Trans-HHS Workshop:
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Regulation of Mammalian Liver Methionine Adenosyltransferase[1,2]

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ABSTRACT *S*-adenosylmethionine (SAM) is an essential metabolite in all cells. SAM is the most important biological methyl group donor and is a precursor in the synthesis of polyamines. Methionine adenosyltransferase (MAT; EC 2.5.1.6) catalyzes the only known SAM biosynthetic reaction from methionine and ATP. In mammalian tissues, three different forms of MAT (MAT I, MAT III and MAT II) have been identified that are the product of two different genes (*MAT1A* and *MAT2A*). Although *MAT2A* is expressed in all mammalian tissues, the expression of *MAT1A* is primarily restricted to adult liver. In mammals, up to 85% of all methylation reactions and as much as 48% of methionine metabolism occurs in the liver, which indicates the important role of this organ in the regulation of blood methionine. Recent evidence indicates that not only is SAM the main biological methyl group donor and an intermediate metabolite in methionine catabolism, but it is also an intracellular control switch that regulates essential hepatic functions such as liver regeneration and differentiation as well as the sensitivity of this organ to injury. Therefore, knowledge of factors that regulate the activity of MAT I/III, the specific liver enzyme, is essential to understand how cellular SAM levels are controlled. J. Nutr. 132: 2377S–2381S, 2002.

KEY WORDS: • *methionine adenosyltransferase* • *S-adenosylmethionine* • *nitric oxide* • *liver* • *glutathione* • *enzyme regulation* • *free radicals*

INTRODUCTION

Methionine adenosyltransferase (MAT; EC 2.5.1.6) is a central metabolic enzyme that has been exceptionally well conserved through evolution (1). MAT is the enzyme responsible for the synthesis of *S*-adenosylmethionine (SAM) from L-methionine and ATP in a two-step catalyzed reaction (2,3). SAM and tripolyphosphate (PPPi) initially are formed and remain bound to the enzyme until PPPi is hydrolyzed to inorganic phosphate (Pi) and the pyrophosphate ion (PPi) before product release (4). In mammalian tissues, three forms of MAT have been identified (5,6). MAT II, primarily expressed in extrahepatic tissues and fetal liver (7,8), is a heterotetramer formed of catalytic $\alpha 2$ and regulatory β subunits (9,10). In adult liver, MAT exists as a tetramer (MAT I) and as a dimer (MAT III) of a single $\alpha 1$ subunit of 43.7 kDa (11,12). The function of these two isoforms of MAT in the liver has not been yet elucidated. Similar to MAT in other tissues, the hepatic isoforms are responsible for supplying the liver with SAM and polyamines under physiological concentrations of methionine. However, MAT I/III also accounts for the catabolism of the majority of the methionine after feeding a meal rich in protein (13,14). The regulation of this essential hepatic enzyme may facilitate the adaptation of hepatocytes to different metabolic conditions. In this context, it has been shown that the elevation of serum methionine levels in rats fed with methionine-rich diets results in increased MAT I/III activity (15), which may contribute to the clearance of this amino acid. However, the activity of MAT I/III is decreased in cirrhotic patients (16,17) as well as in different experimental models of liver injury, such as alcohol intoxication in baboons (18) or carbontetrachloride (CCl_4) (19), paracetamol (20),

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[4] Abbreviations used: CCl_4, carbontetrachloride; GSH, glutathione (reduced); GSNO, *S*-nitroglutathione; GSSH, glutathione disulfide (oxidized glutathione); H_2O_2, hydrogen peroxide; k_{cat}, turnover number; MAT, methionine adenosyltransferase; *MAT1A*, methionine adenosyltransferase 1A gene; *MAT2A*, methionine adenosyltransferase 2A gene; NO, nitric oxide; Pi, inorganic phosphate; PPi, pyrophosphate ion; PPPi, tripolyphosphate; ROS, reactive oxygen species; SAE, *S*-adenosylethionine; SAM, *S*-adenosylmethionine.

