THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 24, Issue of June 11, pp. 17075–17079, 1999

Printed in U.S.A.

Methionine Adenosyltransferase *S*-Nitrosylation Is Regulated by the Basic and Acidic Amino Acids Surrounding the Target Thiol*

(Received for publication, December 18, 1998, and in revised form, April 12, 1999)

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_S_-Adenosylmethionine serves as the methyl donor for many biological methylation reactions and provides the propylamine group for the synthesis of polyamines. _S_-Adenosylmethionine is synthesized from methionine and ATP by the enzyme methionine adenosyltransferase. The cellular factors regulating _S_-adenosylmethionine synthesis have not been well defined. Here we show that in rat hepatocytes _S_-nitrosoglutathione monoethyl ester, a cell-permeable nitric oxide donor, markedly reduces cellular _S_-adenosylmethionine content via inactivation of methionine adenosyltransferase by _S_-nitrosylation. Removal of the nitric oxide donor from the incubation medium leads to the denitrosylation and reactivation of methionine adenosyltransferase and to the rapid recovery of cellular _S_-adenosylmethionine levels. Nitric oxide inactivates methionine adenosyltransferase via _S_-nitrosylation of cysteine 121. Replacement of the acidic (aspartate 355) or basic (arginine 357 and arginine 363) amino acids located in the vicinity of cysteine 121 by serine leads to a marked reduction in the ability of nitric oxide to _S_-nitrosylate and inactivate hepatic methionine adenosyltransferase. These results indicate that protein _S_-nitrosylation is regulated by the basic and acidic amino acids surrounding the target cysteine.

In the liver *S*-adenosylmethionine (AdoMet)[1] serves as the methyl donor for many biological methylation reactions (such as DNA, proteins, phospholipids, and adrenergic, dopaminergic, and serotoninergic molecules) and provides the propylamine group for the synthesis of polyamines (1–3). AdoMet is synthesized from methionine and ATP by the enzyme methionine adenosyltransferase (MAT). There are two MAT genes; one is expressed only in the liver, and the other is expressed in extrahepatic tissues and fetal liver (2–4). Up to 85% of all methylation reactions and as much as 50% of methionine metabolism occur in the liver (5), which agrees with this tissue having the highest specific activity of MAT (6). Moreover, in the liver the half-life of AdoMet is of only about 5 min (6).

Reduced levels of AdoMet and/or MAT activity, resulting in the abnormal metabolism of methionine, have been found in human cirrhosis and in a variety of experimental models including liver injury induced by ethanol, CCl_4, and galactosamine (3, 7). The importance of this alteration in AdoMet synthesis in the pathogenesis of a variety of liver disorders is suggested by the finding that exogenous AdoMet administration protects from the hepatotoxic effect induced by a variety of agents, such as ethanol, CCl_4, paracetamol, tumor necrosis factor, and galactosamine (3, 7). The cellular factors regulating hepatic AdoMet levels are beginning to be defined. One such factor is nitric oxide (NO). In previous studies we demonstrated that conditions that induce NO synthesis, such as septic shock and hypoxia, induce the inactivation of hepatic MAT without affecting the expression of the liver-specific MAT gene (8, 9). Further, we have reported previously that incubation of rat hepatocytes with *S*-nitrosoglutathione monoethyl ester (EGSNO), a cell-permeable NO donor, induces MAT inactivation (10). We have also shown that purified rat liver MAT is inactivated by incubation with NO donors (3-morpholinosydnonimine, *S*-nitroso-*N*-acetylpenicillamine, and *S*-nitrosoglutathione) (8, 10). In addition, we have recently demonstrated that liver MAT is *S*-nitrosylated both *in vitro* and *in vivo* (10), and further, we have identified cysteine 121 as the site of molecular interaction of NO and liver MAT (8). Because these results indicate that NO (or related molecules) is a critical regulator of liver MAT activity, we were interested in whether AdoMet content in hepatocytes is regulated by NO.

