Differential Regulation of Methionine Adenosyltransferase in Superantigen and Mitogen Stimulated Human T Lymphocytes*

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Superantigens interact with the T cell receptor for antigen (TCR) and are, therefore, more physiological stimulators of T lymphocytes than nonspecific polyclonal T cell mitogens. The effects of these two classes of T cell stimulators on methionine adenosyltransferase (MAT) and S-adenosylmethionine (AdoMet) levels were investigated. Activation of resting human peripheral blood T lymphocytes by the mitogen phytohemagglutinin (PHA) or the superantigen staphylococcal enterotoxin B (SEB) caused a 3- to 6-fold increase in MAT II specific activity. Although the proliferative response was higher in cultures stimulated with PHA compared with SEB, MAT II activity was comparable in both cultures. Both stimuli caused down-regulation of the MAT protein and induced a comparable increase in the expression of the catalytic α2β2 subunit mRNA and protein. However, in superantigen-stimulated cells, the expression of the noncatalytic β subunit was down-regulated and virtually disappeared by 72 h post-stimulation; whereas, no change in the expression of this subunit was noted in PHA-stimulated cells. Thus, at 72 h following stimulation, PHA-stimulated cells expressed MAT II α2β2 and β subunits while SEB-stimulated cells expressed the α2β2 subunits only; the β subunit was no longer expressed in superantigen-stimulated cells. Kinetic analysis of MAT II in extracts of PHA- and SEB-stimulated cells using reciprocal kinetic plots revealed that in the absence of the β subunit the Km of the enzyme for L-methionine (L-Met) was 3-fold higher than in the presence of the β subunit. Furthermore, AdoMet levels were 5-fold higher in cell extracts lacking the β subunit (SEB-stimulated cell extract) compared with extracts containing MAT II α2β2 and β subunits. We propose that the increased levels of AdoMet in superantigen-stimulated cells may be attributed to the absence of the β subunit, which seems to have rendered MAT II less sensitive to product feedback inhibition by (−)AdoMet. The data suggest that the β subunit of MAT II, which has no catalytic activity, may be a regulatory subunit that imparts a lower Km for L-Met but increases the sensitivity to feedback inhibition by AdoMet. The down-regulation of the β subunit, which occurred when T cells were stimulated via the TCR, may be an important mechanism to regulate AdoMet levels at different stages of T cell differentiation under physiological conditions.

Methionine adenosyltransferase (MAT)1 (ATP:L-methionine S-adenosyltransferase, EC 2.5.1.6) is a key enzyme in cellular metabolism because it catalyzes the formation of S-adenosylmethionine (AdoMet) from L-methionine (L-Met) and ATP. In addition to being the major methyl group donor and a precursor for polyamine biosynthesis, AdoMet regulates several important intracellular enzymatic reactions including those involved in polyamine synthesis and one carbon metabolism (1–4). In mammals, there are at least two MAT isozymes, designated MAT I/III and MAT II (5–7), that have distinct tissue distribution, subunit composition, and kinetic properties (for review, see Refs. 3 and 8). MAT I/III represents a different oligomeric state of the same 48-kDa catalytic subunit designated α1 (8). MAT I (200 kDa), which is a tetramer, and MAT III (100 kDa), which is a dimer of the α1 subunit, differ considerably in their physical properties and in their Km for L-Met (4–7, 9–13), suggesting that differences in the oligomeric state of the α1 subunit has a profound effect on the enzyme properties (for review, see Refs. 3 and 4).

The second mammalian MAT isozyme, MAT II, appears to have a wider tissue distribution than MAT I/III and has been detected in many tissues including erythrocytes (14), lymphocytes (15), brain (16), kidney (6, 17), testis (10), lens (18), and fetal liver (19, 20) and to a lesser extent in adult liver (21). Although the α catalytic subunits of MAT I/III and MAT II are similar, they are products of distinct genes designated MAT1A and MAT2A that encode for the α1 and α2 catalytic subunits, respectively (8). The α1 and α2 subunits are only 84% homologous at the amino acid level, and each consists of 395 amino acids with a predicted Mr of 43,600; however, both subunits migrate anomalously on SDS-PAGE with an apparent size of 48–53 kDa (for review, see Ref. 3).

