

Analysis of pathological defects in methionine metabolism using a simple mathematical model

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Abstract

Derangements in methionine metabolism are a hallmark of cancers and homocystinuria, an inborn error of metabolism. In this study, the metabolic consequences of the pathological changes associated with the key pathway enzymes, methionine adenosyl transferase (MAT), glycine N-methyl transferase (GNMT) and cystathionine β -synthase (CBS) as well as an activation of polyamine metabolism, were analyzed using a simple mathematical model describing methionine metabolism in liver. The model predicts that the mere loss of allosteric regulation of CBS by adenosylmethionine (AdoMet) leads to an increase in homocysteine concentration. This is consistent with the experimental data on the corresponding genetic defects, which specifically impair allosteric activation but not basal enzyme activity. Application of the characteristics of transformed hepatocytes to our model, i.e., substitution of the MATI/III isozyme by MATII, loss of GNMT activity and activation of polyamine biosynthesis, leads to the prediction of a significantly different dependence of methionine metabolism on methionine concentrations. The theoretical predictions were found to be in good agreement with experimental data obtained with the human hepatoma cell line, HepG2.

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1. Introduction

The methionine cycle plays a central role in cellular metabolism, producing two major cellular reagents, AdoMet and glutathione. AdoMet is a universal donor of methyl groups for multiple methylation reactions, which play important regulatory roles in the cell. AdoMet is also a precursor for the synthesis of polyamines. In most tissues, AdoMet is regenerated in the methionine cycle in which homocysteine is an intermediate (Fig. 1). A non-protein amino acid, homocysteine, has lately been the subject of many studies since elevated plasma homocysteine levels are correlated with multiple pathological conditions, such as cardiovascular diseases, neurodegenerative diseases and neural tube defects [1–3]. In

liver, kidneys and pancreas, homocysteine can also be metabolized through the transsulfuration pathway leading to cysteine, a limiting substrate for the synthesis of the major redox buffer, glutathione (Fig. 1).

Methionine metabolism is intimately involved in maintenance of methylation and redox homeostasis and it is, therefore, tightly regulated. Major perturbations in methionine metabolism, viz., focal promoter CpG island hypermethylation of tumor suppressor genes in the context of global hypomethylation, is a hallmark of many cancers [4]. Furthermore, most cancer cells exhibit methionine dependence, i.e., they are unable to grow in folate-replete media in which methionine is replaced by its precursor, homocysteine, and the basis of this auxotrophy is not understood [5]. One of the characteristic features of methionine metabolism in hepatocytes is the presence of two MAT isoforms, one that is inhibited (MATI) and another that is activated (MATIII) by its reaction product, AdoMet. In addition,

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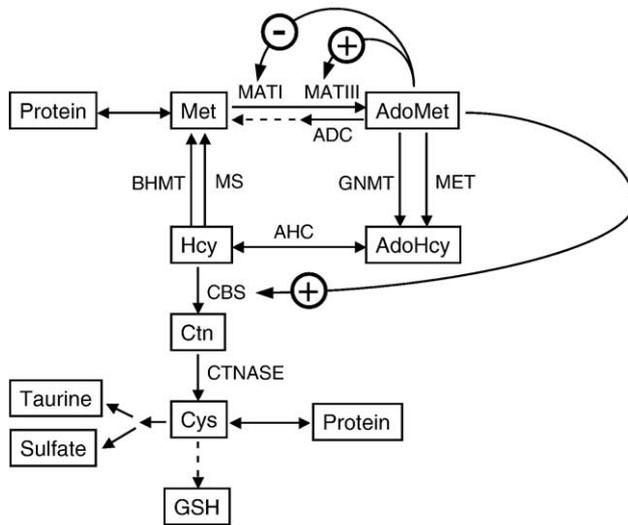


Fig. 1. A simplified scheme of methionine metabolism in liver. Abbreviations: Met—methionine, AdoMet—S-adenosylmethionine, AdoHcy—S-adenosylhomocysteine, Hcy—homocysteine, Ctn—cystathionine, Cys—cysteine, GSH—reduced glutathione, MAT—methionine adenosyltransferase, ADC—S-adenosylmethionine decarboxylase; MET—methylases, GNMT—glycine N-methyltransferase, AHC—S-adenosylhomocysteinase, MS—methionine synthase, BHMT—betaine homocysteine methyltransferase, CBS—cystathionine β -synthase, CTNASE— γ -cystathionase. Curved arrows indicate enzymes, which are subject for allosteric regulation by AdoMet.

liver has a very high activity of GNMT, which utilizes glycine and AdoMet to make sarcosine. Liver also exhibits higher CBS activity compared to other organs.