buthionine and sulfoximine (21) intoxication in rats. Under all these conditions there is an increased production of free radicals. Reactive oxygen and nitrogen species induce the inactivation of MAT I/III (22,23), which may prevent an unnecessary consumption of ATP under stress situations in which the availability of ATP, and therefore the viability of hepatocytes, might be compromised. Additionally, inactivation of MAT I/III would reduce hepatic SAM levels, which might be a key factor in regulating essential hepatic functions, such as liver regeneration, differentiation and sensitivity of liver to injury (24,25). The factors involved in the regulation of the activity of MAT I/III have not been completely elucidated. Our interest has been focused on the investigation of the kinetic and structural principles that govern regulation of MAT I/III activity.

REGULATION OF MAT I/III BY FREE RADICALS

Different studies demonstrate that under oxidative/nitrosative stress, MAT I/III activity is markedly decreased. Inhibition of glutathione (reduced) (GSH) synthesis in rats by the administration of L-buthionine-(*S*,*R*)-sulfoximine (21), a specific inhibitor of γ-glutamyl cysteine synthetase, or the depletion of GSH levels by CCl_4 treatment (19) leads to a marked reduction of MAT I/III activity. Additionally, increased production of nitric oxide (NO) in the liver during septic shock or hypoxia is associated with the inactivation of hepatic MAT; whereas inhibition of nitric oxide synthase by N^G-nitro-L-arginine methyl ester prevented MAT inactivation in response to hypoxia (26–28). Alcoholic cirrhosis in humans, which is accompanied by the formation of free radicals and GSH depletion (29), also is associated with reduced MAT I/III activity (16,17) and impaired methionine metabolism (30,31). The correlation between the formation of free radicals, as well as a depletion of GSH levels, and MAT I/III inactivation agrees with the finding that different thiol-reacting compounds, including fumarylacetoacetate (32), *p*-(cholormercuri)-benzoate (5), oxidized glutathione (33), H_2O_2 (22) and NO (26), reduce MAT activity. This evidence suggests that the redox state of the cysteine residues plays a key role in the control of MAT I/III activity. Although the hepatic MAT α subunit contains 10 cysteine residues, reactive oxygen and nitrogen species, including hydroxyl radical, NO, and peroxynitrite inactivate the enzyme by specific interaction only with one of these cysteines (at position 121), which is conserved in rat, mouse and human MAT I/III (34–36). All other known sequences of MAT contain a glycine instead of a cysteine residue at this position (37). Site-directed mutagenesis studies demonstrate that the enzymatic activity of a recombinant hepatic MAT in which cysteine 121 was substituted by a serine residue is not decreased by H_2O_2 or the NO donors *S*-nitroso-*N*-acetyl-penicillamine and *S*-nitrosoglutathione (GSNO) (22,26). Furthermore, replacement of cysteine 121 by serine had no effect on MAT activity (38), indicating that this residue is not essential for enzyme catalysis. Inactivation, however, was not prevented by replacement by serine with any of the other nine cysteine residues of the MAT I/III subunit (22,26).