In humans, the α2 subunit of MAT II appears to undergo post-translational modification to generate α2′ subunits (15, 22). In addition, the α2β2 subunits have been shown to be associated with a catalytically inactive (21, 23) 38-kDa subunit that has been designated β (15). This hetero-oligomeric form of MAT II (185 kDa), which consists of both α2 and β subunits, has been found in human lymphocytes, bovine brain, Ehrlich ascites tumor, and calf thymus (15, 16). The exact oligomeric state of MAT II is not entirely clear, and the relative ratio of α2α2′ and β subunits in the holoenzyme is not known. However, based on the available Mr and amino acid composition

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** The abbreviations used are: MAT, methionine adenosyltransferase; AdoMet, S-adenosylmethionine; PBMC, peripheral blood mononuclear cells; SEB, staphylococcal enterotoxin B; PHA, phytohemagglutinin; TCR, T cell receptor for antigen; PAGE, polyacrylamide gel electrophoresis; PCA, perchloric acid; DTT, dithiothreitol; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TES, 2-(2-hydroxyethyl)aminoethanesulfonic acid; TBS, Tris-buffered saline; RT-PCR, reverse transcriptase-polymerase chain reaction; bp, base pair(s); MOPS, 4-morpholinopropanesulfonic acid; HPLC, high pressure liquid chromatography.
data, we hypothesize that it may be in the form of $\alpha_2\beta_2$, $\alpha_2\alpha_3\beta_3$, and/or $\alpha_2\alpha_3\beta_4$, where $n$ is either 2 or 3. Thus, for the time being and for simplicity, we shall refer to this form as $(\alpha_2\alpha_2)\beta_2$.

Studies of MAT II from human leukemia cells revealed that the $(\alpha_2\alpha_2)\beta_2$ form has a low $K_m$ for l-Met (3.5–20 $\mu$M) and is strongly and synergistically inhibited by all three of its end products, P$_A$, PP$_A$, and AdoMet (15, 24). However, recent data showed that recombinant $\alpha_2\alpha_2$ subunits expressed in Escherichia coli had a $K_m$ for l-Met of 80 $\mu$M (23), suggesting that the association of the $\alpha_2\alpha_2$ subunits with the $\beta$ subunit lowers the $K_m$ for l-Met.

Despite the availability of detailed information on the kinetic behavior of pure MAT II, the regulation of MAT II in mammalian tissues remains poorly understood, and the relative contribution of the different MAT subunits to enzyme activity and/or regulation is at present not clear. In an effort to address this problem, we have focused our studies on MAT II from human T lymphocytes. We recently reported that in addition to the $\alpha_2\alpha_2$ and $\beta$ subunits, normal resting human lymphocytes have another 68-kDa protein, which has MAT activity, that we have designated $\lambda$ (21). The $\lambda$ protein is also found in human liver and is the only form of MAT in certain types of myelogenous leukemia cells (21). When T lymphocytes are stimulated to divide, $\lambda$ disappears while the $\alpha_2\alpha_2$ subunits, which have higher MAT activity than $\lambda$, concomitantly increase (21). Activation of T cells and the switch in the expression of MAT subunits is accompanied by a significant increase in enzyme activity as well as AdoMet levels and turnover (21, 25, 26). Inasmuch as AdoMet is a strong inhibitor of MAT, the paradoxical simultaneous increase in both AdoMet turnover and AdoMet levels suggests the existence of a cellular regulatory mechanism that allows MAT to remain active in the presence of high concentrations of its products (24). To further investigate this phenomenon, it was necessary to conduct our investigation under more physiological conditions.

Under physiological conditions, T cells are activated via the T cell receptor (TCR) for antigen. The engagement of TCR by antigen transmits important biochemical signals that, together with the fact that superantigen and T cell receptor (TCR) for antigen. The engagement of TCR by antigen transmits important biochemical signals that, together with the fact that normal resting human lymphocytes can be stimulated by a typical antigen. Consequently, this phenomenon, it was necessary to conduct our investigation under more physiological conditions.

In this study, the effects of a nonspecific mitogen and superantigen activation of T cells on MAT activity and the temporal expression of its subunits were compared. We show that, although both mitogen and superantigen induced an increase in MAT activity, they had different effects on MAT II subunit expression and consequently on the kinetic properties of the enzyme. Specifically, we show that the expression of the $\beta$ subunit is down-regulated in superantigen-stimulated T cells and that this phenomenon is associated with a marked increase in intracellular AdoMet concentration and with a form of MAT II that has a reduced affinity for l-Met but is less susceptible to feedback inhibition by AdoMet. These results suggest that the role of the $\beta$ subunit may be regulatory, and the findings underscore the importance of conducting studies of MAT in T cells under physiological conditions.

