Biochemical Basis for the Dominant Inheritance of Hypermethioninemia Associated with the R264H Mutation of the MAT1A Gene

A MONOMERIC METHIONINE ADENOSYLTRANSFERASE WITH TRIPOLYPHOSPHATASE ACTIVITY*

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Isabel Pérez Mato‡, Manuel M. Sánchez del Pino§, Margaret E. Chamberlin, S. Harvey Mudd, José M. Mato, and Fernando J. Corrales**

From the Division of Hepatology and Gene Therapy, University of Navarra, Irunlarrea 1, 31008 Pamplona, Spain, the University of Tennessee Medical School, Veterans Affairs Medical Center/Research 151, Memphis, Tennessee 38104, and the Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, Maryland 20892-4634

Methionine adenosyltransferase (MAT) catalyzes the synthesis of S-adenosylmethionine (AdoMet), the main alkylation agent in living cells. Additionally, in the liver, MAT is also responsible for up to 50% of methionine catabolism. Humans with mutations in the gene MAT1A, the gene that encodes the catalytic subunit of MAT I and III, have decreased MAT activity in liver, which results in a persistent hypermethioninemia without homocystinuria. The hypermethioninemic phenotype associated with these mutations is inherited as an autosomal recessive trait. The only exception is the dominant mild hypermethioninemia associated with a G-A transition at nucleotide 791 of exon VII. This change yields a MAT1A-encoded subunit in which arginine 264 is replaced by histidine. Our results indicate that in the homologous rat enzyme, replacement of the equivalent arginine 265 by histidine (R265H) results in a monomeric MAT with only 0.37% of the AdoMet synthetic activity. However, the tripolyphosphatase activity is similar to that found in the wild type (WT) MAT and is inhibited by PPi. Our in vivo studies demonstrate that the R265H MAT I/III mutant associates with the WT subunit resulting in a dimeric R265H-WT MAT unable to synthesize AdoMet. Tripolyphosphatase activity is maintained in the hybrid MAT, but is not stimulated by methionine and ATP, indicating a deficient binding of the substrates. Our data indicate that the active site for tripolyphosphatase activity is functionally active in the monomeric R265H MAT I/III mutant. Moreover, our results provide a molecular mechanism that might explain the dominant inheritance of the hypermethioninemia associated with the R264H mutation of human MAT I/III.

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** To whom correspondence should be addressed: Division of Hepatology and Gene Therapy, University of Navarra, Irunlarrea 1, 31008 Pamplona, Spain. Tel.: 34-948-425678; Fax: 34-948-425677; E-mail: fjcorrales@unav.es.
1 The abbreviations used are: MAT, methionine adenosyltransferase; AdoMet, S-adenosylmethionine; DTT, dithiothreitol; P_i, orthophosphate; PPi, tripolyphosphate; WT, wild type; PAGE, polyacrylamide gel electrophoresis.

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Characterization of the R265H MAT Mutant

Segregation and mutation analysis revealed that in most of the individuals with MATIA mutations the hypermethioninemic phenotype is transmitted as an autosomal recessive trait (22–25, 28). However, a dominant inherited form of this abnormality has been reported in five families (21, 22, 26, 28–30). In each such family a G-A transition at nucleotide 791 was detected in one MATIA allele. This change results in a MAT in which arginine 264 is replaced by histidine (26). Crystallographic studies of Escherichia coli and recombinant rat liver MAT show that the equivalent arginine 244 or 265, respectively, is located in the interface between the two subunits of the dimeric enzyme and is involved in a salt bridge with glutamic 42, or the homologous 58 in the rat enzyme, of the symmetric subunit, which contributes to the stabilization of the oligomeric state of MAT (31, 32, 40). Moreover, arginine 244 contributes to each active site and is located in the immediate vicinity of the polyphosphate group of ADP (4, 31). It has been shown that replacement of arginine 264 by histidine in human MAT inactivates the enzyme. Moreover, it has been proposed that this mutation hinders normal oligomeric formation (26). However, substitution of arginine 244 by leucine or histidine in E. coli MAT resulted in an inactive enzyme, which, in contrast, remains tetrameric with no apparent changes in the secondary structure (4). To understand the biochemical basis of the dominant inheritance of the phenotype associated with the R264H mutation of human MAT I/III, we have purified and characterized the homologous rat R265H MAT I/III mutant. Our data indicate that the active site for the triphosphatase activity is functionally active in the monomeric R265H MAT I/III mutant and provide a molecular mechanism that might explain the dominant inheritance of the hypermethioninemia associated with the R264H mutation in human MAT I/III.