MAT I/III is regulated by *S*-nitrosylation, through a mechanism similar to that demonstrated for hemoglobin, cardiac calcium release channel, and caspase 3 (39–42). MAT I and MAT III, purified from rat liver, are inactivated in terms of SAM synthesis by the NO donors *S*-nitroso-*N*-acetyl-penicillamine (26) and GSNO (23). Substitution of cysteine 121 by a serine residue protected MAT I/III from inactivation by these NO donors (23,26). Incubation with 100 μM GSNO inactivates MAT I and MAT III by ~70%, but whereas MAT I incorporates one NO per subunit, in MAT III, three *S*-nitroso groups per enzyme subunit are incorporated (23). Different accessibility to *S*-nitrosylation of cysteine residues located in the interaction surfaces between dimers and therefore not accessible in the tetrameric form might account for the different number of the *S*-nitroso groups formed. Incubation with saturating (millimolar) concentrations of GSNO leads to the formation of eight *S*-nitroso groups per subunit in both MAT isoforms (23). This finding agrees with the observation that MAT I/III contains 10 cysteine residues, two of which appear to form an intrasubunit disulfide bridge, probably between cysteine residues 35 and 67 (43). *S*-nitrosylation of both MAT I and MAT III is reversed by millimolar concentrations of GSH (23,26). This observation might raise doubts about the implication of *S*-nitrosylation on the regulation of MAT activity in vivo. However, intraperitoneal injection of bacterial lipopolysaccharide into rats resulted in the accumulation of nitrites and nitrates in serum and in the inactivation of MAT I/III (23). The analysis of MAT III purified from lipopolysaccharide-treated animals revealed a marked increase in the *S*-nitrosylation of this enzyme in the presence of normal cellular concentrations of GSH and GSSG (i.e., oxidized glutathione) (23). Additionally, incubation of isolated rat hepatocytes with *S*-nitrosoglutathione monoethyl ester, an NO donor permeable to the cell membrane, induced a five- to eightfold increase in the hepatocyte NO content that promotes MAT *S*-nitrosylation and inactivation (23). Inactivation of MAT by *S*-nitrosylation induced a fivefold (80%) reduction of the SAM content of hepatocytes within 15 min. (44), which agrees with the observation that the half-life of hepatic SAM is only ~5 min (14). Removal of the NO donor from the incubation media led to the denitrosylation and reactivation of MAT and to the rapid recovery of the cellular SAM levels (44). Reversible inactivation of MAT I/III by *S*-nitrosylation then arises as a mechanism to regulate the hepatic content of SAM. Recent data also demonstrate that incubation of isolated rat hepatocytes with buthionine (*S*,*R*)-sulfoximine or its intraperitoneal injection into rats induced a reduction of hepatic GSH and led to the *S*-nitrosylation and inactivation of hepatic MAT (45). Restoration of GSH levels in hepatocytes and rats by treatment with the monomethyl ester of glutathione reversed MAT *S*-nitrosylation and inactivation (45). These observations suggest that, in the cell, MAT can exist as two forms in equilibrium: active and inactive. This equilibrium can be modified by alteration of the NO levels or by a depletion of the intracellular GSH. Therefore, an increase of the NO levels or a depletion of the cellular GSH will switch the equilibrium toward the inactive, nitrosylated form, whereas a reduction of NO content or replenishment of GSH levels results in the denitrosylation and reactivation of MAT I/III (**Fig. 1**).

As indicated above, inactivation of MAT I/III by oxidation or nitrosylation occurs by the specific and covalent modification of cysteine 121. According to the structural model of hepatic MAT (22) based on the crystal structure of the *Escherichia coli* MAT (46,47), this cysteine is located at a flexible loop over the active-site cleft of the enzyme (46,47). This loop can adopt two different conformations—open and closed—and it has been proposed that in the closed conformation, access of the substrates to the active site is prevented (37). Nitrosylation or oxidation of cysteine 121 might induce a conformational change in the flexible loop, making the active site less accessible for methionine and ATP, probably by switching the loop into the closed conformation. Alternatively, it has been demonstrated that loops located over the