There are few studies trying to identify the active site features that control protein *S*-nitrosylation. It has been recently proposed (11) that protein *S*-nitrosylation involves an acid-base-catalyzed nitrosothiol (SNO)/SH exchange reaction, where the target cysteine residue is localized next to basic and acidic amino acids. Here, we were interested in determining the structural factors that govern liver MAT *S*-nitrosylation and inactivation using liver recombinant enzyme and mutants of MAT where the acidic and basic amino acids in the vicinity of cysteine 121 were replaced by serine by site-directed mutagenesis. Recognition of the topology involved in protein *S*-nitrosylation is likely to prove useful in identifying new targets of protein *S*-nitrosylation.

EXPERIMENTAL PROCEDURES

Isolation and Incubation of Hepatocytes—Hepatocytes were isolated from normally fed Wistar rats (250 g) as described previously (10). Isolated hepatocytes were incubated in the absence or presence of EGSNO at 37 °C. At the indicated periods of time, 2 ml of the cell suspension (2×10^6 cells/ml) were poured into precooled centrifuge tubes and washed twice with phosphate/saline buffer. Hepatocytes were then used for AdoMet or MAT activity determinations. Cell viability was determined before and after incubations by the trypan blue exclusion test. Only preparations with viability over 85% were used.

AdoMet Measurements—AdoMet concentration was determined by high pressure liquid chromatography following the procedure described by Fell *et al.* (12) modified by Miller *et al.* (13). Samples of 2×10^6

* This work was supported by the Plan Nacional de I+D (SAF 98/132), Europharma, and Knoll. 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.

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[1] The abbreviations used are: AdoMet, *S*-adenosylmethionine; NO, nitric oxide; MAT, methionine adenosyltransferase; SNO, nitrosothiol; EGSH, glutathione monoethyl ester; GSNO, *S*-nitrosoglutathione; EGSNO, *S*-nitrosoglutathione monoethyl ester; WT, wild type; NOx, nitrite and nitrate.

hepatocytes washed twice in phosphate/saline buffer were homogenized in 200 μl of 0.4 M perchloric acid. Homogenates were centrifuged at 10,000 × *g* and 4 °C for 15 min. 100 μl of the supernatant were analyzed on a Bio-Sil® ODS-5S column equilibrated in 0.01 M ammonium formate, 4 mM heptanesulfonic acid, pH 4.0. Elution was carried out with a 31-min linear gradient of acetonitrile (0–25%) in the same buffer. Chromatograms were analyzed using the Beckman System Gold software.

Site-directed Mutagenesis; Purification and Characterization of MAT Mutants—Mutants are identified by a number that indicates the residue in the sequence of the enzyme that has been replaced. MAT mutants C121S, D355S, D355E, R357S, R363S, R363K, G359S, and R357S/R363S were obtained by inverse polymerase chain reaction according to the procedure of Serrano *et al.* (14) using the plasmid pSSRL, which includes a 1.2-kilobase pair fragment containing the rat liver MAT coding region (15). The mutants were identified by sequencing of the complete MAT cDNA. Expression and purification of WT (wild type) and mutant proteins were carried out as described previously (15). Protein purity was greater than 95% in all preparations as determined by SDS-polyacrylamide gel electrophoresis. Protein concentration was measured using the Bio-Rad protein assay kit based on the Bradford assay (16). The recombinant WT and MAT mutants analyzed presented similar values of specific activity: 4.36 ± 0.28, 4.19 ± 0.72, 3.97 ± 0.91, 4.22 ± 0.31, 3.89 ± 0.74, 4.23 ± 0.41, 4.89 ± 0.52, 4.01 ± 0.67, and 4.12 ± 0.37 nmol/min/mg protein for WT, C121S, D355S, D355E, R357S, R363S, R363K, G359S, and R357S/R363S, respectively. To exclude that mutant proteins presented any major alteration in their tertiary structure, fluorescence analysis was performed using a Perkin-Elmer LS-5B fluorimeter. Emission spectra were recorded between 290 and 440 nm in 5 mM Tris, pH 7.4, with excitation wavelength set at 280 nm. Protein concentration was 0.02 mg/ml. No differences were observed when the spectra of recombinant WT and MAT mutants were compared.