**EXPERIMENTAL PROCEDURES**

**Materials**—Aprotinin, antipain, chymotrypsin, leupeptin, pepstatin A, phenylmethanesulfonyl fluoride, benzamidine, $\alpha$-phenanithrolone, biocytin acid, SEB, and PMA were from Sigma. All tissue culture reagents were purchased from Cellgro. The ECL chemiluminescence detection system was from Amersham Life Science, Inc. Nitrocellulose membranes were from Fisher Scientific, and the RX medical-x-ray film was from Fuji.

**Cell Culture**—All cells were cultured in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, 50 $\mu$g/ml streptomycin, and 50 units/ml penicillin (referred to as RPMI complete). PEMC were isolated from peripheral blood by Ficoll-Hypaque density centrifugation, washed twice, and then resuspended in RPMI complete. The cells were adjusted to $1\times 10^6$ cells/ml and stimulated with either PHA (Boehringer Mannheim) or the staphylococcal superantigen SEB (Sigma). At specific times after the initiation of culture, the cells were harvested and used for determination of MAT II activity and kinetic properties, MAT $\alpha_2$ subunit mRNA expression, MAT $\lambda$, $\alpha_2\alpha_2$, and $\beta$ subunits protein expression, and AdoMet levels as detailed below. Parallel cultures were set up in 96-well plates and harvested at 48 or 72 h for assessment of the proliferative response.

**Assessment of Cell Proliferation and Measurement of DNA Content**—Preparation of Cell Extracts—MAT activity was assayed in extracts prepared from cell pellets by three cycles of freeze-thawing. The enzyme extraction buffer consisted of 50 mM Tris, pH 7.4, 50 mM NaCl, 5 mM MgCl$_2$, 4 mM DTI, and a mixture of the following proteolytic inhibitors: 50 $\mu$g/ml aprotinin, 25 $\mu$g/ml leupeptin, 10 $\mu$g/ml phenylmethylsulfonyl fluoride (15, 21). The protein concentration in the cellular extracts was determined by the Bradford method (36) with the dye-binding kit from Bio-Rad and/or by the bicinchoninic acid method (37) from Sigma.

**MAT Assay**—MAT activity was assayed as described previously (15, 21). The standard assay contained 20 $\mu$M l-Met, 5 mM ATP in 50 mM TES buffer, pH 7.4, 50 mM KCl, 15 mM MgCl$_2$, 0.3 mM EDTA, and 4 mM DTI. The Met concentration was varied as indicated in the kinetic analysis. One unit of MAT activity is defined as the amount of enzyme that catalyzes the formation of 1 nmol of AdoMet in 1 h.

**Analysis of Kinetic Data**—A plot was performed both manually by the Line-Weaver-Burk and the Slo $versus$ S plots and confirmed by PSI-Plot software (Poly Software International) and the Marquardt algorithm. The kinetic data were fit to nonlinear least squares analysis to the Adair-Scatchard equation (45) for one and two catalytic sites.

**SDS-PAGE and Western Blotting**—The same cell extracts assayed for MAT activity as described above were diluted in loading buffer (60 mM
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Tris-Ci, pH 6.8, 2% SDS, 5% 2-mercaptoethanol, and 5% glycerol), heated in a boiling water bath for 4 min, and analyzed by SDS-PAGE (10% total acrylamide, 2.7% bisacrylamide) as described previously (21). After electrophoretically the proteins onto the nitrocellulose for 1 h at 25 V/cm, the blots were blocked overnight with 6% nonfat dry milk in TBS (0.025% Tween 20, pH 7.5, and 150 mM NaCl) and incubated with primary polyclonal anti-holoenzyme antisera, or polyclonal antisera generated against synthetic peptides of the α or the β subunits (22). The blots were developed with secondary anti-rabbit antibodies conjugated to horseradish peroxidase and the luminol-chemiluminescence reagents (Amersham ECL) (21). The processed blots were exposed to x-ray film, and the autoradiograms were analyzed. For some experiments, the autoradiograms were scanned using a Hoeftek Scanner-3 scanner (Protein Data Base, Inc., Huntington Station, NY), and the intensity of the desired band was integrated and expressed in arbitrary units.