Mutations have been reported along the entire length of the CBS gene that are correlated with a significant increase in plasma homocysteine levels and are associated with pathologies of the cardiovascular, skeletal, ocular and nervous systems [6,7]. While a subclass of these mutations impair basal catalytic activity, a second subclass is characterized by normal to high basal activity but is insensitive to the allosteric regulator, AdoMet [8–10]. In wild type CBS, AdoMet increases the reaction rate 2- to 4-fold [8,11–14]. Plasma homocysteine levels in a patient with a regulatory domain mutation (D444N) is reported to be elevated as with mutations in the catalytic or heme domains [8]. This subclass of regulatory domain mutations, including P422L, I435T, D444N and S466L, represents a conundrum since biochemical studies on these mutations have not adequately explained why they are pathogenic [9,10].

Upon malignant transformation, hepatocytes undergo significant changes including suppression of MATI/III and GNMT expression paralleled by induction of the MATII isoenzyme [15–19]. Compared to normal cells, transformed cells also have significantly activated polyamine metabolism [20,21]. Mathematical modeling is a useful and perhaps, even necessary tool for studying mechanisms of regulation in complex metabolic systems. We have previously reported a simplified mathematical model to describe methionine metabolism in hepatocytes [22]. Appli-

cation of this model provided insights into the role of MATI/III, GNMT and CBS in regulation of methionine metabolism. The model revealed that at low methionine concentrations, MAT activity in hepatocytes is due exclusively to the MATI isoform function and that the metabolic flux through MATIII, GNMT and CBS is small. Under these conditions, methionine is metabolized predominantly via the remethylation pathway. However, at high methionine concentrations, MATIII, GNMT and CBS are activated and provide an overflow route for removal of excess methionine via conversion to cysteine, which can be used for GSH synthesis, or oxidized to taurine and sulfates. The model predicted that disposal of excess organic sulfur via the transsulfuration pathway is associated with a sharp increase in AdoMet concentration [22].

In the present study, we have employed this mathematical model to understand the pathological consequences of the seemingly innocuous regulatory domain mutations in CBS, which are nevertheless associated with hyperhomocysteinemia. We have also analyzed the consequence of changes in expression of the methionine pathway enzymes that accompany malignant transformation, on the response to fluctuations in methionine levels. In addition, we have complemented the modeling studies with experimental evaluation of the changes in metabolic responses to varying methionine concentrations in the human hepatoma cell line, HepG2. Our results underscore the utility of even a simple mathematical model for explaining cellular responses to aberrations in methionine metabolism.

2. Mathematical model

The current mathematical model is based on an earlier version [22], which describes methionine metabolism in hepatocytes and consists of two differential and one algebraic equation as described below.

$$\frac{d[\text{AdoMet}]}{dt} = V_{\text{MAT}} - V_{\text{MET}} - V_{\text{ADC}} \quad (1)$$

$$\frac{d[\text{AdoHcy}]}{dt} = \frac{V_{\text{MET}} - V_{\text{CBS}}}{1 + K_{\text{AHC}}/[\text{Ado}]} \quad (2)$$

$$[\text{Hcy}] = \frac{K_{\text{AHC}}}{[\text{Ado}]} [\text{AdoHcy}] \quad (3)$$

Here, V_{MAT} , V_{MET} , V_{ADC} and V_{CBS} denote the rates of MAT, methylases, adenosylmethionine decarboxylase (ADC) and CBS, respectively. For hepatocytes, V_{MAT} constitutes a sum of the rates of MATI and MATIII, and V_{MET} is a sum of rates of GNMT and other cellular methylases. K_{AHC} is the equilibrium constant for the reaction catalyzed by AdoHcy hydrolase. [Ado] represents the concentration of adenosine, which is kept constant at 1 μM . The model takes into account the fact that the activity of AdoHcy hydrolase is much higher than activities of other enzymes of methionine metabolism.

Thus, one can exclude homocysteine concentration from the model using Eq. (3). Methionine concentration is one of the model parameters. That is why one can exclude from the model the reactions of homocysteine remethylation as well as methionine production in polyamine metabolism. Equations describing the rates of enzymatic reactions, and the values of parameters for normal hepatocytes are the same as employed previously [22], except for the equations describing the CBS and ADC reactions.

