM ATP, 50 mM KCl, 15 mM MgCl₂, 0.3 mM EDTA, 4 mM DTT in 50 mM TES buffer, pH 7.4. The concentration of 1-Met was varied between 2 and 80 μM using 14C-1-Met (57.9 mCi/mmol) to a concentration of up to 20 μM and supplemented with cold 1-Met for higher concentrations. Enzyme velocity was expressed as units/mg or units/mg protein, where 1 unit is defined as the formation of 1 nmol of AdoMet in 1 h. Calculation of Vₘₐₓ and Vₚₐₜ was also done using the PSI-plot software (Poly Software International) and the Marquardt algorithm.

Analysis of the Expressed MAT II α2 and β Subunit Interaction

Experiments were designed to determine whether the expressed α2 and β subunits of MAT II associate and if their association alters the kinetic properties of the enzyme. Two methods were used to detect subunit association.

Analysis by Native Gel Electrophoresis—Cell extracts were separated on 6% polyacrylamide gel in 1.5× Tris, pH 8.8, under native conditions (without SDS). After blotting onto nitrocellulose membrane, the blots were probed with anti-α2 or anti-β antibodies, stripped, and reprobed with anti-β antibodies. Overlapping signals were taken as an indication of co-migration of both subunits.

Analysis by Nickel-Agarose Bead Affinity Capture—The association between the His-tagged α2 subunit and the nontagged β subunit was analyzed by affinity purification on nickel-agarose beads (Qiagen). In a 15-ml tube, 2 ml of the 50% nickel-agarose slurry was spun down at 1000 rpm for 2 min, and then the pellet was equilibrated with a buffer containing 300 mM NaCl and 50 mM sodium phosphate, pH 8. Cellular extract containing about 10 mg of proteins was added and incubated for 30 min at 4 °C, with mixing on a vertical rotator. The equilibrated gel was poured into the column and allowed to settle. The column was washed several times with 5 ml of equilibration buffer until the absorbance at 280 nm of the go-through material was undetectable. Bound proteins were eluted with 5 ml of 300 mM imidazole, dialyzed against 20 mM Tris, pH 8, and lyophilized. The lyophilized proteins were reconstructed in 25 mM Tris buffer, pH 8, and the protein content was determined by the bichromonic acid reagent (Sigma). Proteins (2 μg each) were analyzed by SDS-PAGE as described above.

Separation of Recombinant and Endogenous MAT II Subunits from COS-1 Cell Extracts and Determination of AdoMet Feedback Inhibition of Enzyme Activity

Experiments were designed to test the effect of β subunit on the feedback inhibition of MAT II (α2 subunit) by AdoMet. However, due to association of α2 with endogenous β, as well as the association of the β with the endogenous α2 protein, it was necessary to purify the subunits away from each other and to test them separately and in combination for kinetic properties and inhibition by AdoMet. Protein extracts from COS-1 cells co-expressing α2 and β subunits was fractionated on nickel-agarose column (Qiagen) as described above. The purified proteins were loaded onto a preparative 7.5% SDS-PAGE and after the separation was complete, and the protein bands were visualized by impregnation in cold 300 mM KCl. The α2 or β bands were excised, and proteins were electroeluted from the gel separately into Tris-glycine buffer, pH 8, and dialyzed against 10 mM ammonium bicarbonate and then lyophilized. The lyophilized subunits were reconstituted, and the MAT assay was performed at 20 μM 1-Met (as mentioned above) for α2 subunit alone or α2 plus β subunits (combined at a molar ratio of 1:1) in the presence of 25–50 μM AdoMet.

