9 Metabolic trigger: control of methionine metabolism

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Introduction

Biological transmethylation reactions have attracted the attention of biochemists since they are important in a variety of cellular processes such as methylation of nucleic acids, proteins, phospholipids, and other molecules. Most of these reactions utilize S-adenosylmethionine (AdoMet) as a methyl donor. In the liver there are two isoforms of methionine-adenosyltransferase (MATI and MATIII), that catalyze the synthesis of AdoMet from methionine and ATP. AdoMet activates MATIII and inhibits MATI [1]. What is the rationale for the coexistence of the two MAT isoforms in the liver with reciprocal responses to the product, AdoMet? This is the first question that we have sought to answer in this study by developing a mathematical model.

In liver and kidney cells, dietary methionine can be metabolized to cysteine. This source of cysteine derived from methionine is very important in the maintenance of the intracellular glutathione pool. The question thus arises, how do liver cells control flow through a single methionine metabolite, AdoMet, to provide two different consumers (AdoMet for methyl transfer reactions and glutathione for redox buffering)? This is the second question that we have sought to answer with the model described in this study.

We propose a simple mathematical model for methionine metabolism in liver cells. The model qualitatively fits the experimental data concerning metabolic fluxes and intermediate concentrations under physiological conditions. Analysis of this model shows that methionine metabolism can exist in two steady states. The first corresponds to the physiological values of metabolites, whereas the second occurs at a 10-fold higher AdoMet concentration and leads to a 2–3-fold faster rate of cysteine synthesis from methionine. Under a certain range of parameters

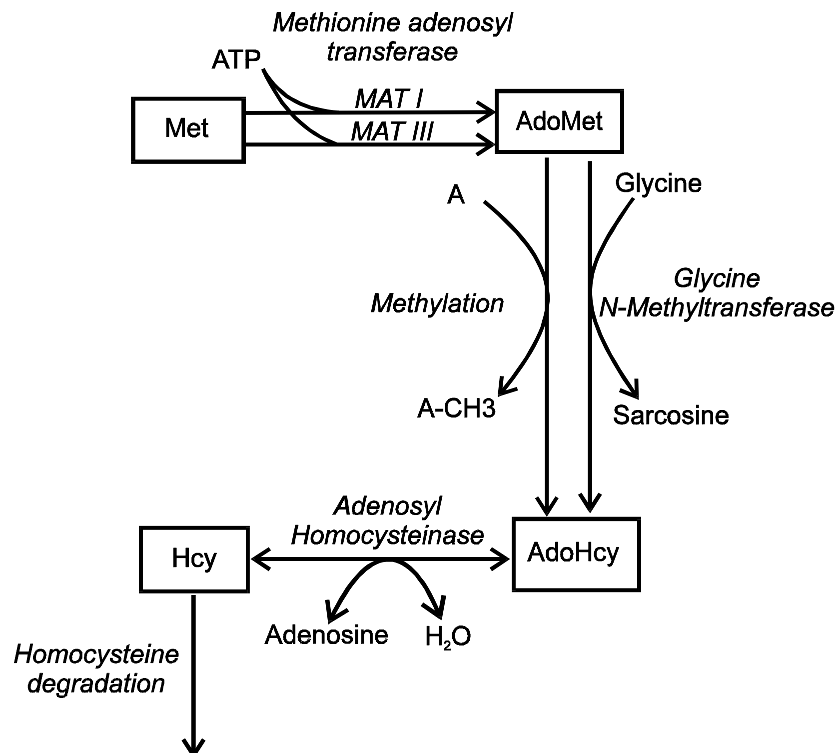

Fig. 9.1 Simplified metabolic pathways of methionine in liver cell employed for modeling in this study. Abbreviations: Met: methionine, AdoMet: S-adenosyl methionine, AdoHcy: S-adenosyl homocysteine, Hcy: homocysteine, A: methylation substrates; A-CH_3: methylated substrates, ATP: adenosine triphosphate.

both steady-states can coexist.

Model description

The model is based on reactions scheme shown on Fig. 9.1. Methionine metabolism starts from the conversion of methionine to AdoMet. We consider methionine concentration as a model parameter. In the liver there are two isoforms of methionine-adenosyltransferase (MATI and MATIII), that catalyze the synthesis of AdoMet from methionine and ATP. AdoMet activates MATIII and inhibits MATI. AdoMet is a substrate for many methylases (MET), which represent the second step in this pathway. Except methylases liver cells have the additional enzyme, glycine-N-methyltransferase (GNMT), that converts AdoMet into S-adenosylhomocysteine and is strongly activated by AdoMet [2]. We assume that the concentrations of adenosine, ATP, glycine and substrates for methylation (A in Fig. 9.1) are constant and that the Michaelis constants are the same for all methylases except GNMT. Adenosylhomocysteinase (AHC) catalyzes the hydrolysis of AdoHcy, the