Determination of MAT S-Nitrosylation—MAT *S*-nitrosylation was measured according to the procedure described by Ruiz *et al.* (10). Briefly, samples of purified recombinant MAT (0.4 mg/ml) were incubated with *S*-nitrosoglutathione (GSNO) at 37 °C for 10 min. The excess GSNO was removed by DEAE chromatography. *S*-Nitrosylated proteins were incubated with 2.2 mM $HgCl_2$, and the amount of NO released was measured by the chemiluminescence derived from its reaction with ozone using a Sievers NOA 280 nitric oxide analyzer. Standard curves were obtained with known concentrations of GSNO.

MAT Activity Measurements—MAT activity was measured as described by Cabrero *et al.* (17) using saturating concentrations of the substrates, 5 mM methionine and 5 mM ATP, in the absence of any reducing agent. Samples of 2×10^6 hepatocytes, prepared as mentioned above, were washed twice in phosphate/saline buffer and homogenized by freezing and thawing in 10 mM Tris/HCl, 0.3 M sucrose, and 1 mM EGTA, pH 7.5, containing 0.1% phenylmethylsulfonyl fluoride and 0.1% benzamidine. Cytosol was obtained by centrifugation at 100,000 × *g* at 4 °C. Excess EGSNO was removed by DEAE chromatography on a 1-ml column (10), and the enzyme activity was measured. Samples of purified WT and mutant recombinant rat liver MAT were incubated for 10 min at 37 °C in the presence or absence of GSNO or peroxynitrite. After incubation, excess GSNO or peroxynitrite was removed by DEAE chromatography (10), and the enzyme activity was measured.

Preparation of GSNO, EGSNO, and Peroxynitrite—GSNO and EGSNO were prepared according to Ruiz *et al.* (10). Briefly, equimolar concentrations of an aqueous solution of $NaNO_2$ and a freshly prepared GSH or EGSH solution in 250 mM HCl and 0.1 M EDTA, pH 1.5, were mixed. The resulting mixture was incubated at room temperature for 5 min and then neutralized with NaOH. Peroxynitrite synthesis was carried out by the method described by Beckman *et al.* (18) modified by Pannala *et al.* (19). Only freshly prepared solutions of GSNO, EGSNO, and peroxynitrite were used.

Cytosolic Nitrite and Nitrate Measurements—Cytosolic nitrite and nitrate (NOx) were reduced to NO by incubation with 1 N HCl containing 50 mM VCl_3 (10). The resulting NO was measured by the chemiluminescence derived from its reaction with ozone using a Sievers NOA 280 nitric oxide analyzer (10).

RESULTS

NO Reduces AdoMet Content in Hepatocytes—The effect of NO on the content of AdoMet was examined in isolated rat hepatocytes. Incubation of rat hepatocytes with the cell-permeable NO donor EGSNO (5 mM) induced a time-dependent reduction of the cellular AdoMet content (Fig. 1). Within 15 min of the addition of EGSNO to the incubation medium, the hepatocyte content of AdoMet decreased about 80%, *i.e.* from 1.03 ± 0.08 nmol of AdoMet/10^6 cells in control hepatocytes to 0.22 ± 0.035 nmol of AdoMet/10^6 cells in hepatocytes treated with the NO donor (Fig. 1). The reversibility of the depletion of AdoMet by EGSNO in isolated rat hepatocytes was examined by removing the NO donor from the incubation medium and resuspending the cells in fresh buffer without EGSNO (Fig. 1). Within 15 min of the removal of the NO donor and resuspension of the cells in fresh medium without EGSNO, cellular AdoMet content returned close to that of untreated hepatocytes (Fig. 1). When hepatocytes were maintained in the presence of the NO donor, the depletion of the cellular content of AdoMet was maintained during the next 15 min (Fig. 1). As previously demonstrated (10), incubation with EGSNO within 15 min induced the inactivation of MAT (from 36.4 ± 0.5 pmol/min/mg of protein in control hepatocytes to 10.95 ± 2.8 pmol/min/mg of protein in the presence of 5 mM EGSNO). Removal of the NO donor from the incubation medium returned MAT activity to values similar to those of the untreated hepatocytes (29.18 ± 1.1 pmol/min/mg of protein) within 15 min.