The current model describes the rate of the reaction catalyzed by CBS in greater detail. The reaction proceeds via formation of a ternary complex with ordered binding of substrates (first serine and then homocysteine) [23]. The equation for this mechanism was obtained using a quasi-stationary approach and is as follows.

$$V_{\text{CBS}} = \frac{V_{\text{max}}^{\text{CBS}}}{1 + \frac{K_{\text{m1}}^{\text{CBS}}}{[\text{Ser}]} + \frac{K_{\text{m2}}^{\text{CBS}}}{[\text{Hcy}]} \left(1 + \frac{K_{\text{d}}^{\text{CBS}}}{[\text{Ser}]}\right)} \quad (4)$$

Here, $V_{\text{max}}^{\text{CBS}}$ is CBS activity (maximal rate), $K_{\text{m1}}^{\text{CBS}}$ and $K_{\text{m2}}^{\text{CBS}}$ are the Michaelis constants for serine and homocysteine, respectively, and $K_{\text{d}}^{\text{CBS}}$ is the dissociation constant for serine. Values of kinetic parameters for CBS that have been employed in this model are listed in Table 1. A serine concentration [Ser] of 590 μM was employed [24].

As described in the literature, AdoMet is an allosteric activator of CBS. According to different reports, the mechanism of activation involves either decreasing the Michaelis constant for homocysteine [9,13], or increasing the maximal rate of the reaction [12]. However, under physiological conditions, the two mechanisms are indistinguishable since the following conditions are true: $K_{\text{m1}}^{\text{CBS}}/[\text{Ser}] \approx 4$, $K_{\text{d}}^{\text{CBS}} \ll [\text{Ser}]$, $K_{\text{m2}}^{\text{CBS}} \gg [\text{Hcy}]$. This allows significant simplification of the Eqs. (4) to (5) to describe the rate of the CBS reaction.

$$V_{\text{CBS}} \approx \frac{V_{\text{max}}^{\text{CBS}}}{1 + 4 + \frac{K_{\text{m2}}^{\text{CBS}}}{[\text{Hcy}]}} \approx \frac{V_{\text{max}}^{\text{CBS}}}{K_{\text{m2}}^{\text{CBS}}} [\text{Hcy}] \quad (5)$$

It follows from Eq. (5) that under physiological conditions, a decrease in the Michaelis constant for homocysteine or an increase in the maximal reaction rate will increase the CBS

reaction rate to the same extent. In our model, we assume that AdoMet increases the maximal rate of the reaction and describe this dependence in Eq. (6).

$$V_{\text{max}}^{\text{CBS}} = V_0^{\text{CBS}} \left(1 + \frac{K_{\text{A2}}^{\text{CBS}} - 1}{1 + (K_{\text{A1}}^{\text{CBS}}/[\text{AdoMet}])}\right) \quad (6)$$

Here, V_0^{CBS} is the maximal rate of the reaction in the absence of AdoMet, $K_{\text{A1}}^{\text{CBS}}$ is the activation constant of CBS by AdoMet and therefore, reflects the regulation of CBS by AdoMet; $K_{\text{A2}}^{\text{CBS}}$ determines the value of the maximal activation of the enzyme. Since the flux of AdoMet into the polyamine pathway is small in normal cells [25,26] for simplicity, the rate of the ADC reaction (the intensity of polyamine metabolism) was set at zero.

To simulate methionine metabolism in HepG2 cells, the initial model was modified to accommodate changes occurring in hepatocytes upon malignant transformation. The sum of the rates of MATI and MATIII in Eq. (1) was substituted with the rate of the reaction catalyzed by MATII ($V_{\text{MAT}} = V_{\text{MATII}}$). The additional consumption of AdoMet by activated polyamine metabolism was included in the model as the rate of the ADC reaction in Eq. (1). The contribution of GNMT was removed from the total rate of methylation processes to reflect repression of this enzyme in hepatomas [27].

The equation describing the rate of the MATII reaction was written based on previously published data [28]. MATII kinetics is characterized by a Hill coefficient of 0.76 for methionine and inhibition by AdoMet as described in Eq. (7).

$$V_{\text{MATII}} = \frac{V_{\text{max}}^{\text{MATII}}}{1 + \left(\frac{K_{\text{m}}^{\text{MATII}} \left(1 + \frac{[\text{AdoMet}]}{K_{\text{i}}^{\text{MATII}}}\right)}{[\text{Met}]}\right)^{0.76}} \quad (7)$$

Values for the kinetic parameters for MATII are listed in Table 1.