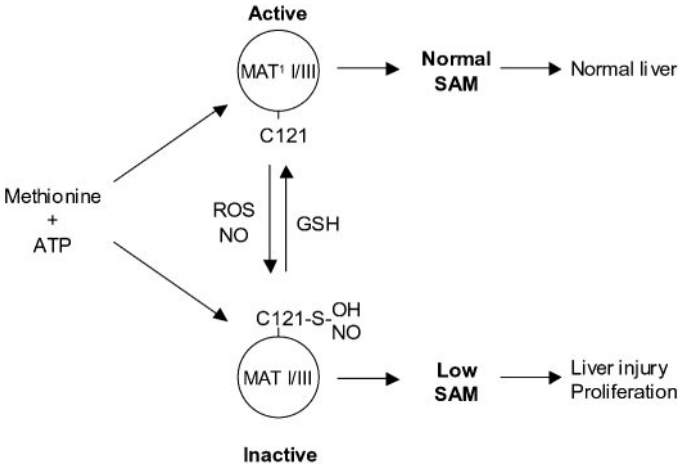

FIGURE 1 Regulation of MAT I/III by reactive oxygen species and NO. Regulation of MAT I/III by oxidation or nitrosylation of Cys121 might have important physiopathological consequences. With hypoxia or septic shock, the hepatic production of NO and/or reactive oxygen species will be increased, switching MAT into the less active conformation. This would contribute to regulating the hepatic utilization of ATP and therefore to preventing nicotinamide adenine dinucleotide depletion and mitochondrial denergization (i.e., a drop of ATP levels) during the stress imposed by either oxygen- or nitrogen-reactive species. Additionally, because SAM is associated with such hepatic functions as regeneration, differentiation and liver injury, the inactivation of MAT I/III by ROS and NO might be a key signal to respond to injury. C121-S-OH/NO, oxidation or nitrosylation of cysteine residue at position 121. GSH, glutathione (reduced); MAT, methionine adenosyltransferase; NO, nitric oxide; ROS, reactive oxygen species; SAM, S-adenosylmethionine.

active site of different enzymes play a central role in the binding of substrates or in catalysis (48–51). Therefore, it is worth considering that the flexible loop of MAT may be involved in the synthesis of SAM or in the association of methionine and/or ATP with the enzyme. According to this work, oxidation or nitrosylation of cysteine 121 may compromise the functionality of this loop, resulting in a reduced capacity of SAM synthesis. Studies to determine which hypothesis can explain these observations are in progress. As previously mentioned, it has been proposed that protein *S*-nitrosylation involves an acid-base-catalyzed nitrosothiol *S*-nitrosothiol/thiol exchange reaction, in which the target cysteine residue is localized next to basic and acidic amino acids that reduce the pKa of the thiol group (52). In MAT I/III, cysteine 121 is not flanked by acidic and basic amino acids. However, arginine residues 357 and 363 as well as aspartic acid 355 configure the tridimensional microenvironment of cysteine 121 (44). Replacement of these residues by serine markedly reduces the capacity of GSNO to *S*-nitrosylate and inactivate MAT (44). According to these observations, the guanidino groups of arginine 357 and 363 may facilitate the deprotonation of the sulfur group of cysteine 121. This will increase the nucleophilicity of this residue (by lowering its pKa) and consequently facilitate the nitrosylation of its sulfur group. The function of the γ-carboxylic acid group of the aspartic acid 355 may be to facilitate the protonation of GSNO and, accordingly, facilitate the donation of the NO group. Recognition of this topology is likely to prove useful in identifying new targets of protein *S*-nitrosylation. Additionally, the definition of this structural motif might facilitate the design of new *S*-nitrosylation sites in proteins that are not regulated by NO. As previously mentioned, cysteine 121 is specific to human, rat and mouse MAT I/III, whereas all other known sequences of this enzyme contain a glycine instead of a cysteine residue at this position (37). However, arginine 357, arginine 363 and aspartic acid 355, which facilitate the *S*-nitrosylation of cysteine 121 in MAT I/III, are conserved in MAT II. Replacement of glycine 120 by cysteine results in a MAT II mutant protein that incorporates ~1 mol of SNO/mol of MAT subunit and is 80% inactivated after incubation with micromolar concentrations of GSNO. The wild-type enzyme is not nitrosylated or inactivated after incubation with GSNO. As is the case with the liver enzyme, the nitrosylation and inactivation of the MAT II mutant by GSNO is reversed by 2 mM GSH (53).

MAT I/III is responsible for the catabolism of as much as 48% of the ingested methionine that is converted into SAM at the expense of ATP. Regulation of MAT I/III by oxidation or nitrosylation of cysteine residue 121 might have important physiopathological consequences. With hypoxia or septic shock, the hepatic production of NO and/or reactive oxygen species will be increased, switching MAT into the less active conformation. In this state, the consumption of ATP by MAT would be reduced. This would contribute to regulating the hepatic utilization of ATP and, therefore, to preventing nicotinamide adenine dinucleotide depletion and mitochondrial de-energization during the stress imposed by either oxygen- or nitrogen-reactive species. Indeed, overexpression of rat MAT I/III cDNA in Chinese hamster ovary cells led to ATP and nicotinamide adenine dinucleotide depletion and increased the sensitivity of the cells to oxidative stress (22,54). Additionally, because SAM is associated with such hepatic functions as regeneration, differentiation and liver injury (24,25,55,56), the inactivation of MAT I/III by reactive oxygen species (ROS) and NO might be a key signal in responding to injury.