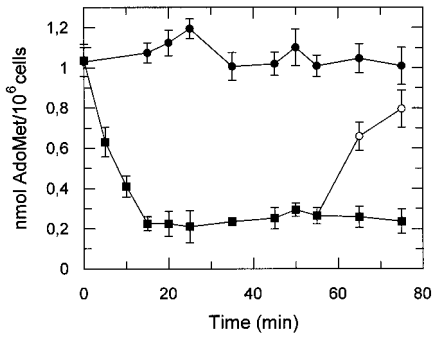

FIG. 1. **Effect of EGSNO on AdoMet content in isolated rat hepatocytes.** Isolated rat hepatocytes were incubated in the absence (●) or in the presence (■) of 5 mM EGSNO. After incubation with EGSNO for 55 min, The NO donor was removed from the medium by centrifugation, and after washing the cells twice, the hepatocytes were resuspended in the absence (○) or presence (■) of 5 mM EGSNO. AdoMet content was determined as described under "Experimental Procedures" at the times indicated in the figure. Values are expressed as means ± S.E. of three independent experiments in duplicate.

We next analyzed whether the effect of EGSNO on AdoMet content in hepatocytes was dose-dependent (Fig. 2). The addition of EGSNO (0.5–5 mM) to isolated rat hepatocytes induced a dose-dependent accumulation of NOx, which led to a progressive depletion of AdoMet content (Fig. 2). Significant reduction of the AdoMet content in hepatocytes was already observed at 1 mM EGSNO, a condition where intracellular NOx increased about 5-fold. The addition of EGSNO to isolated rat hepatocytes induced, as described previously (10), a dose-dependent inactivation of MAT activity (data not shown).

The Structural Factors That Govern Liver MAT S-Nitrosylation—*S*-Nitrosylation of recombinant WT rat liver enzyme and mutants of MAT was measured after reaction with various concentrations of GSNO (5–100 μM). In WT rat liver, MAT incubation for 10 min with 100 μM GSNO produced the *S*-nitrosylation of about 1.3 thiol residues/enzyme subunit (Fig. 3). Incubation with GSNO also induced the inactivation of the enzyme. As shown in Fig. 4, there is a close inverse correlation between the extent of *S*-nitrosylation and loss of activity induced by GSNO ($r = 0.95$, $p < 0.001$). Incorporation of 1 mol of SNO/mol of MAT subunit led to about 80% inactivation of MAT. Maximal *S*-nitrosylation and inactivation were obtained within 10 min of incubation with GSNO (data not shown). We have previously shown (8) that replacement of cysteine 121 by serine (referred to as C121S) prevented the ability of NO donors to

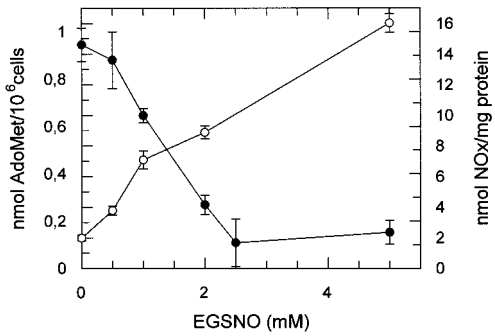

FIG. 2. **Effect of EGSNO on AdoMet content and NOx levels.** Hepatocytes were incubated with various concentrations of EGSNO. Cytosolic AdoMet (●) and NOx (○) content were determined after 20 min of incubation with the NO donor as described under "Experimental Procedures." Values are expressed as means ± S.E. of three independent experiments in duplicate.