The rate of the ADC reaction in the model was described by a simple Michaelis–Menten function of AdoMet:

$$V_{\text{ADC}} = \frac{V_{\text{max}}^{\text{ADC}}}{1 + \frac{K_{\text{m}}^{\text{ADC}}}{[\text{AdoMet}]}} \quad (8)$$

with the Michaelis constant for AdoMet $K_{\text{m}}^{\text{ADC}} = 50 \mu\text{M}$ [29–31]. We ascribe the maximal rate of the ADC reaction to the rate of polyamine metabolism that in turn is determined by the activity of the key enzyme, ornithine decarboxylase, and does not depend on methionine metabolism intermediates except AdoMet. $V_{\text{max}}^{\text{ADC}}$, the maximal rate of the ADC reaction, was set at 400 $\mu\text{mol/h}$ 1 cells, which is the rate of AdoMet production as described previously in our model [32]. Based on the published data, polyamine metabolism consumes about 1% of AdoMet that is produced in normal tissues and the consumption can be increased up to 100-fold

Table 1
Values of CBS and MATII parameters employed in the model

Parameter	Units	Value		References
		Model	Experiment (liver)	
V_0^{CBS}	mmol/ h 1 cells	527	60–290	[23,24,46]
$K_{\text{m1}}^{\text{CBS}}$	μM	2500	1150–4000	[23,46,47]
$K_{\text{m2}}^{\text{CBS}}$	μM	590	590–25,000	[13,23,46,47]
$K_{\text{d}}^{\text{CBS}}$	μM	1	–	–
$K_{\text{A1}}^{\text{CBS}}$	μM	60	34	[14]
$K_{\text{A2}}^{\text{CBS}}$		2	2–4	[13,14]
$V_{\text{max}}^{\text{MATII}}$	$\mu\text{mol/ h 1 cells}$	507	–	–
$K_{\text{m}}^{\text{MATII}}$	μM	4	3–8	[28,48]
$K_{\text{i}}^{\text{MATII}}$	μM	50	32–50	[28]

in tumor cells so that AdoMet consumption in methylation reactions versus polyamine biosynthesis become approximately equal (i.e., $V_{\text{ADC}} + V_{\text{MET}} = 400 \mu\text{mol/h}$ 1 cells) [25,26,33].

The equation describing the total rate of the cellular methylases remains the same as in the earlier version of the model [22]:

$$V_{\text{MET}} = \frac{V_{\text{max}}^{\text{MET}}}{1 + \frac{K_{\text{m1}}^{\text{MET}}}{[\text{AdoMet}]} + \frac{K_{\text{m2}}^{\text{MET}}}{[\text{A}]} + \frac{K_{\text{m1}}^{\text{MET}}}{[\text{AdoMet}]} \frac{K_{\text{m2}}^{\text{MET}}}{[\text{A}]}} \quad (9)$$

For normal hepatocytes, $K_{\text{m1}}^{\text{MET}} = 1(1 + [\text{AdoHcy}]/4) \mu\text{M}$, and $[\text{A}]$ is a sum of the concentrations of methylation substrates, which is set to be constant in the model. However, two parameters were changed to better fit the experimental data for the increase in AdoMet levels observed in HepG2 cells at high methionine concentrations. The maximal rate of the reaction ($V_{\text{max}}^{\text{MET}}$) was increased from 4.54 mmol/h 1 cells to 5.45 mmol/h 1 cells. In addition, inhibition of methylation by AdoHcy in the HepG2 cell model was increased as follows: $K_{\text{m1}}^{\text{MET}} = 50(1 + [\text{AdoHcy}]/4) \mu\text{M}$.

3. Materials and methods

3.1. Materials

Eagle's minimum essential media either lacking or containing 100 μM methionine were purchased from Sigma. Fetal bovine serum was from HyClone. HepG2 cells (human hepatocellular carcinoma) were from American Type Culture Collection. Methionine (cell culture tested grade) was purchased from Sigma. Cell labeling grade [^{35}S]-methionine (1000 Ci/mmol) was purchased from Amersham Biosciences. Unless noted otherwise, all other chemicals used in this study were from Sigma.