third step in the pathway. The AHC reaction is at equilibrium because its activity is much higher than the activity of all other reactions in this pathway. The pathway bifurcates at the next step since homocysteine has two metabolic destinies. It can be transformed into cystathionine in a reaction catalyzed by cystathionine-β-synthase (CBS). Cystathionine is subsequently converted to cysteine, and can either be degraded to sulfate or support protein and glutathione biosyntheses. Alternatively, homocysteine can be reconverted to methionine in a reaction catalyzed by methionine synthase (MS) or betaine methyltransferase. Both the transmethylation catalyzed by MS and the transsulfuration catalyzed by CBS are irreversible. MS rate is included in total Hcy utilisation rate, but not included in methionine synthesis and in the scheme because methionine is considered as the model parameter. We assume that the total rate of homocysteine utilization (V_D) depends linearly on homocysteine concentration. After excluding the homocysteine concentration we have the system of two differential equations that describe the kinetics of AdoMet and AdoHcy concentrations:

$$\frac{d[AdoMet]}{dt} = (V_{MATI} + V_{MATIII}) - (V_{GNMT} + V_{MET}) = V^+ - V^- \tag{9.1}$$

$$\frac{d[AdoHcy]}{dt} = \frac{(V_{GNMT} + V_{MET}) - (V_{MS} + V_{CBS})}{D} = \frac{V^- - V_D}{D} \tag{9.2}$$

where $D = (1 + K_{AHC}/[\text{Adenosine}])$, $V^+ = V_{MATI} + V_{MATIII}$, $V^- = V_{GNMT} + V_{MET}$, and $V_D = V_{MS} + V_{CBS}$.

MATI The enzyme exhibits Michaelis-Menten dependence on methionine concentration and is inhibited by AdoMet. Since the ATP concentration is kept as a constant in our model, there is no dependence on this substrate.

$$V_{MATI} = V_{max}^{MATI} \Big/ \left(1 + \frac{K_m^{MATI}}{[Met]}\left(1 + \frac{[AdoMet]}{K_i^{MATI}}\right)\right) \tag{9.3}$$

where $V_{max}^{MATI} = 0.56$ mmol/h/l cells, $K_m^{MATI} = 41\mu$M, and $K_i^{MATI} = 50\mu$M.

MATIII This enzyme exhibits sigmoidal dependence on methionine concentration. AdoMet activates the enzyme by decreasing K_m for methionine.

$$V_{MATIII} = V_{max}^{MATIII} \Big/ \left(1 + \frac{K_{m1}^{MATIII} K_{m2}^{MATIII}}{[Met]^2 + [Met] \cdot K_{m2}^{MATIII}}\right) \tag{9.4}$$

where $K_{m1}^{MATIII} = 20000 \Big/ \left(1 + 5.7\left(\frac{[AdoMet]}{[AdoMet]+600}\right)^2\right)$, $V_{max}^{MATIII} = 22.9$ mmol/h/l cells, $K_{m1}^{MATIII} = 19.1 \times 10^3\mu$M (at 60 μM of AdoMet), and $K_{m2}^{MATIII} = 21.1\mu$M.

Methylation Many methyltransferases exist that utilize AdoMet as a methyl donor. All of them are inhibited by AdoHcy and exhibit low Michaelis constant for AdoMet (except glycine N-methyltransferase)

$$V_{MET} = V_{max}^{MET} \Big/ \left(1 + \frac{K_{m1}^{MET}}{[AdoMet]} + \frac{K_{m2}^{MET}}{[A]} + \frac{K_{m2}^{MET}}{[A]} \cdot \frac{K_{m1}^{MET}}{[AdoMet]}\right) \tag{9.5}$$

where $K_{m1}^{MET} = 10 \times \left(1 + \frac{[AdoHcy]}{4}\right)$, $V_{max}^{MET} = 4.54$ mmol/h/l cells, $K_{m1}^{MET} = 17\mu$M (at 3 μM AdoHcy), [A] is methylation substrate concentration, and $[A]/K_{m2}^{MET} = 0.1$.

GNMT Glycine N-methyltransferase exhibits kinetic parameters that are quite different from other methyl transfer reactions, i.e., sigmoidal dependence on AdoMet. The enzyme is also inhibited by AdoHcy, which is common for most methyltransferases.

$$V_{GNMT} = V_{max}^{GNMT} \Big/ \left(1 + \left(\frac{K_m^{GNMT}}{[AdoMet]}\right)^{2.3}\right) \left(1 + \frac{[AdoHcy]}{K_i^{GNMT}}\right) \tag{9.6}$$

where $V_{max}^{GNMT} = 10.6$ mmol/h/l cells, $K_m^{GNMT} = 4500$ μM, and $K_i^{GNMT} = 20$ μM.