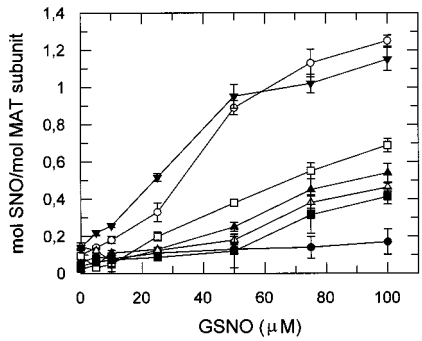

FIG. 3. **Dose-dependent *S*-nitrosylation of WT and mutant rat liver MAT by GSNO.** Recombinant MAT purified from *Escherichia coli* transformed with WT and mutant cDNA were incubated in the presence of various concentrations of GSNO for 10 min. The formation of SNO groups was determined as described under "Experimental Procedures." Values are expressed as means ± S.E. of three experiments in triplicate using different preparations of the enzyme. ○, WT; ●, C121S; ■, R363S/R357S; △, R357S; ▲, R363S; □, D355S; ▼, R363K. Mutants are identified by a number that indicates the amino acid residue in the sequence of the enzyme that has been replaced.

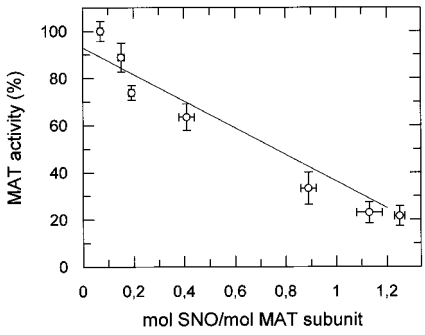

FIG. 4. **Correlation between extent of MAT *S*-nitrosylation and loss of activity.** MAT purified from *E. coli* transformed with WT mutant cDNA was incubated in the presence of various concentrations of GSNO (5–100 μM) for 10 min at 37 °C. The enzyme activity and the formation of SNO groups were then determined as described under "Experimental Procedures." MAT activity is expressed as the percent of activity remaining after treatment with GSNO. Values are the mean ± S.E. of three different enzyme preparations ($r = 0.95$; $p < 0.001$).

inactivate hepatic MAT. Consistently, incubation of C121S with GSNO (5–100 μM) resulted in the incorporation of only about 0.2 mol of (SNO) per mol of MAT subunit. The mechanism of protein *S*-nitrosylation has been recently proposed (11) to involve an acid-base-catalyzed SNO/SH exchange reaction, where the target cysteine residue is located next to basic and acid amino acids. In human and rat liver MAT, cysteine 121 is

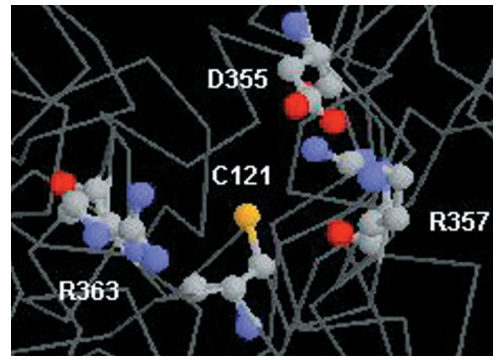

FIG. 5. **A model structure of rat liver MAT.** The structural detail of the microenvironment of cysteine 121 is based on a model structure for the rat liver MAT subunit published previously (32) using the data available for the x-ray crystal structure of *E. coli* MAT (33, 34). This figure was prepared with Ras Mol (35). The position of cysteine 121 (*C121*), arginine 357 (*R357*), arginine 363 (*R363*), and aspartic acid 355 (*D355*) are indicated. According to this model, the distances between the SH group of cysteine 121 and the guanidino groups of arginine 357 and arginine 363 are 3.3 and 4.4 Å, respectively. The distance between the SH group of cysteine 121 and the γ-COOH of aspartic acid 355 is 4.9 Å.