**Results**

Detection and Kinetic Analysis of rMAT II α2 Subunit Expressed in COS-1 Cells—COS-1 cells were transfected with pTarget/T/MAT2A plasmid DNA. Protein extracts from untransfected, mock-transfected, and MAT2A-transfected cells were analyzed by Western blots. In untransfected and mock-transfected cells, small amounts of endogenous α2, α2', and β subunits were detected (Fig. 1, lanes 1 and 2); however, cells transfected with pTarget/T/MAT2A expressed abundant...
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Fig. 1. Expression of rMAT II α subunit in COS-1 cells. COS-1 cells were transfected with pTarget/MAT2A vector DNA as described under "Materials and Methods." The cells were harvested after 48 h, the proteins were extracted, and 40 μg of protein were applied to 7.5% SDS-PAGE. The separated proteins were blotted onto a nitrocellulose membrane, which was probed with anti-MAT II α2 and β antibodies. Lane 1, untransfected COS-1 cells; lane 2, mock-transfected cells; lane 3, MAT II α2-transfected cells. The expressed α2 protein migrated at higher than the native α2, since it contains an additional six His residues and an enterokinase site at the N-terminal end.

Fig. 2. Effect of overexpression of MAT II α2 on the enzyme kinetics in COS-1 cells. Cellular protein extracts from untransfected, mock-transfected or pTarget/MAT II A-transfected COS-1 cells were assayed for MAT activity as described under "Materials and Methods," at different concentrations of L-Met. A Lineweaver-Burk plot (1/V vs 1/[L-Met]) was generated. A, normal cells; B, mock-transfected cells; C, α2 cDNA-transfected cells. Enzyme velocity is expressed as units/ml or units/mg of protein, where 1 unit is defined as the formation of 1 nmol of S-adenosylmethionine (AdoMet) in 1 h. Calculation of K_m and V_max was also done using the PSI-plot software (Poly Software International) and the Marquardt algorithm.

amounts of the α2 protein, which migrated with a higher molecular weight due to the additional N-terminal polyhistidine tag and the enterokinase site (Fig. 1, lane 3). To study the effect of overexpression of α2 on MAT kinetics, extracts from untransfected and transfected cells were assayed for MAT activity at different concentrations of L-Met ranging from 2 to 80 μM. Transfection with pTarget/MAT2A caused a 2–3-fold increase in MAT specific activity compared with untransfected cells (50 versus 20 units/mg of protein). In addition, expression of the α2 affected the enzyme K_m for L-Met. MAT II in untransfected and mock-transfected cells had K_m of 15 and 16 μM, respectively (Fig. 2, A and B), which are within the range found in resting human lymphocytes (23, 25). However, in cells expressing high levels of rMAT II α2 protein, two kinetic forms with K_m for L-Met of 17 and 75 μM could be discerned (Fig. 2C).

Detection and Kinetic Analysis of MAT II β Expressed in COS-1 Cells—COS-1 cells were transfected with pTarget/T/ MAT2B plasmid DNA, and the protein extracts from untransfected or transfected cells were analyzed by Western blots. Cells transfected with pTarget/T/MAT2B expressed higher amounts of the β protein compared with untransfected cells (Fig. 3, inset). Expression of MAT II β protein did not significantly affect the kinetic behavior of MAT II in normal cells (Fig. 3) inasmuch as the K_m for L-Met was 20 μM; however, expression of β caused an increase in V_max and a ~2-fold increase in the MAT specific activity at different concentrations of L-Met (35 versus 20 units/mg of protein).

Co-expression of MAT II α2 and β Subunits—COS-1 cells were co-transfected with pTarget/MAT2A and pTarget/T/ MAT2B plasmid DNA, and the protein extracts from untransfected and transfected cells were analyzed by Western blots. As shown in Fig. 4, the cells that were co-transfected with both vectors expressed abundant amounts of the α2 and β proteins compared with untransfected or mock-transfected cells.

Evidence for Association of MAT II α2 and β Subunits—The association between the α2 and β subunits was assessed by different means. First, extracts from COS-1 cells co-transfected with vectors encoding α2 and β subunits were subjected to PAGE gel separation under native conditions and blotted onto a nitrocellulose membrane that was sequentially probed with antibodies to α2 followed by antibodies to the β subunit, with stripping in between the probing. Both antibodies recognized the same bands on the transblot (Fig. 5) and this indicated the co-migration of both subunits under native conditions.