EXPERIMENTAL PROCEDURES

Materials—Columns and chromatography media were from Amersham Pharmacia Biotech. AdoMet was from Boehringer Ingelheim (Knoll). All other reagents were from Sigma.

Site-directed Mutagenesis—A 1.2-kilobase fragment containing the rat MATIA coding region (35) was subcloned into a pET vector. The resulting plasmid includes a 5′-sequence that encodes for 6 histidine residues and a thrombin cleavage site in frame with the rat liver MATIA coding region. Mutants were obtained by inverse polymerase chain reaction according to the procedure of Perez-Mato et al. (34). The mutants were identified by sequencing the complete MAT cDNA.

Expression and Purification of His-tagged WT and MAT I/III Mutants—WT and MAT I/III mutant proteins were overexpressed in E. coli BL21(DE3) as described previously (33). Recombinant His-tagged WT and MAT I/III mutant proteins were purified from the bacterial cytosolic extracts by affinity chromatography on a Ni²⁺-Sepharose column equilibrated in 50 mM Tris/HCl, pH 8, 0.5 mM NaCl, 75 mM imidazole. Elution was performed using a linear gradient from 75 to 500 mM equilibrated in 50 mM Tris/HCl, pH 8, 0.5 mM NaCl, 75 mM imidazole, and 0.5-mL fractions were collected. Protein elution was monitored by measuring the absorbance at 280 nm. The protein-containing fractions were pooled, and aliquots were analyzed by size exclusion chromatography and SDS-PAGE (35). Heterodimer formation was also tested in vitro in two ways. In the first, MAT III purified from rat liver was incubated with equimolar concentration of the R265H mutant His-tagged protein (0.2 mg/mL) in 50 mM Tris/HCl, pH 7.5, 150 mM KCl, 10 mM MgCl₂, 5 mM DTT for 30 min at 25 °C. Alternatively, MAT III purified from rat liver was denatured by incubation with 5 M urea in 50 mM Tris/HCl, pH 7.5, 150 mM KCl, 10 mM MgCl₂, 5 mM DTT, and then refolded by 20-fold dilution (the final concentration of WT MAT subunits was 0.6 μM) in the same buffer containing a 3-fold molar excess of His-tagged R265H MAT I/III mutant subunit, followed by incubation for 30 min at 25 °C. The two mixtures were then re-purified on a Ni²⁺ column as indicated above. Imidazole was removed from the retained fractions using a 5-mL Hi-trap desalting cartridge. Protein was then denatured by adding urea to a final concentration of 5 M and chromatographed again on a 1-mL Ni²⁺-Sepharose column equilibrated with 50 mM Tris/HCl, pH 8, 0.5 mM NaCl, 75 mM imidazole. Flow-through and imidazole eluted fractions were analyzed, in both experiments, by SDS-PAGE (35).

Purification of MAT from Rat Liver—MAT III was purified from rat liver according to the procedure described previously (33). Protein purity was more than 95% as estimated by SDS-PAGE (35).

Size Exclusion Chromatography—Protein samples were analyzed using a Superdex 200 HR 10/30 column equilibrated with 50 mM Tris/HCl, pH 7.5, 150 mM KCl, 10 mM MgCl₂ in an AKTA fast protein liquid chromatography (Amersham Pharmacia Biotech). After sample injection, proteins were isocratically eluted at a flow rate of 0.8 mL/min. Fractions of 0.2 mL were collected. Protein elution was monitored by measuring the absorbance at 280 nm. The elution volume (in mL) of the standard proteins were: tiroglobulin (669 kDa), 8.1; ferritin (440 kDa), 10.22; catalase (232 kDa), 12.11; ovalbumin (43 kDa), 14.73; chymotrypsin A (25 kDa), 18; ribonuclease A (13.7 kDa), 19. According to the elution volume of the standard proteins, the estimated molecular mass of the R265H MAT I/III rat mutant protein was 41 kDa. AdoMet synthetic as well as triphosphatase activities were measured in the collected fractions.