not flanked by acid and basic amino acids but by glutamine and valine (20–22). However, when the three-dimensional structure of liver MAT is analyzed, one residue of aspartic acid (Asp-355) and two residues of arginine (Arg-357, Arg-363) are all found in the vicinity of the sulfur group of cysteine 121 (Fig. 5). Replacement of aspartic acid 355 by serine (D355S) markedly reduced the *S*-nitrosylation of the mutant enzyme in the presence of GSNO (Fig. 3). Replacement of arginine 357 (R357S) or of arginine 363 (R363S) by serine led also to a marked reduction of the *S*-nitrosylation of the mutant enzyme as compared with the WT liver MAT (Fig. 3). Further, a double mutant, where arginine 357 and arginine 363 were both replaced by serine (R357S/R363S) incorporated only about 0.4 mol of SNO/mol of MAT subunit after incubation with 100 μM GSNO (Fig. 3). As negative controls, we have replaced arginine 363 by lysine (R363K) and aspartic acid 355 by glutamic acid (D355E) and observed that these conservative changes had no effect on the *S*-nitrosylation of the mutant enzymes by GSNO as compared with the WT liver MAT. Fig. 3 shows the results obtained with the mutant enzyme R363K; similar results were observed with the mutant enzyme D355E (data not shown). As an additional control we have changed glycine 359, a noncharged residue that is separated by 5.3 Å from the thiol group of cysteine 121, to serine (G359S) and observed that this mutation had no effect on the *S*-nitrosylation of the mutant enzyme by GSNO (data not shown). These controls provide evidence that the effects on MAT *S*-nitrosylation of changing arginine 357, arginine 363, or aspartic acid 355 to serine are due to loss of charge and not to alterations in the position of cysteine 121. Finally, similarly to the WT recombinant MAT, incubation of mutants D355S, D355E, R357S, R363S, R363K, G359S, and R357S/R363S with millimolar concentrations of GSNO resulted in the incorporation of about 10 mol of SNO/mol of MAT subunit, indicating that all cysteine residues present in the protein (21) were *S*-nitrosylated.

We next analyzed whether these mutants showing impaired MAT *S*-nitrosylation were resistant to GSNO-induced enzyme inactivation. Whereas incubation of the WT recombinant enzyme with 50 μM GSNO led to 70% reduction of the enzyme activity, replacement of cysteine 121 by a serine residue prevented the ability of GSNO to inactivate liver MAT (Fig. 6). Replacement of aspartic acid 355, arginine 357, or arginine 363 by serine led to a marked reduction of the ability of GSNO to inactivate liver MAT (Fig. 6). Further, replacement of both

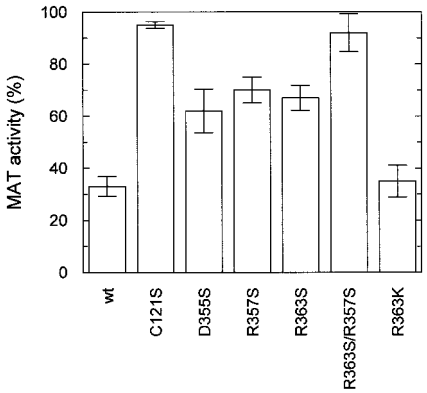

FIG. 6. **Inactivation of WT and mutant rat liver MAT by GSNO.** MAT purified from *E. coli* transformed with WT and mutant cDNA were incubated in the absence or presence of 50 μM GSNO for 10 min. The enzyme activity was then measured as described under "Experimental Procedures." Results are expressed as the percent of activity remaining after treatment with GSNO for each individual mutant. Values are expressed as mean ± S.E. of three different preparations. Mutants are identified by a number that indicates the amino acid residue in the sequence of the enzyme that has been replaced.