**Semiquantitation of MAT II α2 Subunit mRNA Expression by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)—Normal, resting PBMC (2×10^6) were stimulated with PHA or SEB for specific time intervals. At the desired times, the cells were harvested and counted, and total RNA was extracted using RNAzol-B (Tel-Test Inc., Friendswood, TX) as described previously (38). The RNA was treated with RNase-free DNase to remove contaminating DNA that could interfere with the PCR analysis. First-strand cDNA was prepared from 2 μg of total RNA using superscript reverse transcriptase (Promega, Madison, WI), and random hexanucleotides (Boehringer Mannheim). The reaction was performed in 20 μl: 2 μl of 10 × PCR buffer (0.2 mM each deoxynucleoside triphosphate, 2.5 mM MgCl2, and 100 μg/ml nuclease-free bovine serum albumin); 2 μl of 10 mM dNTPs mix; 2 μg of random hexanucleotides; 2.5 mM DTT; 1 unit of RNAse inhibitor; and 200 units of DNA polymerase and [α-32P]ATP (Dupont) (38). One microdrop of the PCR reaction was precipitated with 0.5 vol of 10% trichloroacetic acid (TCA), washed in TCA, and counted in a β-counter (21). The absorbance at 260 and 280 nm, and the peaks were identified and integrated using an on-line digital computer and Waters HPLC software. Biologically active (-)-AdoMet, used as the HPLC standard, was prepared using E. coli MAT (10 μg/ml) incubated with 1 mM L-Met in 100 mM Tris, pH 8.3, 100 mM KCl, 20 mM MgCl2, 2.5 mM ATP, 2 mM DTT, and 150 μg/ml of bovine serum albumin, for 1 h at 37 °C and was purified chromatographically on an HPLC C18 column (Waters microBondapak) as described previously (15).

**Statistical Analyses—**Experiments were conducted a minimum of three times, and statistical differences were evaluated by Student's t tests.

**RESULTS**

Effect of Mitogen and Superantigen Activation of T Cells on MAT Activity—Polyclonal mitogens and superantigens activate T cells by distinct mechanisms. Both types of T cell activators stimulate large numbers of T cells to proliferate; however, PHA usually stimulates at least twice as many cells as SEB (34). The difference in the mode of interaction of mitogen and superantigens with T cells can be reflected in the biochemical pathways used to program the cells to divide and/or differentiate (32). To investigate whether mitogen and superantigen may have different effects on the induction of MAT activity, PBMC were stimulated with optimal concentrations of either PHA or SEB, and we then measured the proliferative response and MAT activity. As expected, the proliferative response to PHA was significantly higher than that to SEB (Fig. 1). However, despite this difference in proliferative responses, there was no significant difference in MAT activity in PBMC cultures stimulated with PHA or SEB (p > 0.8, Fig. 2). Both stimuli induced a similar if not a significant increase in MAT activity compared with unstimulated cells. After 48 h in culture with PHA or SEB, MAT activity in PBMC increased by 2.4- and 3.2-fold, respectively, compared with unstimulated PBMC (p < 0.05).

Effect of Mitogen and Superantigen Activation of T Cells on MAT mRNA Expression—Inasmuch as PHA stimulates 80–
90% of resting T cells, and whereas SEB is only capable of stimulating T cells that bear the appropriate Vβ elements (usually 20–40% of resting T cells) (34), the finding that MAT activity was similar in both cultures suggests that, on a per cell basis, SEB may be inducing higher levels of MAT activity than that induced by PHA. Superantigen-stimulated cells may have higher MAT activity than PHA-stimulated cells due to a higher induction of MAT subunit expression or differences in MAT regulation elicited by SEB stimulation. To distinguish between these possibilities, the expression of MAT mRNA and protein was examined in PHA- and SEB-stimulated cells. PBMC were incubated with specific stimulators for 0, 8, 12, 24, 36, 48, and 72 h. At each time point, replicate cultures were harvested, and MAT α2 subunit mRNA expression was determined by RT-PCR and Northern blots. As shown in Fig. 3, the kinetics of α2 subunit mRNA induction by PHA and SEB was similar, peaking at 4–8 h after addition of the stimulus and declining to near base-line levels by 12 h (Fig. 3). Both PHA and SEB induced a 5–8-fold increase in the amount of α2 subunit mRNA compared with unstimulated cells (Fig. 3).