Homocysteine cleavage The description of homocysteine (Hcy) cleavage is simplified in the present model. The rate of Hcy degradation (V_D) depends linearly on substrate concentration.

$$V_D = \alpha_D \cdot [Hcy] \tag{9.7}$$

where $[Hcy] = [AdoHcy]\frac{k_{AHC}}{[\text{Adenosine}]}$, $\alpha_D = 1333$ 1/h, $k_{AHC} = 0.1$ μM, and [Adenosine] = 1 μM.

Results

We found out that AdoHcy is the fast variable of the model (time constant is in the seconds time regime), whereas AdoMet is the slow variable (time constant is about tens of minutes). The interplay between the overall rates of AdoMet production (V^+) and consumption (V^-) and their dependence on methionine and AdoMet concentrations determines the kinetic behaviour of whole metabolic system. Fig. 9.2 shows the dependence of V^+ (solid lines) and V^- (dashed line) on the concentration of AdoMet. As follows from reactions scheme, changes in methionine concentration affects V^+ only without altering V^-. Curves 1–3 in Fig. 9.2 represent V^+ under three different concentrations of methionine. One steady state exists if methionine concentration is lower than 50 μM (Fig. 9.2, curve 1). This steady-state is stable. This represents the "low" mode of methionine metabolism, at a

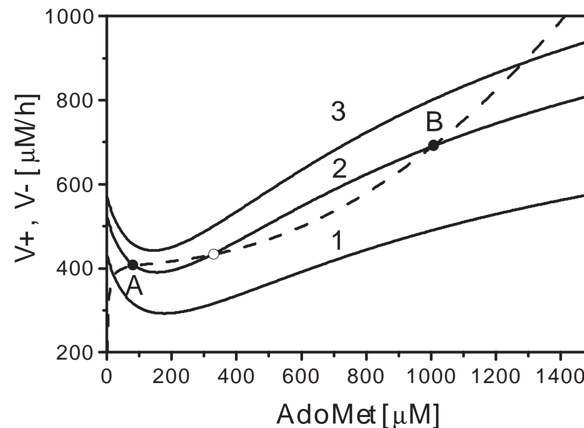

Fig. 9.2 The total rates of AdoMet production (V^+, solid lines) and consumption (V^-, dashed line) as a function of AdoMet concentration. V^+ is shown at three different methionine concentrations: 1 is at 42.5 μM, 2 is at 52.5 μM, and 3 is at 57.5 μM. Solid circles indicate stable steady states with low (A) and high (B) AdoMet concentrations, the open circle indicates an unstable steady state.

rather low AdoMet concentration and low metabolic rate. If the concentration of methionine is higher than 54 μM (Fig. 9.2, curve 3), the system achieves a second stable steady state in which the concentration of AdoMet and the metabolic flux are much higher. This represents the "high" mode for methionine metabolism. When the methionine concentration lies within a narrow range between 50 and 54 mM we can see three points of intersection, i.e., three steady states (Fig. 9.2, curve 2). The middle point is a saddle and is unstable. The other two represent the steady states at low and high concentrations of AdoMet. These data imply that for the same concentration of methionine, the rates of AdoMet degradation and synthesis can either be low (Fig. 9.2, steady state A) or high (Fig. 9.2, steady state B). Thus, the system is bi-stable.

The dependence of the rates of individual enzymes as a function of methionine concentration is shown in Fig. 9.3. At the "low" steady-state the total rate of AdoMet synthesis (Fig. 9.3A, curve 1) is largely determined by MATI (Fig. 9.3A, curve 2) while the flux through MATIII is small (Fig. 9.3A, curve 3). The rate of AdoMet synthesis is balanced by its consumption as determined by the total methylase activity (Fig. 9.3B, curves 1 and 2 respectively). Under these conditions, the flux through GNMT is negligible (Fig. 9.3B, curve 3). When the system is in the "high" steady state, the rate of AdoMet production is governed mostly by MATIII (Fig. 9.3A, curve 3). Under these conditions the AdoMet production exceeds its consumption by the methyltransferases and could render the system unstable (Fig. 9.3B curve 2). However, this is averted by the compensatory increase in the degradation of AdoMet through GNMT.