Enzymatic Activity Measurements—Activity assays were performed in a final volume of 100 μL, at 37 °C for 10 min in 50 mM Tris/HCl, pH 8, 4 mM MgCl₂, 250 mM KCl, 3.6 mM DTT, 2 mM ATP/MgCl₂ and methionine or 2 mM triphosphate were used as substrates to determine AdoMet synthetase or triphosphatase activities, respectively. Both activities were monitored by following the formation of inorganic phosphate according to the method of Lanzetta (37). The effect of NO on the triphosphatase activity of the R265H mutant was determined in the absence of DTT. Activation of the triphosphatase activity by substrates was studied by enzyme preincubation with 2 mM methionine and 2 mM ATP.

RESULTS

Oligomeric State of the R265H MAT I/III Mutant Protein—The molecular mass and catalytic properties of a purified R265H MAT I/III mutant protein have been studied by size exclusion chromatography. The elution volume of the R265H MAT was 16 mL (Fig. 1), which corresponds to a molecular mass of 41 kDa according to the elution profile of standard proteins. This result suggests that this mutant protein is a monomeric MAT.

Kinetics of the Monomeric R265H MAT I/III Mutant—To test the enzymatic activity of the R265H MAT I/III mutant protein, fractions were collected, and AdoMet synthetic activity and triphosphatase activity were measured. Interestingly, the analysis of the R265H fractions revealed that, although no AdoMet synthetic activity was detected, a triphosphatase activity was measured at the same elution volume matching the absorbance peak (Fig. 1). This result indicates that triphosphatase hydrolysis can be catalyzed by a single MAT subunit. In contrast, AdoMet synthetic activity requires at least a dimeric enzyme, since this active site is constituted between two monomers.

The catalytic properties of the R265H MAT mutant were
Characterization of the R265H MAT Mutant

Fig. 1. Analysis by size exclusion chromatography of the R265H MAT I/III mutant. The molecular mass of the His-tagged R265H MAT I/III mutant, that had been purified by Ni²⁺-Sepharose column chromatography, was estimated by size exclusion chromatography on a Superdex 200 HR 10/30 column. —, absorbance profile at 280 nm. Fractions of 0.2 ml were collected and tripolyphosphatase (open circles) and AdoMet (closed circles) activities were measured as described under “Experimental Procedures.” The estimated molecular mass of the R265H MAT mutant is 41 kDa.