**Effect of Mitogen and Superantigen Activation of T Cells on the Expression of MAT Subunit Proteins**—PBMC were incubated with PHA or SEB for 0, 8, 12, 24, 36, 48, and 72 h. At each time point, cell extracts were analyzed by immunobots to determine the expression of MAT subunit proteins. As shown in Fig. 4, stimulation with either mitogen or superantigen caused a time-dependent decrease in α protein, which nearly disappeared by 48 h after stimulation, while the amount of both α2 and α2′ subunits increased concomitantly, relative to unstimulated cells cultured in medium alone. The relative increase in the α2′ compared with the α2 subunit correlated with a higher MAT activity in SEB-stimulated cultures. However, the most striking difference between extracts of PHA- and SEB-stimulated cells was the finding that stimulation with superantigen induced a dramatic down-regulation of the β subunit expression, which was no longer detectable by 72 h (Fig. 4A). These findings were reproducible when PBMC from different individuals were stimulated with this superantigen (Fig. 4B).

**Kinetic Properties of MAT from Mitogen- and Superantigen-activated Cells**—We reported recently that PHA activated PBMC that expressed high levels of α2/α2′ and β subunits appeared to have two kinetically different forms of MAT with a Km for L-Met of 16 and 80 μM (21). In addition, we reported that recombinant α2/α2′ expressed in E. coli without the β subunit has a Km for L-Met of 80 μM (23). It was of interest, therefore, to determine the consequence of SEB-induced down-regulation of the β subunit on MAT kinetic properties. The Km for L-Met in extracts from 72-h cultures stimulated with either PHA or SEB was compared by the Lineweaver-Burk and the S/V versus S plots, which are commonly used to determine the presence of multiple enzymes catalyzing the same reaction (46). As shown in Fig. 5, MAT in the 72-h PHA-stimulated cell extracts, which consisted of α2/α2′ and β subunits (Fig. 4),
appeared to exist in two kinetic forms. These observations were also confirmed by computer curve fitting by nonlinear least squares. For example, when MAT data from PHA-stimulated cell extracts, which has the $a_2/a_2^9/b$ subunits, were fit to both single- and two-site Adair-Scatchard equations, the data clearly fit the two-site model better as indicated by improvement in correlation coefficient, coefficient of determination and $\chi^2$. By analyzing the values for low (2.5–20 $\mu$M) and high (20–80 $\mu$M) L-Met concentrations separately, the data were consistent with the co-existence of two enzyme species with calculated $K_m$ values for L-Met of 53–74 $\mu$M ($V_{max}$ 9.7–12, correlation coefficient 5 0.998, sum of squares 5 0.071) and 23 $\mu$M ($V_{max}$ 5.3, correlation coefficient 0.999, sum of squares 0.013), respectively. Conversely, the kinetic data of MAT from SEB-stimulated cell extracts that lacked the $b$ subunit clearly fit the single-site model based on the same criteria, with a $K_m$ value of 55–67 $\mu$M and a $V_{max}$ of 12–13.5, respectively (correlation coefficient 0.997). Thus, in the absence of the $b$ subunit, the $K_m$ for L-Met is higher, suggesting that $b$ may be a regulatory subunit of MAT.

Effect of Mitogen and Superantigen Activation of T Cells on Intracellular AdoMet Levels—Intracellular AdoMet levels were determined by HPLC at different times following stimulation of PBMC with either mitogen or superantigen. Levels of AdoMet were higher in PHA- and SEB-stimulated cells compared with unstimulated cells (Fig. 6); however, the levels of AdoMet were markedly higher in SEB-stimulated compared with PHA-stimulated cells (Fig. 6). At 72 h, steady-state levels of AdoMet were 10-fold higher in SEB-stimulated cells compared with control cells ($p < 0.01$) and 4–6-fold higher than in PHA-stimulated cells ($p < 0.02$). The difference between AdoMet levels in cells stimulated with SEB or PHA was highest and more significant at the time point of total disappearance of the MAT II $b$ subunit in SEB-stimulated cells. Based on an estimated volume of 0.69 ml/10$^6$ stimulated PBMC (26, 25), these data indicate that AdoMet concentrations can exceed 100 $\mu$M in cells stimulated via a physiological route.

