Applying Computational Systems Biology Techniques to Explore Disease-Related Methionine Cycle

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ABSTRACT

Methionine is an essential amino acid, meaning that it must be provided in the diet. Its derivatives play a significant role in the health of humans. It has been proven that malfunctions in the methionine cycle lead to many severe diseases. In this paper, we have constructed a complete model of the methionine cycle based on computational systems biology techniques.

Our results show that alterations of the methionine cycle can lead to many severe diseases. An excess of methionine intake causes an increase in cystathionine, resulting in diseases such as Down Syndrome, hepatic cancer, and hepatic disease. Methionine deficiency can dramatically decrease the concentration of AdoMet and cystathionine. Decreased AdoMet leads to cirrhosis and hepatocellular carcinoma, while a decrease in cystathionine can result in cardiovascular disease.

In the case of MATI/III deficiency, methionine accumulates quickly, blocks the synthesis of all subsequent intermediates, and eventually results in hypermethioninemia and cirrhosis. Deficiency of SM or AH dramatically decreases homocysteine and cystathionine, leading to vascular disease. When folate is removed, homocysteine accumulates to an elevated level that in turn causes colorectal cancer, neural tube defects, and an increased risk of vascular occlusion.

Finally, deficiency of betaine and MS result in the increase of cystathionine and homocysteine, which can lead to megaloblastic anemia and neurological abnormalities. Our study has demonstrated the utility of using a program based model to predict various effects on the methionine cycle.

Keywords: pathway, methionine cycle, systems biology

1: INTRODUCTIONS

Methionine is an essential amino acid, meaning that it cannot be produced by the body and it must be obtained in the diet. Its derivatives play significant roles in the regulation of the human body. In humans, the methionine cycle consists of methionine (Met), S-adenosylmethionine (AdoMet), S- adenosyl-homocysteine (AdoHcy), homocysteine (Hcy), and cystathionine (Cys). This cycle is highly complicated and supplies sulfur and other compounds required by the body for normal metabolism and growth. Normal function of the methionine cycle is essential for growth and development of human body. If the methionine cycle is not functioning or temporarily blocked, the effect manifests itself in the human body as Alzheimer's disease [1], Down Syndrome [2], cardiovascular disease [3], liver disease [4], neural tube defects [5], and various cancers [6, 7]. Therefore, with the importance of this cycle in mind, we chose to model this particular pathway.

Foods, such as fruits, meat, vegetables, nuts, and legumes all contain methionine. There are several enzymes that exist in the cycle including MAT, SM GNMT, AH, CBS, BHMT, and MS (Figure 1). Upon consumption, methionine can first be changed to AdoMet by an enzyme with isoforms, MATI and MATIII. After this reaction, SM and GNMT work to produce AdoHcy via methyl group transfer. Enzyme AH helps a reversible conversion of AdoHcy to Homocysteine, which has an alternative fate in vivo. Homocysteine can be converted back to methionine, consequently making a closed loop, or it can undergo transulfuration by CBS to make cystathionine. Positive and negative feedback throughout the cycle provides a further level of sophistication. The first intermediate, AdoMet serves as an activator for MATIII and a repressor for MATI; however, its activity is not limited solely to the upstream reaction. AdoMet can also activate CBS and repress BHMT in the cycle. AdoHcy can repress GNMT, SM, and BHMT; however, it also activates CBS.

2: DESCRIPTION OF OUR MODIFIED MATHEMATICAL MODEL

In this paper, we introduce kinetic equations into every step of the methionine cycle. The model consists of four differential equations, based on known reaction kinetics. We have modified the original equations [8, 9] by adding the three important effects of activation, inhibition, and degradation into the balanced equations. As shown in Figure 1, balances around the four substrates are depicted by the following four differential equations individually.