then further studied. MAT and triphosphatase activities of the MAT mutant R265H were measured at 2 mg of methionine and ATP or triphosphate (Fig. 2). Both activities were determined as the accumulation of inorganic phosphate (P_i) after incubation at 37 °C for different periods of time. Our data indicate that while the V_max for the triphosphatase activity of the R265H mutant was very similar to that determined for a WT enzyme, AdoMet synthetic activity of this MAT variant was decreased by more than 99% (Table I). Replacement of arginine 265 by serine instead of histidine resulted in a monomeric MAT enzyme, AdoMet synthetic activity of this MAT variant was measured in the presence of ATP (Fig. 3). However, a decrease of the enzymatic activity from 128 to 67 nmol min⁻¹ was observed when the tripolyphosphatase activity of the R265H MAT I/III mutant was dependent on Mg²⁺ concentration, but Kᵣ was not required (not shown). No change of triphosphatase specific activity was observed by increasing the protein concentration (0.026–0.345 mg/ml). Additionally, to verify that the monomer does not reassociate under assay conditions, a gel filtration molecular mass determination in a column equilibrated with 2 mM P Pi was performed. Under these conditions the estimated molecular mass of the R265H MAT mutant was 41.6 kDa (not shown). These two lines of evidence indicate that the monomeric state of this MAT mutant is maintained after incubation with P Pi, and therefore, the tripolyphosphatase activity of the R265H MAT mutant is not a consequence of subunit dimerization. In contrast to WT protein, preincubation with methionine and ATP did not stimulate the triphosphatase activity (not shown). R265H triphosphatase activity is specific for P Pi. Less than 2% of the hydrolytic activity measured in the presence of P Pi was observed when ATP, P P, or metatriphosphate were used as substrates (Table II). No significant changes of enzymatic activity or Kᵣ were detected when the tripolyphosphatase activity of the R265H MAT I/III mutant was tested in the presence of ATP (Fig. 3). However, a decrease of the enzymatic activity from 128 to 67 nmol min⁻¹ was observed when the triphosphatase activity was measured in the presence of 2 mM P Pi, a classical inhibitor of the tripolyphosphatase activity of MAT (Fig. 3). Since the Hill coefficient was always close to 1.0, the experimental data were fitted to the Michaelis-Menten equation. The Kᵣ for P Pi was increased from 84 to 143 μM when the triphosphatase activity was measured in the presence of P Pi, NO regulates hepatic MAT activity through specific interaction with cysteine residue 121 (33, 38). To analyze the effect of NO on the triphosphatase activity of the R265H MAT I/III mutant, the enzyme was nitrosylated by incubation with 250 μM nitrosylated glutathione before determining its capacity to hydrolyze triphosphatase. No variations of the activity were observed after nitrosylation of this enzyme. The enzymatic activities were 125 and 129 nmol P_i min⁻¹ for the non-nitrosylated and nitrosylated forms, respectively.

Table I

| Substrate concentration | V_max | V_max/K_m | K_m |
|-------------------------|-------|-----------|-----|
| PPPi (mM)               |       |           |     |
| 0.026                   | 156 ± 21 | 20 ± 5    |
| 0.05                    | 128 ± 13 | 8 ± 3     |
| 0.12                    | 29 ± 7.4 | 122 ± 5   |

Table II

| Substrate concentration | Enzymatic activity |
|-------------------------|--------------------|
| PPPi (mM)               | nmol min⁻¹ mg⁻¹ |
| 0.026                   | 128 ± 13          |
| 0.05                    | 1.2 ± 0.73        |
| 0.12                    | 1.9 ± 0.68        |
| 0.2                    | 0.4 ± 0.22        |

Fig. 2. AdoMet synthetic and triphosphatase activity of the R265H MAT I/III mutant. Tripolyphosphatase (open circles) and MAT (closed circles) activities of a purified R265H MAT I/III mutant were measured by following the formation of P_i after incubation with the substrates for different periods of time. Tripolyphosphatase and MAT activity values were 129 and 2 nmol min⁻¹ mg⁻¹, respectively.

Fig. 3. Inhibition of the triphosphatase activity of the R265H MAT I/III mutant by PPi. The inhibition of triphosphatase activity of the R265H MAT I/III mutant by ATP and P Pi was examined. Tripolyphosphatase activity of the R265H mutant was measured in the absence (open circles) and in the presence of 5 mM ATP (closed circles) or 5 mM P Pi (triangles). The experimental data were fitted to the Michaelis-Menten equation. Values of V_max and Kᵣ for PPPi were 128 nmol min⁻¹ and 83 μM, respectively, in the absence of any inhibitor; 67 nmol min⁻¹ and 143 μM, respectively, in the presence of ATP; and 129 nmol min⁻¹ and 78 μM, respectively, in the presence of ATP.
Association between WT and R265H Mutant MAT I/III Subunits—The capacity of the R265H MAT I/III mutant to form hetero-oligomers with a WT subunit has been examined both in vivo and in vitro, taking advantage of the N-terminal His-tag of the mutant protein. If such an interaction occurred, mutant and WT subunits would combine in a hetero-oligomeric form that would be retained on a Ni²⁺-Sepharose column. When MAT purified from bacteria overexpressing WT and R265H MAT subunits was chromatographed on a Ni²⁺-Sepharose column, the resulting MAT III hetero-oligomeric MAT, the flow-through and retained fractions resulted from a Ni²⁺-Sepharose column, the presence of MAT in the flow-through fraction of the affinity column was indicative of association between MAT III and His-tagged mutant subunits, which were eluted with 500 mM imidazole. Proteins were identified by SDS-PAGE. FT, flow-through fraction from the Ni²⁺-Sepharose column; R, fraction retained on the Ni²⁺-Sepharose column; Mr, molecular mass standards (bovine serum albumin, 61 kDa; ovalbumin, 47.7 kDa; carbonic anhydrase, 34.6 kDa).