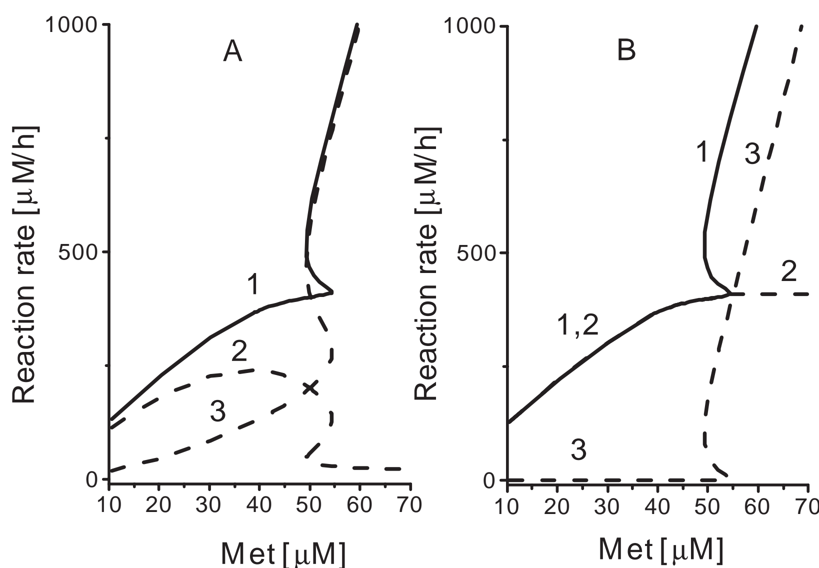

Fig. 9.3 The dependence of steady-state levels of AdoMet and reaction velocities of key enzymes on methionine concentration. (A): Dependence of AdoMet production (9.1) and the activities of MATI (9.2) and MATIII (9.3) on methionine concentration. (B): Kinetics of AdoMet consumption (9.1) and of its components, total methylation (9.2) and glycine-N-methyltransferase (9.3) on methionine concentration.

Discussion

The methionine metabolic pathway serves two major roles in liver cells which are production of methyl groups to support a variety of methyl transfer reactions and the conversion of methionine to cysteine. According to our model, to fulfill these physiological functions the pathway can operate in two modes. The first mode represents a low metabolic rate, occurs at low methionine concentrations, results in low AdoMet concentrations, and serves to maintain the cells supply of AdoMet which is the major substrate for methylation. The second mode represents a high metabolic rate, occurs at high methionine concentrations, results in high AdoMet concentrations, serves to enhance cysteine synthesis if it is necessary, and provides a degradative pathway for excess methionine.

In the “low” mode the system functions predominantly to produce the methylation substrate, AdoMet. The regulatory mechanism under these conditions is the same as that seen in many metabolic pathways where the product, AdoMet in this instance, inhibits its own production. This strategy permits relatively independent regulation of AdoMet levels by its various consumers, the methylases. In this steady state, both the AdoMet concentration and the flux through this metabolic pathway are low. In the “high” mode, AdoMet levels and the rates of its synthesis and degradation are greatly increased. The flow of methionine through AdoMet under these conditions greatly exceeds the demand of the methylases (Fig. 9.3B).

Under these conditions, the AdoMet levels are critically dependent on the methionine concentration. The behaviour predicted by this model is supported by experimental studies in which the concentrations of AdoMet and AdoHcy and the activity of GNMT were found to increase during adaptation to excess dietary methionine [3]. From the biochemical point of view GNMT may be viewed as performing a futile task since it converts glycine to sarcosine which is not a useful metabolite. Sarcosine can either be converted back to glycine by sarcosine dehydrogenase or transported out of the cell. Our mathematical model suggests a rationale for the existence of this enzyme. It endows the cell with the ability to greatly increase the flow from methionine to homocysteine under conditions of excess methionine. Thus, as has been noted previously, the liver which alone contains MATIII, may be unique in its ability to adapt quickly to high levels of methionine [4]. Our model is consistent with the observation that the concentration of methionine in rat liver is relatively insensitive to changes in dietary protein intake, in contrast to the hepatic concentrations of AdoMet and AdoHcy which increase with augmented dietary protein [5].

A mode of "bistability" exists in a narrow parameter range between "high" and "low" mode of methionine pathway's operation. In this range both modes can coexist. We do not think that bistability itself is an important feature of methionine metabolism. We believe that it is the best way to achieve a triggering in this pathway. The existence of bistability leads to a sharp change of AdoMet concentration and methionine flux under variation of methionine concentration.

The existence of two states in a system is a common phenomenon in biology. The switching mechanism in many cases is dependent on a signal transduction pathway and involves covalent modification via phosphorylation/dephosphorylation reactions. Analysis of the methionine metabolic pathway reveals the existence of a different type of metabolic switch, one which is accomplished by changes in the concentration of the substrate.

Acknowledgements

This work was supported by grants from the National Institutes of Health (HL58984) to R.B.

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