3.2. Cell culture and treatment conditions

Cells were grown in 60 mm \times 15 mm tissue culture dishes in regular (methionine-replete) Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 2.2 g/l sodium bicarbonate and 10 ml/l antibiotic/antimycotic solution. Cells were maintained at 37 $^{\circ}\text{C}$ in a 5% CO_2 atmosphere. When 60–80% confluent, cells were washed with phosphate-buffered saline and transferred to a methionine-deficient medium (4 ml/dish) and incubated for 30 min. Experiments were initiated by addition of freshly prepared sterile stock solution of methionine (10 mM) to obtain the final desired methionine concentration. For each experimental time point, 2–3 dishes were set up in parallel. For experiments in which incorporation of radioactive methionine into the glutathione pool was followed, [^{35}S]-methionine was diluted with phosphate-buffered saline to a concentration of 1.25 μM . Radioactivity (5 $\mu\text{Ci}/4$ ml dish) was added together with the unlabeled methionine stock

solution. The change in methionine concentration due to addition of the label (i.e., an increase by 1.25 nM) was negligible and disregarded. The concentration of and radioactivity associated with glutathione were measured at 10 h following introduction of radioactive methionine to cells. The rate of [^{35}S]-methionine incorporation into glutathione is linear over this time period [32].

3.3. Metabolite analysis

Dishes were placed on ice in a dark room and the culture medium was removed by aspiration, followed by two washes of the cells with ice-cold phosphate-buffered saline. Cells were collected by scraping and the resulting suspension was divided into several tubes for protein concentration determination (using the Bradford assay, BioRad) and metabolites analysis. Metabolite concentrations were recalculated in units of $\mu\text{mol/l}$ cells as described previously [32].

To measure the concentration of reduced and oxidized glutathione, cells were lysed and deproteinized with metaphosphoric acid solution (16.8 mg/ml HPO_3 , 2 mg/ml EDTA and 300 mg/ml NaCl) and then centrifuged to remove the precipitate. Following derivatization with 2,4-dinitrofluorobenzene, samples were analyzed by HPLC on an anion-exchange column ($\mu\text{Bondapak-NH}_2$, 3.9×300 mm, Waters), as described previously [32]. Glutathione fractions were collected, mixed with scintillation liquid, and the observed disintegrations per minute were normalized for protein concentration in each sample. Differences in dilution of the label between samples due to differences in the final concentration of methionine between them were taken into account by applying the corresponding coefficients reflecting the ratio of labeled/unlabeled methionine in each sample.

The concentration of AdoMet was measured following a modification of the procedure described previously [34]. The cells were deproteinized by mixing with an equal volume of 10% trichloroacetic acid solution and centrifugation. The supernatant was analyzed by HPLC on a 5 μ Ultrasphere ODS column (4.6 mm \times 25 cm, Beckman Coulter) under isocratic conditions at a flow rate of 1 ml/min with monitoring at 260 nm. The mobile phase contained 40 mM NaH_2PO_4 , 10 mM heptanesulfonic acid sodium salt (Fisher), 17% methanol, pH 4. AdoMet eluted as a single peak with a retention time of 18 min. The concentration of AdoMet in the samples was determined using a calibration curve generated for the compound.

4. Results

4.1. Loss of allosteric regulation of CBS increases homocysteine levels

We have applied our model to determine the effect of decreased sensitivity of CBS to AdoMet on the steady state

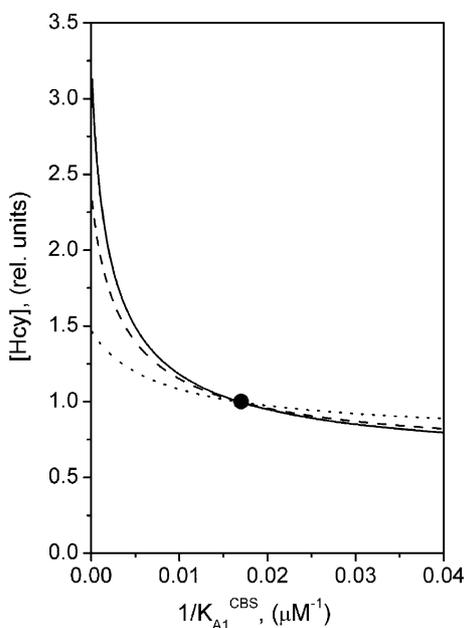


Fig. 2. Steady-state homocysteine concentration as a function of CBS sensitivity to AdoMet, calculated using the model for normal hepatocytes. In this model, CBS sensitivity to AdoMet decreases with increasing K_{A1}^{CBS} . At $K_{A1}^{CBS} = \infty$ ($1/K_{A1}^{CBS} = 0$) the enzyme completely loses its ability to be activated by AdoMet. The values of K_{A1}^{CBS} (which determines the maximal possible extent of CBS activation by AdoMet) were 2, 4, and 6 for the dotted, dashed and solid lines, respectively. The intersection point corresponds to the physiological state in normal hepatocytes.