Hetero-oligomeric formation was also demonstrated in vitro. When MAT III was incubated with equimolar concentrations of R265H mutant, the Ni²⁺-Sepharose column did not retain any MAT III subunit, suggesting that there was no association between the mutant and the rat liver protein after 30-min incubation (Fig. 5A). Alternatively, hetero-oligomer formation was attempted under conditions more nearly approaching those that might exist in vivo by refolding a urea-denatured MAT III in the presence of the R265H mutant. After purification and urea denaturation of the resulting His-tagged protein, the flow-through and Ni²⁺-retained fraction of a second Ni²⁺-Sepharose chromatography were analyzed by SDS-PAGE. Protein was detected in both fractions (Fig. 5B), indicating that the His-tagged purified protein was a hetero-oligomer consisting of refolded MAT III and mutant (His-tagged) subunits.

Hybrid MAT Characterization—To estimate the molecular mass of the hetero-oligomers resulting from cotransformed bacteria, size exclusion chromatography was performed, 0.4-ml fractions were collected, and AdoMet synthetic and triphosphatase enzymatic activities were measured. The estimated molecular mass of MAT III purified from rat liver, which was used as a control, was 91 kDa, according to the dimeric state of this protein. Peaks of AdoMet synthetic and triphosphatase activities were detected when fractions of MAT III were analyzed under conditions (A) or by refolding of MAT III in the presence of mutant subunits (B). The obtained protein mixtures were then denatured with 5 M urea and chromatographed on a Ni²⁺-Sepharose column. The presence of MAT in the flow-through fraction of the affinity column was indicative of association between MAT III and His-tagged mutant subunits, which were eluted with 500 mM imidazole. Proteins were identified by SDS-PAGE. FT, flow-through fraction from the Ni²⁺-Sepharose column; R, fraction retained on the Ni²⁺-Sepharose column; Mr, molecular mass standards (standard proteins were the same as in Fig. 4).

Characterization of the R265H MAT Mutant

Fig. 4. In vivo association between WT and His-tagged R265H MAT mutant subunits. Proteins were obtained and purified as described under “Experimental Procedures.” Hetero-oligomers were chromatographed on a Ni²⁺-Sepharose column before (A) or after (B) denaturation with 5 M urea. The presence of MAT protein in the flow-through fraction of the affinity column was indicative of association between WT and His-tagged mutant subunits, which were eluted with 500 mM imidazole. Proteins were identified by SDS-PAGE. FT, flow-through fraction from the Ni²⁺-Sepharose column; R, fraction retained on the Ni²⁺-Sepharose column; Mr, molecular mass standards (bovine serum albumin, 81 kDa; ovalbumin, 47.7 kDa; carbonic anhydrase, 34.6 kDa).

Fig. 5. In vitro association between MAT III purified from rat liver and recombinant His-tagged R265H mutant subunits. Association between purified MAT III and R265H MAT mutant subunits was analyzed under native conditions (A) or by refolding of MAT III in the presence of mutant subunits (B). The obtained protein mixtures were then denatured with 5 M urea and chromatographed on a Ni²⁺-Sepharose column. The presence of MAT in the flow-through fraction of the affinity column was indicative of association between MAT III and His-tagged mutant subunits, which were eluted with 500 mM imidazole. Proteins were identified by SDS-PAGE. FT, flow-through fraction from the Ni²⁺-Sepharose column; R, fraction retained on the Ni²⁺-Sepharose column; Mr, molecular mass standards (standard proteins were the same as in Fig. 4).