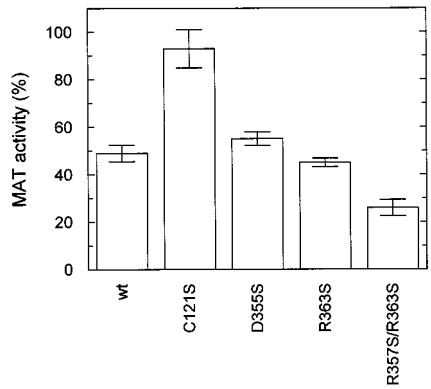

FIG. 7. **Inactivation of WT and mutant rat liver MAT by peroxynitrite.** MAT purified from *E. coli* transformed with WT and mutant cDNA were incubated in the absence or presence of 50 μM peroxynitrite for 10 min. The enzyme activity was then measured as described under "Experimental Procedures." Results are expressed as the percent of activity remaining after treatment with peroxynitrite for each individual mutant. Values are expressed as mean ± S.E. of three different preparations. Mutants are identified by a number that indicates the amino acid residue in the sequence of the enzyme that has been replaced.

arginine 357 and arginine 363 by serine was as effective as the substitution of cysteine 121 by serine to prevent GSNO-induced MAT inactivation (Fig. 6). On the contrary, replacement of arginine 363 by lysine, aspartic acid 355 by glutamic acid, or glycine 359 by serine did not affect the ability of GSNO to inactivate liver MAT. Fig. 6 shows the results obtained with the mutant enzyme R363K; similar results were observed with the mutant enzymes D355E and G359S (data not shown).

Incubation of WT liver MAT for 10 min with 50 μM peroxynitrite also induced the inactivation of the enzyme (Fig. 7). As expected, incubation of WT liver MAT with peroxynitrite did not result in the *S*-nitrosylation of the enzyme (data not shown). Whereas replacement of cysteine 121 by serine prevented the ability of peroxynitrite to inactivate liver MAT, replacement of aspartic acid 355 or arginine 363 by serine, or replacement of both arginine 357 and arginine 363 by serine had no effect on the inactivation of the enzyme by peroxynitrite (Fig. 7). These results indicate that whereas arginine 357, arginine 363, and aspartic acid 355 are needed for transnitrosation of cysteine 121, these residues are unneeded for the interaction of this thiol with peroxynitrite. Moreover, these results provide further evidence that in these mutants cysteine 121 is not buried in the protein.

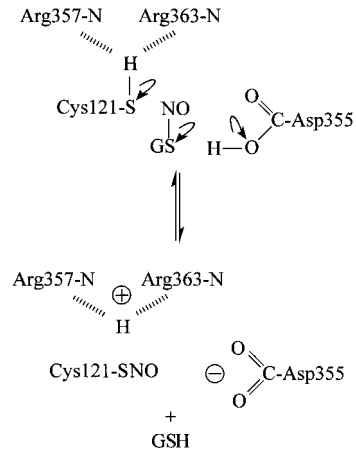

FIG. 8. **A model for the reaction of cysteine 121 with GSNO.** Whereas the guanidino moiety of arginines 357 and 363 facilitates the deprotonation of the sulfur group of cysteine 121 and thus increases its nucleophilicity, the γ-COOH group of aspartic acid 355 facilitates the protonation of GSNO and consequently enhances the donation of its NO group.

DISCUSSION

Our results indicate that NO or related molecules can regulate AdoMet content in hepatocytes via reversible NO-mediated *S*-nitrosylation and inactivation of hepatic MAT. An increase in the hepatic levels of NO induces a rapid *S*-nitrosylation and inactivation of hepatic MAT (10), which leads to a rapid depletion of the AdoMet content. Conversely, the elimination of the NO source leads to the rapid denitrosylation and activation of hepatic MAT (10) and to the rapid increase of the AdoMet content. This agrees with the observation that the half-life of hepatic AdoMet is only about 5 min (6). Further, our results indicate that a moderate increase in the hepatocyte levels of NO, as observed during incubation with 1 mM EGSNO, is sufficient to have a significant effect on AdoMet content. We have previously established (9) that in rat hepatocytes kept under low oxygen levels MAT is inactivated. Further we also found that in rat hepatocytes hypoxia induced the expression of NO synthase and that the inactivation of MAT during hypoxia was prevented by the NO synthase inhibitor N^G-monomethyl-L-arginine methyl ester (9). Elevated levels of NO or related molecules may contribute to the abnormal metabolism of methionine in patients with liver cirrhosis. Hypermethioninemia, reduced hepatic MAT activity, and decreased content of liver AdoMet have been detected in patients with liver cirrhosis and/or in experimental models of chronic liver injury (23–28). In patients with liver cirrhosis and in experimental models of chronic liver disorders serum levels of NOx are elevated (29, 30). The serum NOx levels in patients with liver cirrhosis have been reported to increase up to 10-fold (from 3 μM in control subjects to 34 μM in cirrhosis) (30). Although it is not known what may be the concentration of NOx in the cirrhotic liver, the present results indicate that 50 μM GSNO inactivate liver MAT by about 60%. These data are evidence for a pathophysiological interruption of AdoMet metabolism by NO.