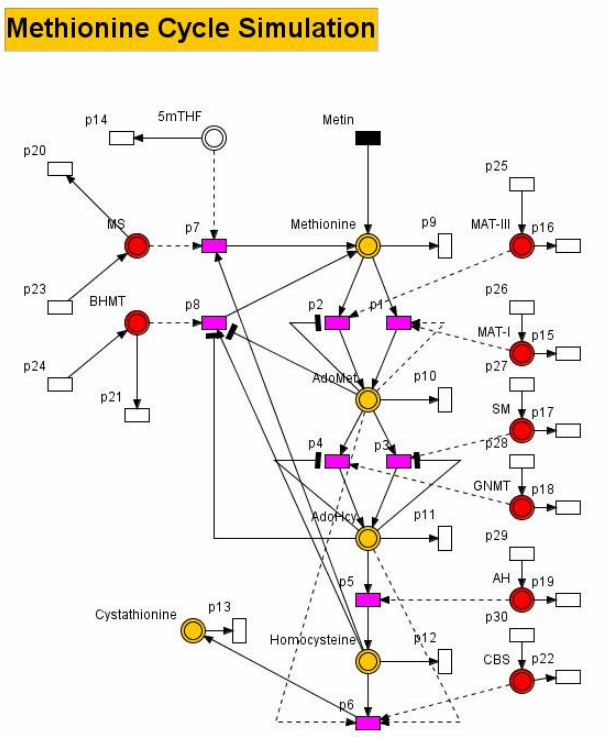

Figure 1 A simple structure of methionine cycle established by HFPN

$$\frac{d[Met]}{dt} = \frac{dm1}{dt} = p7 + p8 + Metin - p2 - p1 - p9 \quad (1)$$

$$\frac{d[AdoMet]}{dt} = \frac{dm2}{dt} = p2 + p1 - p3 - p4 - p10 \quad (2)$$

$$\frac{d[AdoHcy]}{dt} = \frac{dm3}{dt} = p3 + p4 - p5 - p11 \quad (3)$$

$$\frac{d[Hcy]}{dt} = \frac{dm4}{dt} = p5 - p6 - p7 - p8 - p12 \quad (4)$$

The terms on the right-hand sides are the rates of the reactions.

3: CONSTRUCTING THE COMPLETE METHIONINE CYCLE WITH GENOMIC OBJECT NET

After constructing an initial model of the methionine cycle, we then applied the key parameters in order to simulate the diseases that would result from an abnormal methionine cycle. Similar to how entities represent enzymes and metabolites that participate in this pathway, continuous processes represent the specific reactions that are occurring in this pathway. For example, we have shown a simple structure in Figure 1. After the main structure has been established, we applied parameters provided by Reed [9] and Martinov [8], as listed in Table 1 and Table 2, to complete the whole model of the methionine cycle.

Table 1 Properties of each entry in our constructed model

Name	Type	Variable	Initial Value
Methionine	Double	m1	3.33
AdoMet	Double	m2	0
AdoHcy	Double	m3	0
Homocysteine	Double	m4	0
Cystathionine	Double	m5	0
MAT-III	Double	m6	5
MAT-I	Double	m7	5
SM	Double	m8	5
GNMT	Double	m9	5
AH	Double	m10	5
MS	Double	m11	5
BHMT	Double	m12	5
CBS	Double	m13	5
5mTHF	Double	m14	5.2
	Double	a1	100
	Double	a2	10
	Double	b1	1.7
	Double	b2	30
	Double	VmaxMATI	0.155833333
	Double	VmaxMATIII	6.352777778
	Double	VmaxSM	1.255833333
	Double	VmaxGNMT	2.944444444
	Double	VAH	a1*(m3-a2*m4)/3600
	Double	VCBS	(b1*(m2+m3)-b2)*m4/3600
	Double	VmaxMS	0.138888889
	Double	VmaxBHMT	0.694444444
	Double	KmMATI	41
	Double	KmMATIII	2000/(1+5.7*(m2/(m2+600))*(m2/(m2+600)))
	Double	KmSM	1.0*(1+(m3/4))
	Double	KmGNMT	4500
	Double	KmBHMT	12
	Double	KmMS	0.1

The detailed steps including equations applied are shown below.

Step1: First, we set the input rate of methionine as:

$$Metin = 3.33/60\,\text{sec} \quad (5)$$

Step2: Methionine is catalyzed to turn to AdoMet by MATI and MATIII, the equations are:

$$p1 = V_{MATI} = \frac{V\max MATI}{1 + \frac{KmMATI}{m1} \times \left(1 + \frac{m2}{KiMATI}\right)} \quad (6)$$