Fig. 6. Size exclusion chromatography of MAT III and hetero-oligomeric MAT. The molecular mass of the hetero-oligomeric MAT was estimated by size exclusion chromatography of the protein purified by Ni²⁺-Sepharose chromatography. —, absorbance profile at 280 nm, Fractions of 0.4 ml were collected, and triphosphatase (open circles) and AdoMet synthetic (closed circles) activities were measured as described under “Experimental Procedures.” A, rat liver MAT III; B, hetero-oligomeric MAT. The estimated molecular mass for MAT III and the hetero-oligomer was 90 kDa.
Characterization of the R265H MAT Mutant

Flow-through was 41 and 90 kDa, respectively. Activities were measured as described under "Experimental Procedures." The estimated molecular mass of the retained fraction and flow-through was 41 and 90 kDa, respectively.

Substrates methionine and ATP (36). To test whether activation occurs also upon preincubation of the WT R265H heterodimeric MAT, the protein was pretreated with 2 mM methionine and 2 mM ATP, and triphosphatase activity was then measured. No activation was observed after incubation of the heterodimer with methionine and ATP. The activity values were 132 and 134 nmol P_i min⁻¹ mg⁻¹ for the non-preincubated and preincubated forms, respectively. The absence of activation of the heterodimeric MAT might be explained by a deficient or non-productive interaction with the substrates. This hypothesis would agree with the previous result, indicating that the synthesis of AdoMet is impeded in the heterodimer.

**DISCUSSION**

Methionine adenosyltransferase catalyzes the synthesis of AdoMet, the main alkylating agent in living cells (1). In the liver, MAT is also responsible for the catabolism of up to 50% of the dietary methionine (15). Humans with mutations in the gene MAT1A have decreased MAT activities in the liver, resulting in persistent hypermethioninemia without homocystinuria (22, 23). The hypermethioninemic phenotype associated with all mutations tested to date is inherited as an autosomal recessive trait, the only known exception being the hypermethioninemia due to the G-A transition at nucleotide 791 of exon VII (21–30). This change results in a mutant MAT1A-encoded protein in which arginine 264 has been replaced by histidine (26). To investigate the biochemical basis of the dominant inheritance of hypermethioninemia of individuals with this mutation, we have studied the enzyme kinetics and the oligomerization capacity of a purified MAT protein containing the homologous R265H mutation encoded by rat MAT1A.

Our results indicate that the molecular mass of the R265H MAT I/III rat mutant protein is 41 kDa, establishing its monomeric state. Although previous studies using cell extracts also suggested that the equivalent R264H MAT I/III mutant protein cannot form homo-oligomers (26, 39), it has been reported that the tetrameric conformation of the *E. coli* MAT is not altered by the replacement of the equivalent arginine 244 by histidine (4). Our results demonstrate that the R265H MAT I/III rat mutant has impaired ability to form homo-oligomers. Arginine 265, similarly to arginine 244 in the *E. coli* MAT, is involved in a salt bridge formation with symmetrical glutamic 58 (31, 32), which is important for dimerization (40). Therefore, the absence of this arginine might compromise the dimerization capacity of this MAT mutant.

The synthetic reaction catalyzed by MAT occurs through two consecutive steps. AdoMet and PPP, are first synthesized from methionine and ATP, PPP, is subsequently hydrolyzed to PP, and P, to allow product release from the active site of the enzyme (2, 3). The function of the triphosphatase activity of MAT is still under discussion (4, 41). MAT activity of the R265H MAT I/III mutant was less than 1% of the activity of the WT enzyme, in agreement with previous data, which indicate that the active site of a dimeric MAT is configured by amino acid residues from both subunits (31, 32, 40). However, the triphosphatase activity was not modified by this mutation, indicating that the active site for this activity is fully functional. Therefore, the hydrolytic site for the triphosphatase activity must be configured by residues held on a single subunit. Triphosphatase activity of this MAT mutant is similar to that determined for a WT enzyme in the resting, less active state (36). Additionally, we found that triphosphatase activity of the R265H MAT I/III mutant is not regulated by NO, which further agrees with the mutant enzyme being in the resting state. We have shown previously that triphosphatase activity of MAT III is stimulated by preincubation with methionine and ATP (36). However, no activation of the triphosphatase activity was found when R265H MAT was preincubated with methionine and ATP, suggesting that the regulation of this activity by the natural substrates of the enzyme has been lost. The absence of AdoMet synthetic activity, and the failure of methionine and ATP to activate the triphosphatase activity, suggest that binding of the substrates is impaired. Substitution of arginine 265 by serine instead of histidine resulted in a 5-fold decrease of triphosphatase activity. Thus, it seems that, although the presence of a positive charge at position 265 is involved, it is not an absolute requirement for the PPP, hydrolytic activity. This result might be explained by previous evidence, which indicates the positive charge of the equivalent arginine 244 in the *E. coli* MAT is responsible for the correct orientation of the PPP, in the active site of the enzyme (4, 42).