NO inhibits many enzymes by *S*-nitrosylation of active site and regulatory thiols (31). It is not yet known, however, what structural factors govern the modification of a specific protein cysteine residue by NO. An acid-base-catalyzed SNO/SH exchange reaction, where the target cysteine residue is located next to basic and acid amino acids, has been recently proposed

(11). The NO-mediated inactivation of hepatic MAT appears to occur through *S*-nitrosylation of the residue of cysteine 121 of the enzyme (8, 10). Our results indicate that the substitution of cysteine 121 by serine prevents MAT *S*-nitrosylation and inactivation by GSNO. Further we show that there is a close inverse correlation between the extent of *S*-nitrosylation and loss of activity. Cysteine 121 is localized at a "flexible loop" over the active site cleft of MAT (32–34). This loop can adopt two different conformations, open and closed, and it has been proposed that in the closed conformation prevents the entrance of the substrates to the active site (34). Cysteine 121 is not essential for activity because the substitution of this amino acid residue by serine has no effect on MAT activity (15). The present results suggest that formation of a SNO group in cysteine 121 induces a conformational change in the "flexible loop" making less accessible the active site of the enzyme for the substrates, probably by switching the loop into the closed conformation. Replacement of the acidic amino acid (Asp-355) or of any of the two basic amino acids (Arg-357, Arg-363) localized in the proximity of cysteine 121 by serine markedly reduced the capacity of GSNO to *S*-nitrosylate and inactivate liver MAT. Further, our results indicate that GSNO-induced MAT *S*-nitrosylation and inactivation are greatly prevented in a double mutant enzyme where arginine 357 and arginine 363 were both replaced by serine. When the changes did not involve loss of charge (arginine 363 by lysine and aspartic acid 355 by glutamic acid) these mutations had no effect on the *S*-nitrosylation and inactivation of the mutant enzymes by GSNO. Our results also indicate that the change of a non-charged amino acid (glycine 359) in the vicinity of cysteine 121 by serine did not affect *S*-nitrosylation and inactivation of the mutant enzyme by GSNO. We also show that peroxynitrite-induced MAT inactivation involves the reaction of this oxidizing agent with cysteine 121. In this case, however, replacement of arginine 357, arginine 363, or aspartic acid 355 by serine did not affect peroxynitrite-induced MAT inactivation. These results indicate that the decreased GSNO-mediated MAT *S*-nitrosylation and inactivation seen following replacement of acidic and basic residues in the vicinity of cysteine 121 with serine are because of loss of charge and are not because of structural changes that alter the position or otherwise protect the thiol. One possible explanation of these findings is that the function of the guanidino groups of arginine 357 and arginine 363 may be to facilitate the deprotonation of the sulfur group of cysteine 121 (Fig. 8). This will increase the nucleophilicity of cysteine 121 (by lowering its pK_a) and consequently facilitate the nitrosylation of its sulfur group. The function of the γ-COOH group of aspartic acid 355 may be to facilitate the protonation of GSNO and, accordingly, facilitate the donation of its NO group. These results provide the first experimental evidence that, as proposed by Stamler *et al.* (11), the *S*-nitrosylation of protein thiol residues is governed by the basic and acidic amino acids surrounding the target thiol. Recognition of this topology is likely to prove useful in identifying new targets of protein *S*-nitrosylation.

Acknowledgments—We thank Prof. Manuel Martín Lomas and Dr. Dacil Zurita for the synthesis of EGSH.

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