HYSTERETIC BEHAVIOR OF METHIONINE ADENOSYLTRANSFERASE. REGULATION OF MAT ACTIVITY BY METHIONINE CONCENTRATIONS

MAT catalyzes two consecutive reactions in vivo, the synthesis of SAM and tripolyphosphate and the hydrolysis of the latter to PPi and Pi (2). This tripolyphosphatase activity can be studied in vitro by using tripolyphosphate as substrate. Information about the catalytic mechanism and its regulation can thus be obtained by using different substrates—ATP and methionine or tripolyphosphate.

MATIII shows different responses over time with different substrates. It is linear with tripolyphosphate, but a lag phase is present with ATP and methionine. After the lag phase, the k_{cat} or turnover of the enzyme is faster than the time needed to reach steady-state activity. This result indicates that the lag phase is due to a slow transition, probably a change in conformation of the enzyme, rather than to the accumulation of a reaction intermediate. The lag phase is not affected by SAM and does not depend on the protein concentration, which rules out a change in the oligomeric state of MAT. On the other hand, the lag phase is decreased by increasing concentrations of substrate, suggesting induction of a conformational change after substrate binding rather than binding to one of the species of a pre-existing equilibrium.

Comparison of the time course of both SAM synthetase activity and tripolyphosphatase activity indicates that they are very similar initially, but whereas the tripolyphosphatase activity remains the same, the SAM synthetase activity increases with time (lag phase). This result suggests that ATP and/or

methionine induces a stimulation of MAT that does not take place with tripolyphosphate alone. To test this hypothesis, MATIII was incubated in the presence of ATP and methionine before the measurement of the tripolyphosphatase activity. Both ATP and methionine are required to stimulate the enzyme, whereas ADP and methionine or SAM are unable to produce any significant effect. A fourfold stimulation in enzyme, with a half-time of 1.5 min, is obtained. The increase in activity, similar to the ratio of steady-state SAM synthetase to tripolyphosphatase activities, as well as the half-time, suggest that the stimulation of tripolyphosphatase activity and the lag phase of SAM synthetase activity are the same process. After stimulation, tripolyphosphatase and SAM synthetase activity are the same. Because SAM synthetase activity measures the rate-determining step, identical activities indicate that hydrolysis of tripolyphosphate is the rate-determining step. Otherwise, tripolyphosphatase activity should be faster than SAM synthetase activity.

As mentioned above, *S*-nitrosylation of MATIII inhibits SAM synthetase activity (23). To understand the mechanism of inhibition, characterizing the NO effect on tripolyphosphatase activity as well as its stimulation should be of great value. The basal nonstimulated tripolyphosphatase activity of MATIII is not inhibited by *S*-nitrosylation. Thus, NO must affect the synthetic reaction, the stimulation process and/or catalysis of the stimulated enzyme. The tripolyphosphatase activity is the same when the enzyme is *S*-nitrosylated after being stimulated than when it is stimulated after *S*-nitrosylation. In both cases the activity is intermediate between nonstimulated and stimulated MATIII. These results indicate that NO directly inhibits tripolyphosphatase activity of the stimulated enzyme but not the stimulation process itself (57). After *S*-nitrosylation, stimulated tripolyphosphatase and SAM synthetase activities remain the same, indicating that NO does not change the rate-determining step. Thus, if NO inhibits the synthesis of SAM, this reaction still is faster than is the hydrolysis of tripolyphosphate.