Effect of AdoMet on MAT Activity in Extracts of Mitogen- and Superantigen-activated Cells—According to the kinetic analysis of $a_2/a_2^9/b$ MAT II from human T cells, AdoMet is a potent inhibitor of its own synthesis, particularly in the presence of Pi and PPi (24). The finding that both MAT activity and AdoMet levels were higher in superantigen-activated compared with mitogen-activated cells suggested that the enzyme may be differentially regulated in cells stimulated by these distinct stimuli. Guided by the knowledge that AdoMet activates hepatic MAT III ($a_1$)$^2$ and moderately inhibits MAT I ($a_1$)$^4$ (6), we hypothesized that in the absence of $b$, the $a_2/a_2^9$ polymeric forms of MAT II may be less susceptible to feedback inhibition by AdoMet. We, therefore, investigated whether the SEB-in-
duced down-regulation of the β subunit has an effect on the feedback inhibition of MAT by AdoMet. As shown in Table I, MAT from 72-h activated SEB extracts (α2/α2β but no β subunits) was almost 2-fold less inhibited by AdoMet compared with MAT from PHA-stimulated cells (α2/α2β and β subunits).

DISCUSSION

Activation of resting T cells triggers biochemical signals that initiate a cascade of events leading to cellular differentiation and proliferation. AdoMet plays a key role in these events, and as the major methyl group donor, it regulates protein activity, RNA stability, and gene expression in addition to acting as a cofactor for several enzymatic reactions and as a precursor for the polyamines (39–41). It is reasonable, therefore, to assume
that the levels of AdoMet need to be tightly regulated to meet specific cellular demands at the different stages of T cell activation and differentiation.

The regulation of cellular AdoMet concentrations can be achieved at the level of its synthesis by MAT and/or its utilization via transmethylation and polyamine synthesis pathways. Our studies have focused on the regulation of MAT activity in human lymphocytes as a means for regulating AdoMet levels. In previous studies, we reported that activation of T cells is accompanied by an increase in MAT activity that was associated with a decline in the \( \gamma \) subunit and a concomitant increase in the \( \alpha_2 \) and \( \alpha_2 \gamma \) catalytic subunits (21). In this study, the use of a superantigen as a T cell stimulator revealed that stimulation of T cells via the physiological route induces specific changes in MAT subunit expression and kinetic and regulatory properties that were not observed in nonphysiologically stimulated, mitogen-activated T cells.

Superantigens are potent T cell stimulators that stimulate T cells in a manner similar to that of conventional antigen because they interact with the T cell via the \( \alpha \beta \) TCR (for review, see Refs. 34 and 35). However, superantigens interact with specific V\( \beta \) elements found on the outside of the variable region of the TCR \( \beta \) chain (V\( \beta \) elements) and, thus, are less specific than antigen. Each superantigen has a characteristic affinity for 1–5 of the 25 human TCR V\( \beta \) elements and can stimulate all T cells expressing them. Consequently, a given superantigen can stimulate from 5 to 40% of resting T cells compared with \( \leq 0.001 \% \) stimulated by an antigen (34). The ability of superantigen to stimulate large numbers of T cells, plus the fact that stimulation is via the TCR, provides an ideal system for studying biochemical events triggered in T cells under conditions that mimic antigenic stimulation (32).

In previous studies, we have shown that the signaling and molecular requirements for T cell stimulation by nonspecific mitogens are different from those for superantigen (31–33). Here we showed that like PHA, superantigen induces down-regulation of \( \lambda \) and up-regulation of \( \alpha \) and \( \alpha' \) expression; however, unlike PHA, superantigen seems to down-regulate the expression of the \( \beta \) subunit. We have reported that \( \lambda \) and \( \alpha_2 \alpha' \) subunits are capable of catalyzing the synthesis of AdoMet; whereas, \( \beta \) subunit has no known catalytic function (21). The data from this study suggest that the \( \beta \) subunit may exert a regulatory effect on the enzyme activity.