Second, we took advantage of the selective His tag on the α2 subunit but not on the β subunit and used nickel-agarose to affinity-purify the α2 subunit from extracts of COS-1 cells co-expressing α2 and β subunits to test whether the β subunit associates with the α2 subunit. As shown in Fig. 6A, analysis of the protein purified on nickel-agarose columns by SDS-PAGE showed that the β subunit co-purified with the α2 subunit. Interestingly, when extracts from cells, which were individually expressing α2 or β proteins, were simply mixed in a protein ratio of 1:1, the α2 and β subunits also associated and co-purified on the nickel-agarose column (Fig. 6B), thereby indicating the spontaneous association of these proteins.

Together, the data provide evidence that the α2 subunit associates with the β subunit of MAT II. Interestingly, some of the native α2 and α2' subunits were also captured by the His-tagged α2 protein, suggesting heterologous oligomerization of α2 and α2' subunits with each other as well as with the β subunit.

Kinetic Analysis of MAT in Extracts from COS-1 Cells Co-expressing rMAT II α2 and β Subunits—MAT activity was assayed in extracts of COS-1 cells expressing α2 only or co-expressing both α2 and β subunits. MAT activity was increased by approximately 5-fold in the co-transfected cells as compared with untransfected or mock-transfected cells (Fig. 7). Further, co-expression of β subunit caused an increase in MAT activity over that found in cells transfected with α2 alone. The K_m for L-Met in extracts from COS-1 cells co-expressing both subunits indicated the presence of a single kinetic form of MAT II with K_m of 20 μM (data not shown).

Next we investigated whether the β subunit alters the feedback inhibition of MAT II by the product, AdoMet. The activity of the purified α2 with and without β was tested in the absence
or in the presence of AdoMet. To rule out the possible association of either α2 or β subunits with the endogenous MAT II subunits, we performed nickel-agarose column purification of extracts from COS-1 cells co-transfected with both subunits, separated the products on SDS-PAGE, excised the α2 and β bands from the gel, electroeluted the protein, and purified each subunit separately. The purified subunits were mixed in equimolar ratios, and assayed for MAT activity in the presence or in the presence of AdoMet. While the addition of the β subunit significantly increased the catalytic activity of α2 by almost 2-fold, the presence of the β subunit rendered the enzyme more susceptible to AdoMet inhibition, with more than a

![Image](https://example.com/image1.png)

**FIG. 3.** Effect of overexpression of MAT II β on the enzyme kinetics in COS-1 cells. Cellular protein extract from pTargetT/MAT2B-transfected COS-1 cells was assayed for MAT activity as described under "Materials and Methods." Cells were harvested after 48 h, proteins were extracted, and 40 μg were applied to 7.5% SDS-PAGE followed by transblotting onto nitrocellulose membrane, which was probed with anti-MAT II α2 and β antibodies. Lane 1, untransfected COS-1 cells; lane 2, β cDNA-transfected cells.

![Image](https://example.com/image2.png)

**FIG. 4.** Co-expression of rMAT II α2 and rMAT II β subunits in COS-1 cells. COS-1 cells were co-transfected with pTargetT/MAT2A (with His tag and enterokinase site) and pTargetT/MAT2B DNA as described under "Materials and Methods." Cells were harvested after 48 h, proteins were extracted, and 40 μg were applied to 7.5% SDS-PAGE followed by transblotting onto nitrocellulose membrane, which was probed with anti-MAT II α2 and β antibodies. Lane 1, untransfected COS-1 cells; lane 2, α2 and β cDNA-transfected cells.

![Image](https://example.com/image3.png)

**FIG. 5.** Co-migration of MAT II α2 and β subunits on native PAGE. Forty micrograms of protein extracts from untransfected normal or rMAT II α2- or rα2β-expressing cells were applied to 6% native polyacrylamide gel. The transblot was probed with antibody to the rα2 protein (lane 1), developed with ECL, stripped, and then reprobed with antibody to rβ protein (lane 2). A, untransfected cells; B, α2-expressing cells; C, α2β-expressing cells.