Triphosphatase activity of the R265H MAT I/III mutant is specific of PPP, and depends on the presence of Mg2⁺ in the assay mixture, but K⁺ is not required. These findings suggest that while Mg2⁺ is directly involved in the binding of PPP, (31), K⁺, which binds to the interface between monomers (40), might contribute to the stabilization of a functional conformation of dimeric MAT, which is not accessible to the monomeric R265H mutant. This mutant showed no cooperativity when its triphosphatase activity was assayed with different PPP, concentrations. Triphosphatase activity of WT MAT is not altered by the presence of saturating concentrations of ATP (36). This finding might be explained by assuming that ATP and PPP, bind to different sites, or, alternatively, binding of the PPP, moiety of ATP might be sterically restricted in the dimeric enzyme. To further assess this question, we studied the effect of ATP on the triphosphatase activity of the monomeric R265H MAT mutant. We found that ATP had no effect on the triphosphatase activity of the R265H MAT I/III mutant.

![Graph showing absorbance at 280 nm](image)
Since accessibility should not be compromised in the monomer, we propose that ATP and PPP, have different binding sites. In contrast, PP₃, a classical inhibitor of MAT triphosphatase activity, induces a 1.5- and 2.3-fold decrease of the enzymatic activity and affinity of the enzyme, respectively. Similarly to E. coli MAT (43), PP₃ and PPP, might compete for the same binding site. Our data suggest that the active site of MAT has two coordinated subsites: the synthetic site, configured by amino acid residues from both subunits (31, 32), is responsible for the binding of methionine and ATP and performs the AdoMet synthetic reaction. The hydrolytic site, configured by amino acid residues from one single subunit, accounts for the binding of PPP, and performs the PPP, hydrolytic reaction.

Dominant inheritance of the hypermethioninemia of humans carrying the R264H mutation in MAT1A might be explained by our findings, which indicate that replacement in the rat homologue of arginine 265 by histidine produces a monomeric MAT that interacts with the WT MAT subunit, in vivo and in vitro, resulting in a hybrid enzyme with impaired AdoMet synthetic activity. Evidence suggesting the capacity of the MAT mutant R264H to associate with WT MAT was first proposed for the human enzyme by Chamberlin et al. (26) on the basis of MAT activity recovered in COS cell extracts after cotransfection with residues from one single subunit, accounts for the binding of the synthetic site, configured by amino acid residues from both subunits (31, 32), is responsible for the binding of methionine and ATP and performs the AdoMet synthetic reaction. The hydrolytic site, configured by amino acid residues from one single subunit, accounts for the binding of PPP, and performs the PPP, hydrolytic reaction.

In conclusion, our data provide a molecular explanation for the dominant inheritance of the persistent hypermethioninemia associated with the human R264H MAT I/III mutation. We demonstrate that the equivalent R265H mutation in rat MAT I/III results in a monomeric MAT, which can associate with the WT enzyme to form a dimeric R265H-WT MAT lacking AdoMet synthetic activity. We have also shown that the active site for the triphosphatase activity is functionally active in the monomeric R265H MAT I/III mutant. Our data suggest that the active site of MAT has two coordinated subsites: the synthetic site, configured by amino acid residues from both subunits, that performs the AdoMet synthetic reaction, and the hydrolytic site, configured by amino acid residues from one single subunit, that performs the PPP, hydrolytic reaction.

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Isabel Pérez Mato, Manuel M. Sánchez del Pino, Margaret E. Chamberlin, S. Harvey Mudd, José M. Mato and Fernando J. Corrales

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