concentration of homocysteine. Fig. 2 demonstrates the predicted response in normal hepatocytes at different values for the constant K_{A2}^{CBS} that determines the magnitude of CBS activation by AdoMet. An increase in the constant K_{A1}^{CBS} leads to a decrease in CBS responsiveness to AdoMet, which is completely lost at $K_{A1}^{CBS} = \infty$ ($1/K_{A1}^{CBS} = 0$). The model predicts that as CBS loses its sensitivity to AdoMet, the steady state homocysteine concentration increases 1.5–3.0-fold depending on the value of K_{A2}^{CBS} value. Homocysteine concentration is predicted to increase further at high methionine concentration (70 μM) when liver cells activate the disposal route involving GNMT and CBS.

4.2. Regulation of methionine metabolism in transformed cells

Since malignant transformation is accompanied by major changes in the expression of methionine cycle enzymes and activation of polyamine biosynthesis, we have determined the effect of varying methionine on its clearance via the transsulfuration pathway. In HepG2 cells, incorporation of labeled methionine into the glutathione pool is roughly proportional to methionine concentrations in the range of 20–50 μM and plateaus at concentrations >100 μM (Fig. 3). In contrast, glutathione concentration in the cells was not dependent on the methionine concentration. The average glutathione concentration in HepG2 cells obtained in ten independent experiments was 6.3 ± 1.5 mmol/l cells. Earlier

results indicated that $\sim 10\%$ of intracellular glutathione is synthesized from methionine in 10 h in HepG2 cells [32]. This implies that the loss of label from the glutathione pool is insignificant and the label incorporation into glutathione reflects the flux through the transsulfuration pathway under our experimental conditions.

A strong dependence of AdoMet concentration on extracellular methionine ranging from 50 to 500 μM in concentration as predicted for normal cells [22] was not observed experimentally with the transformed HepG2 cells. The intracellular AdoMet concentration was found to be 50–75 $\mu\text{mol/l}$ cells at 50 μM methionine. When grown in the presence of higher methionine concentrations, AdoMet levels increased steadily for several hours with the average increase being ~ 1.5 -fold at an extracellular methionine concentration of 500 μM (Fig. 4).

The experimental results were well described by the model for transformed hepatocytes. As shown in Fig. 3, the theoretical transsulfuration rate increases with methionine concentration between 0 and 50 μM and then remains constant. This prediction correlates well with the experimental data obtained in HepG2 cells on the dependence of the transsulfuration rate on methionine concentrations. In contrast, the model developed for normal hepatocytes predicts a sharp increase in the transsulfuration rate when methionine concentrations exceed 50 μM , reflecting “disposal” of excess methionine via the transsulfuration pathway.

The model describing methionine metabolism in transformed cells also predicts a slight increase in AdoMet

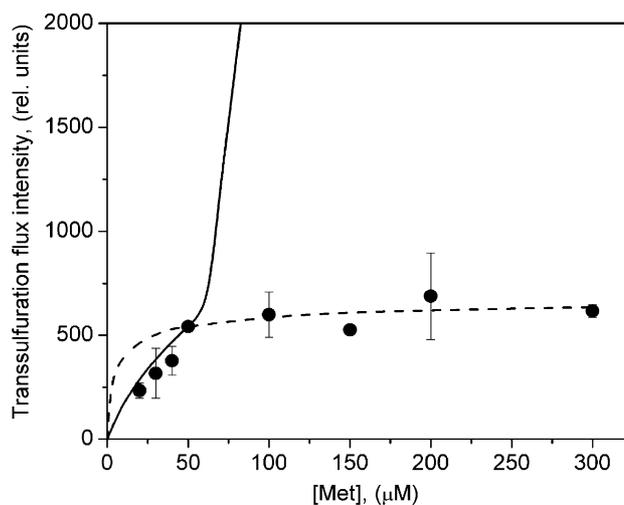


Fig. 3. Dependence of the transsulfuration flux on methionine concentration. The results calculated from the models for normal and transformed hepatocytes are depicted as solid and dashed lines, respectively. An integral of CBS rate for 10 h at each methionine concentration was calculated. Experimental data points (solid circles) describe incorporation of radioactive label from methionine into the glutathione pool in cultured HepG2 cells. Each data point is an average of two independent measurements. The data set is representative of 9 independent experiments. The theoretical and experimental data were normalized to the corresponding values obtained at 50 μM methionine.