![Image](https://example.com/image4.png)

**FIG. 6.** MAT II α2 and β subunits co-purify on nickel-agarose gel. rMAT II α2, but not rMAT II β, was expressed as a poly-His-tagged protein. Cellular protein extracts from untransfected and α2-, β-, and α2β-transfected COS-1 cells were loaded onto nickel-agarose columns. The captured proteins were eluted in 300 mM imidazole, dialyzed, and lyophilized. The reconstituted proteins were applied to SDS-PAGE and transblotted onto nitrocellulose membranes. Transblots were probed with anti-MAT II α2 and β antibodies. Fig. 6A, lane 1, untransfected cell extract; lane 2, untransfected cell extract after separation on nickel-agarose; lane 3, α2-expressing cell extract; lane 4, α2-expressing cell extract after separation on nickel-agarose; lane 5, β-expressing cell extract; lane 6, β-expressing cell extract after separation on nickel-agarose; lane 7, αβ-co-expressing cell extract; lane 8, αβ-co-expressing cell extract after separation on nickel-agarose columns. The same purification procedure was followed for the mixed extracts from the α2- expressing cells and the β-expressing cells, and this was followed by analysis on SDS-PAGE (Fig. 6B). Lane 1, α2-expressing cell extract; lane 2, α2-expressing cell extract after separation on nickel-agarose; lane 3, β-expressing cell extract; lane 4, β-expressing cell extract after separation on nickel-agarose; lane 5, mixed extracts from α2- and β-expressing cells; lane 6, mixed extracts from α2 and β-expressing cells after separation on nickel-agarose column.

![Image](https://example.com/image5.png)

**FIG. 7.** Effect of co-expression of MAT II α2 and β on MAT specific activity in COS-1 cells. Cellular protein extracts were assayed for MAT activity, as described under "Materials and Methods," at different concentrations of L-Met. Normal, untransfected COS-1 cells; mock, mock-transfected; α, α2 cDNA-transfected; β, β cDNA-transfected; αβ, α2 cDNA- and β cDNA-co-transfected COS-1 cells. MAT activity is expressed as units/mg of protein, where 1 unit is defined as the formation of 1 nmol of AdoMet in 1 h.

2-fold increase in the inhibitory effect of AdoMet seen when α2 and β were combined (Fig. 8).

**DISCUSSION**

The essential role of AdoMet in cellular metabolism is underscored by the fact that it participates in as many reactions...
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as ATP and regulates the function of many key molecules and pathways (2, 3, 5, 6). It follows that understanding the regulation of synthesis of this pivotal compound is important. AdoMet is synthesized by MAT, which in mammalian cells exists as at least two isozymes; MAT I/III are confined to hepatic tissue, and MAT II is found in all tissues (3, 5). An emerging theme in the regulation of MAT activity in mammalian cells is that the differential oligomerization of the enzyme subunits can profoundly alter the enzyme physical properties, activity, and kinetic regulation. For example, the α catalytic subunit of the hepatic form of MAT exists either as a dimer (MAT III) or a tetramer (MAT I). This difference in oligomeric forms results in profound changes in the hydrophobic properties of the enzyme; MAT I is nonhydrophobic, whereas MAT III is strongly hydrophobic (7, 29). Furthermore, MAT I is inhibited, while MAT III is activated by AdoMet, and the $K_m$ for L-Met for these two forms is 3–14 μM and 100–200 μM, respectively (16, 23, 30, 31). Although several elegant studies (9, 29, 31–33) have described means for the interconversion of MAT I and III, the physiologic relevance of the need to have these two forms of MAT in the liver remains unclear.

Unlike the hepatic forms of MAT, MAT II, which is present in all mammalian tissues, consists of nonidentical subunits $\alpha_2$ and $\beta$ (13). Previously, we reported that the $\alpha_2$ subunit is catalytic and suggested that the $\beta$ subunit has regulatory properties (13, 23–25). To directly assess the role of each MAT subunit in enzyme activity, it was essential to express these subunits in mammalian cells to ensure proper post-translational modification and to study whether these subunits associate and determine the consequence of this association on the kinetic properties of MAT II.