Regulation of MAT activity by differential subunit oligomerization has been reported for the hepatic isozymes MAT I/III (4, 6). The tetrameric form, MAT I (\( \alpha_1 \gamma \)) has a \( K_{m} \) for L-Met of 3–14 \( \mu M \), whereas, the \( \alpha_1 \) dimer, MAT III, has a \( K_{m} \) of 200 \( \mu M \) (4, 6, 13). The physiologic relevance for the existence of different oligomeric states of the hepatic form of MAT is not entirely clear; however, these isozymes are starkly different in their kinetic and physical properties as well as in their regulation by AdoMet (4, 6). At physiological concentrations of L-Met, MAT I was reported to be 10-fold more active than MAT III (7). In addition, MAT I is feedback-inhibited by AdoMet, whereas, MAT III shows positive cooperativity and is markedly activated by concentrations of AdoMet between 50–500 \( \mu M \) (6). Whether a balance between MAT I and MAT III is important for normal liver function and metabolism is not entirely clear; however, studies by Mato and coworkers (4, 42–44) revealed that the ratio of MAT I to MAT III can be regulated, in part, by oxido/reduction of the enzyme sulfhydryl group and that the ratio of reduced form (GSH) of glutathione/oxidized form (GSSG) of glutathione can affect the enzyme oligomeric state.

The data presented here, together with several previous observations (21, 23), suggest that MAT II may also be regulated by differential oligomerization of its subunit proteins \( \alpha_2 \), \( \alpha_2 \gamma \), and \( \beta \). In our earlier studies, we reported that MAT II purified from leukemic cells, which consist of the \( \alpha_2 \), \( \alpha_2 \gamma \), and \( \beta \) subunits, had a \( K_{m} \) for L-Met of 3.8 \( \mu M \) (24). Detailed kinetic analysis of this pure enzyme allowed us to develop a steady-state kinetic model that correctly predicted the in situ MAT activity in human leukemia cells expressing the \( \alpha_2 \alpha_2 \gamma \) and \( \alpha_2 \beta \) subunits (25). However, when the model was applied to resting, freshly isolated T cells from healthy individuals, the predicted value for MAT activity was higher than the measured value (25). This difference was explained by the finding that in resting T cells MAT exists as a less active 68-kDa \( \alpha \) form (21, 25). Activation of T cells resulted in the replacement of \( \lambda \) with the \( \alpha_2 \alpha_2 \beta \) subunits (21). The increase in the \( \alpha_2 \alpha_2 \beta \) subunits was accompanied by an increase in MAT activity, suggesting that the \( \alpha_2 \alpha_2 \beta \) subunits are more catalytically active than the \( \lambda \) subunit. Attempts to separate the \( \beta \) subunit from the holoenzyme...
zyme revealed that this subunit has no MAT catalytic activity (21, 23). The possibility that the β subunit may have a regulatory role in MAT II activity was first suggested from experiments showing that purified recombinant α2/α2′ subunits expressed in E. coli had a $K_m$ for L-Met of 80 μM, which was considerably higher than that of the pure α2/α2′ β holoenzyme form (23).

The data presented in this study support the role of the β subunit in regulating MAT activity. In SEB-stimulated cells, β was no longer detected after 72 h of culture, and kinetic analysis revealed that MAT in extracts of these cells has a $K_m$ for L-Met of 55–67 μM and a $V_{max}$ of 12–13.5. By contrast, PHA-stimulated cells, which expressed the α2, α2′, and β subunits, appeared to have two kinetic forms of MAT with $K_m$ for L-Met of 53–74 μM ($V_{max}$ 9.7–12) and 23 μM ($V_{max}$ 5.3), respectively. We propose that at 72 h, the relative amount of α2/α2′ subunits is higher than the β subunit in PHA-stimulated cells, allowing both homomeric and heteromeric association of MAT subunits, where the α2/α2′ homomer has a $K_m$ of 53–74 μM and the α2/α2′ β heteromer has a $K_m$ of 23 μM. Clearly, these possibilities need to be investigated directly with more detailed structural analyses. Nonetheless, the data presented here suggest that the β subunit lowers the $K_m$ of the enzyme for L-Met while rendering it more susceptible to feedback inhibition by AdoMet. The negative regulatory role of β may explain the finding that AdoMet levels were 3–5-fold higher in SEB compared with PHA-stimulated cells. Down-regulation of the β subunit may be required in physiologically stimulated cells to relieve MAT from being feedback inhibited by its product, thereby allowing a concomitant increase in both synthesis and steady-state levels of this important molecule. Indeed, AdoMet levels reached 100 μM in SEB-stimulated cells. It is tempting to speculate that this mode of regulation of MAT via differential subunit association may be necessary to achieve appropriate levels of AdoMet that are required for physiological activation and functional differentiation of T cells at specific stages of the cell cycle.

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