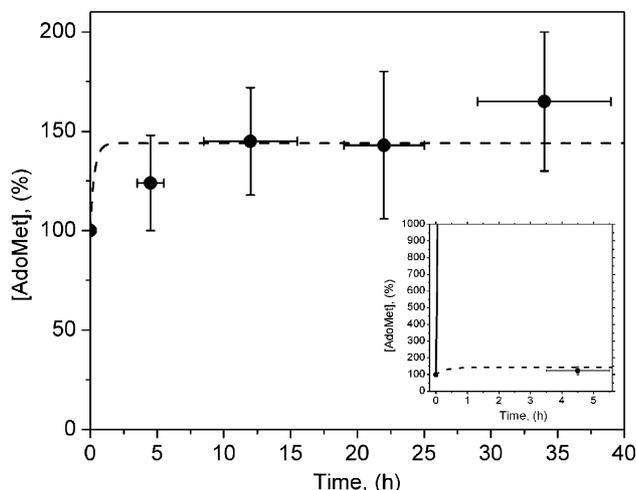


Fig. 4. Comparison of experimental versus theoretical kinetics of change in AdoMet concentration at 500 μM methionine. The dashed lines represent results calculated from the models for HepG2 cells. Deviation in time indicated for each data point represents a time interval encompassing all data points, which contributed to the corresponding averaged value. The number of independent measurements that were averaged for each point was 5, 10, 14, and 13 for the first, second, third and fourth data points, respectively. The AdoMet concentration measured at 50 μM methionine at each time point were taken as 100%. Insert: comparison of the kinetics of change in AdoMet concentration at 500 μM methionine calculated for hepatocytes (solid) versus HepG2 cells (dashed). Due to the steep increase in AdoMet concentration in hepatocytes, only the initial kinetic traces are shown for clarity and include one experimental data point obtained with HepG2 cells.

levels at high methionine concentrations, which was indeed observed in HepG2 cells (Fig. 4) and is clearly distinguished from the steep increase predicted for normal cells (Fig. 4 inset). Indeed, the model for normal hepatocytes predicts an approximately 10-fold jump in AdoMet concentration during the switch to disposal of excess methionine through the transsulfuration pathway [22]. It is important to note that the exclusion of polyamine metabolism from the model describing methionine metabolism in transformed cells ($V_{\text{max}}^{\text{ADC}}=0$) does not affect the regulation per se of AdoMet levels or the transsulfuration flux on methionine concentration (not shown). The amount of methionine incorporated into GSH increases but the dependence itself is unchanged.

5. Discussion

We have employed a simple mathematical model to describe methionine metabolism in liver cells as a basis for understanding a paradoxical set of regulatory domain mutations in CBS, which retain high enzyme activity but are impaired in allosteric activation by AdoMet. We have also used this model to predict and to experimentally validate the changes in the response of transformed versus normal liver cells to methionine excess. Our results demonstrate the success of even this simple model of

methionine metabolism in liver for analyzing pathological aberrations in this pathway.

Defects in CBS are the single most common cause of homocystinuria, an autosomal recessive disorder, which is accompanied by severely elevated levels of plasma homocysteine [7]. Biochemical characterization of pathogenic mutations found in homocystinuric patients is beginning to shed light on the catalytic penalties associated with individual mutations [9,10,35–37]. However, the pathogenicity of a subclass of these mutants, which exhibit impaired response to AdoMet but otherwise display robust basal catalytic activity, is puzzling. We therefore explored the applicability of our model for gaining insights into the dependence of homocysteine levels to loss of this regulatory feature in CBS.