In this study, we provide evidence that the $\alpha_2$ and $\beta$ subunits of MAT II associate spontaneously and that this association alters the kinetic properties of the enzyme. nickel-agarose capture purification of the His-tagged $\alpha_2\beta$ subunit from COS-1 cells co-expressing $\alpha_2\beta$ and $\beta\beta$ showed that the $\beta$ subunit copurified with the $\alpha_2$ subunit. Interestingly, $\alpha_2$ oligomerized also with the endogenous $\alpha_2$ and $\alpha_2\beta$ subunits, suggesting heterologous oligomerization of $\alpha_2\alpha_2\beta$ and $\beta$ subunits of MAT II. The consequence of the differential oligomerization remains under investigation, but it is noteworthy that in cells expressing abundant amounts of $\alpha_2\beta$ and low levels of endogenous $\beta$ subunit, we could detect two kinetic forms of MAT II with $K_m$ values for 1-Met of 17 and 75 μM. By contrast, in cells co-expressing $\alpha_2\beta$ and $\beta\beta$, only one kinetic form of MAT II was detected with a $K_m$ for 1-Met of 20 μM. We believe that the lower $K_m$ form represents oligomers of $\alpha_2/\alpha_2\beta$ and $\beta$, and the higher $K_m$ form found in cells overexpressing $\alpha_2$ protein represents homo-oligomeric $\alpha_2$ subunits, which are in great excess of the endogenous $\beta$ subunit. This conclusion is consistent with our previous findings that the $K_m$ for rMAT II $\alpha_2$ is 80 μM (24) and that in physiologically stimulated peripheral blood mononuclear cells, where the expression of the $\beta$ subunit is down-regulated and only the $\alpha_2$ subunit is expressed, the $K_m$ for 1-Met shifts from 20 μM to 55–67 μM (25).

Together, the data provide evidence that the MAT II $\beta$ subunit associates with the $\alpha_2$ subunit and lowers the $K_m$ for 1-Met. The association between the $\alpha_2$ and $\beta$ subunit does not appear to require metabolically active cells, since it occurs spontaneously when purified $\alpha_2$ and $\beta$ protein were mixed; however, the molar ratio of each subunit in the holoenzyme remains to be determined. We hypothesize, based on our data, that $\alpha_2$ can exist as homo-oligomers (dimers or tetramers) or as $\alpha_2/\beta$ hetero-oligomers. The relative ratio of either form would depend on the relative molar concentration of $\alpha_2$ to $\beta$ subunits, which as we had previously reported can vary at different stages of lymphocyte activation (25). We believe that this may be an important mechanism by which MAT II can change its kinetic properties to synthesize different amounts of AdoMet, because in addition to its effect on lowering the $K_m$ for 1-Met, the $\beta$ subunit renders the enzyme more sensitive to AdoMet feedback inhibition.

The findings in this study provide a possible explanation for our recent observation that down-regulation of $\beta$ subunit expression in activated lymphocytes results in 5-fold higher AdoMet levels in these cells (25). In these physiologically stimulated cells, the $\beta$ subunit disappears after 72 h, and the $\alpha_2/\beta$ subunits retain MAT activity with a $K_m$ for L-Met of 55–67 μM and are at least 2-fold more resistant to AdoMet feedback inhibition. As a result, there is an accumulation of higher amounts of intracellular AdoMet, reaching up to 100 μM compared with resting lymphocyte levels of 20 μM. Although we have not fully deciphered the physiologic importance of this observation, it is interesting to note that certain methyltransferases, including specific DNA methyltransferases that have a relatively high $K_m$ for AdoMet (26, 34) may be more active at the concentration attained in cells expressing only MAT II $\alpha_2$ subunits.

In conclusion, the data reported here show that the association of MAT II $\alpha_2$ and $\beta$ subunits alters the kinetic and regulatory properties of the enzyme. We believe that this mode of regulation is important for adjusting the levels of AdoMet to meet the cellular requirements at different stages of differentiation and that this may be needed to regulate the expression of certain genes and/or the function of gene products.

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FIG. 8. Effect of rMAT II $\beta$ on the feedback inhibition of MAT II by AdoMet. The MAT assay was conducted as described under “Materials and Methods,” at 20 μM of L-Met. AdoMet was added at a final concentration of 25 or 50 μM to rMAT II $\alpha_2$ in the absence or in the presence of the rMAT II $\beta$ subunit.
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