Although the concentration of ATP in hepatocytes remains more or less constant, methionine levels fluctuate over a wider range of concentrations (58). It is appealing to propose methionine concentration in hepatocytes as a regulatory element of MATIII activity. Supporting this proposal is the fact that stimulation of tripolyphosphatase activity takes place in a physiologically significant methionine concentration. To reach 50% of stimulation, ~100 μM methionine is needed, suggesting that under normal physiological concentrations, 50–80 μM methionine (58), MATIII should have very low activity. To test this hypothesis in vivo, MAT activity was measured in intact hepatocytes as follows: two groups of hepatocytes were suspended in media containing 10 or 500 μM methionine for 20 min. After this time, the cells were washed and fresh media containing 2 mM ethionine, without methionine, was added. Cell samples were taken at several intervals to measure their *S*-adenosylethionine (SAE) content (44). MAT is able to use ethionine to produce SAE (59), which is not metabolized and accumulates in the cell. Thus, SAE accumulation reflects MAT activity without interference by the many enzymes that use SAM. According to the proposed model, even though ethionine concentration is the same, it takes longer to reach steady-state activity in cells that have been incubated in a low methionine concentration (**Fig. 2**). This indicates that MAT in the cells previously incubated with high methionine concentration already was stimulated when ethionine was added. The short delay observed to reach the final activity in these cells is probably due to the time needed for ethionine to accumulate in the hepatocytes.

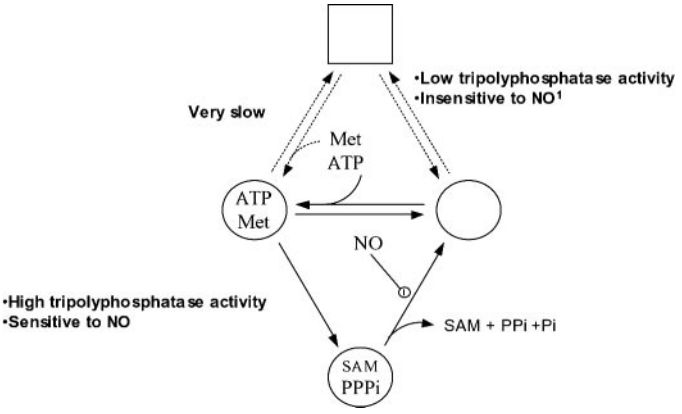

FIGURE 2 Proposed model for MATIII action. Under normal physiological conditions (i.e., low methionine), a low tripolyphosphatase activity MATIII conformation is present that is insensitive to NO. Under this condition, SAM concentration in hepatocytes is probably maintained by the small amount of MATI present. When methionine concentration increases, such as after a protein-rich meal, it binds together with ATP to MATIII and induces a conformational change. The resulting enzyme has a high tripolyphosphatase activity that is inhibited by NO. The step most affected by NO is the hydrolysis of tripolyphosphate. Once excess of methionine is eliminated, MATIII would slowly go back to the low-activity form. Low-activity MAT I/III is represented by a box; high-activity MAT I/III is represented by a circle. del Pino, M. M., Corrales, F. J. & Mato, J. M. (2000) Hysteretic behavior of methionine adenosyltransferase III. Methionine switches between two conformations of the enzyme with different specific activity. J. Biol. Chem. 275: 23476–23482, with permission. NO, nitric oxide; Pi, inorganic phosphate; PPi, pyrophosphate ion; PPPi, tripolyphosphate; SAM, *S*-adenosylmethionine.

The results described here can be summarized in the model depicted in Figure 2. Under normal physiological conditions (i.e., low methionine), a low tripolyphosphatase activity MATIII conformation is present that is insensitive to NO. Under these conditions, SAM concentrations in hepatocytes are probably maintained by the small amount of MATI present. When methionine concentration increases, such as after a protein-rich meal, it binds together with ATP to MATIII and induces a conformational change. The resulting enzyme has a high tripolyphosphatase activity that is inhibited by NO. The rate-determining step, and the one most affected by NO, is the hydrolysis of tripolyphosphate. Once excess of methionine is eliminated, MATIII would slowly go back to the low-activity form (57).

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