Interestingly, the model predicts increasing accumulation of homocysteine in CBS mutants with decreasing sensitivity to AdoMet (Fig. 2). A 1.5- to 3.0-fold increase in homocysteine concentration is predicted for complete loss of AdoMet regulation and is relevant to the subclass of mutations that respond to unphysiologically high concentrations of AdoMet [9,10]. While the calculated increase in homocysteine is less dramatic than what is observed in these patients, it is strongly dependent on the magnitude of maximal activation of wild type CBS by AdoMet. In principle, AdoMet could affect CBS activity in at least two ways: (i) stabilize CBS and render it less sensitive to proteolytic degradation (Prudova and Banerjee, unpublished results) and (ii) function as a V-type allosteric effector that enhances the V_{max} of the reaction. If AdoMet activates CBS in vivo to a greater extent than is assumed in the model, then homocysteine levels would be significantly higher upon loss of CBS sensitivity to AdoMet. Elevation of homocysteine levels would be further exacerbated by differences in stability between wild type and regulatory domain mutants as has been demonstrated for the D444N mutation whose steady-state levels are ~ 4 -fold lower [10].

What is the mechanistic basis of homocysteine accumulation when CBS is unresponsive to AdoMet? At normal methionine concentrations, the regulatory domain mutants of CBS displaying normal catalytic activity support a lower rate of homocysteine consumption versus wild type enzyme due to loss of allosteric activation. An equilibrium between the rates of homocysteine production and consumption is achieved at increased homocysteine concentration. The increase in homocysteine concentration would in turn lead to an increase in the CBS reaction rate because the K_m for homocysteine is significantly higher than its physiological concentration [12]. Homocysteine concentration will increase until the CBS reaction rate reaches its normal value. Thus, a steady-state methionine metabolism in these patients will be characterized by a higher homocysteine concentration and a normal CBS reaction rate. The problem will be exacerbated at high methionine concentrations when hepatic cells respond by increasing flux through the disposal route involving MATIII, GNMT and CBS. Under these conditions, homocysteine concentration in patients with the

AdoMet-insensitive CBS mutations will increase more significantly than in normal individuals.

Upon malignant transformation, hepatocytes cease to express GNMT and MATI/III and induce expression of the MATII isozyme. Additionally, polyamine metabolism is significantly activated in transformed cells. Our experimental observations are consistent with the theoretical predictions that regulation of methionine metabolism is dramatically altered in these cells. As shown in Fig. 3, the transsulfuration rate does not change significantly in hepatoma cells when the methionine concentration is varied between a physiological range to 300 μM and that the concentration of AdoMet exhibits a very weak dependence on methionine levels (Fig. 4).

In contrast, a number of studies with hepatocytes *in vivo* and *in culture* demonstrate a significant increase in the transsulfuration flux and an increase in glutathione synthesis and glutathione concentration with increasing methionine [38–42]. Our model predicts that at methionine concentrations $>54 \mu\text{M}$, AdoMet concentration increases 10-fold and hepatocytes effectively clear excess sulfur through the transsulfuration pathway (Fig. 3). Indeed, a 5–20-fold increase in liver AdoMet concentrations is observed in animal models in response to an increase in methionine concentrations [38,42–44]. Recently, an extended mathematical model was employed to demonstrate that removal of excess methionine by hepatocytes could provide effective stabilization of blood methionine concentration [45].

Our model results suggest that the differences in regulation of methionine metabolism in normal versus transformed liver cells are primarily determined by the difference in MAT reaction kinetics and GNMT expression. The change in polyamine metabolism does not significantly influence the regulation of methionine metabolism.

Methionine is an essential amino acid, albeit a toxic one at high concentrations. The difference in the response of normal and transformed hepatocytes to excess sulfur is related to the inability of transformed cells to switch to a high methionine mode as described previously [22] and the consequent failure to stabilize methionine concentrations at the organismal level. Thus, transformed hepatocytes stop expending energy for a function that is vital for the organism but is apparently useless for these cells, and therefore may receive a short-term growth and survival advantage in comparison to non-malignant cells.

6. Conclusions

We have employed a mathematical model to simulate methionine metabolism in liver cells and demonstrate its utility in explaining the metabolic changes associated with two pathologies: neoplastic transformation and homocystinuria resulting from AdoMet-insensitive mutations in human CBS. The model predicts that substitution of MATI/III isozyme by MATII in addition to loss of GNMT

activity in transformed cells leads to a qualitatively different dependence of methionine metabolism on methionine concentrations, which is validated by our experimental data on the human hepatoma cell line. This study also provides insights into the pathogenicity of a class of seemingly paradoxical patient mutations, which results in robust CBS activity albeit with loss of sensitivity to the AdoMet. This study demonstrates the value of a combined theoretical and experimental approach for understanding metabolic regulation in health and in disease.

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