Table 2 Firing rules and properties of each process

Name	Type	Firing Style	Kinetic Script
p1	continuous	and	VmaxMATI/(1+(KmMATI*(1+(m2/50))/m1))
p2	continuous	and	VmaxMATIII/(1+(KmMATIII*21.1)/(m1*m1+m1*21.1))
p3	continuous	and	VmaxSM/(1+(KmSM/m3)+10+10*KmSM/m3)
p4	continuous	and	(1/(1+(m3/20))*(VmaxGNMT/(1+(KmGNMT/m2)*(KmGNMT/m2))))
p5	continuous	and	a1*(m3-a2*m4)/3600
p6	continuous	and	(b1*(m2+m3)-b2)*m4/3600
p7	continuous	and	VmaxMS*m15*m4/(1*KmMS+KmMS*m15+25*m4+m15*m4)
p8	continuous	and	(0.7-(0.025*(m2+m3-150)))*(VmaxBHMT*m4/(KmBHMT+m4))
p9	continuous	and	m1/10000
p10	continuous	and	m2/10000
p11	continuous	and	m3/10000
p12	continuous	and	m4/10000
p13	continuous	and	m5 / 10000
p14	continuous	and	m14/10000
p15	continuous	and	m6 / 10
p16	continuous	and	m7 / 10
p17	continuous	and	m8 / 10
p18	continuous	and	m9 / 10
p19	continuous	and	m10 / 10
p20	continuous	and	m11 / 10
p21	continuous	and	m12 / 10
p22	continuous	and	m13 / 10
p23	continuous	and	1
p24	continuous	and	1
p25	continuous	and	1
p26	continuous	and	1
p27	continuous	and	1
p28	continuous	and	1
p29	continuous	and	1
p30	continuous	and	1
p31	discrete	and	3.33

$$p2 = V_{MATIII} = \frac{V\max MATIII}{1+\frac{Km1MATIII \times Km2MATIII}{m1^2 + m1 \times Km2MATIII}} \quad (7)$$

$$Km1MATIII = \frac{20000}{1+5.7\times\left(\frac{m2}{m2+600}\right)^2} \quad (8)$$

Step3: AdoMet turns to AdoHcy by SM and GNMT:

$$p3 = V_{SM} = \frac{V\max SM}{1+\frac{Km1SM}{m2}+\frac{Km2SM}{[A]}+\frac{Km2SM}{[A]}\times\frac{Km1SM}{m2}} \quad (9)$$

$$Km1SM = 1.0\times\left(1+\frac{m3}{4}\right) \quad (10)$$

$$p4 = V_{GNMT} = \frac{V\max GNMT}{1+\left(\frac{KmGNMT}{m2}\right)^2}\times\frac{1}{1+\frac{m3}{KiGNMT}} \quad (11)$$

Step4: AdoMet turns to homocysteine by AH:

$$p5 = V_{AH} = \frac{1}{3600}\times a1\times(m3 - a2\times m4) \quad (12)$$

Step5: Homocysteine turns to Cystathionine by CBS:

$$p6 = V_{CBS} = \frac{1}{3600}\times[b1\times(m2+m3)-b2]\times m4 \quad (13)$$

Step6: Finally, homocysteine goes back to methionine by MS and BHMT:

$$p7 = V_{MS} = \frac{V\max MS\times m14\times m4}{KdMS\times KmMSHcy + KmMSHcy\times m14 + KmMS5mTHF + m14\times m4} \quad (14)$$

$$p8 = V_{BHMT} = [0.7-(0.025)\times(m2+m3-150)]\times\frac{V\max BHMT\times m4}{KmBHMT + m4} \quad (15)$$

As can be seen in Figure 2, we have constructed complete model of the methionine cycle with Genomic Object Net, a useful computational system biology tool developed by Masao Nagasaki and his colleague [10]..

4: RESULTS AND DISCUSSION

4.1 Simulation of the normal methionine cycle

By running the simulation of the methionine cycle, one can observe the dynamic changes of each substance over time. The effects of protein self-degradation, feedback activation and inhibition are integrated into the cycle. Among them, degradation is represented by processes with kinetic equations, for example, MATIII (m6) will be degraded during the simulation at a rate of m6/10 per second. Meanwhile, test connectors and inhibitory connectors are used to represent feedback activation and inhibition respectively. Based on known biological knowledge, we also assumed the activation threshold to be 0 and the inhibition threshold due to AdoMet and AdoHcy to be 135 and 13 individually. These assumptions may not reflect the real biological system; however, they can be used initially to explore the dynamic behaviour of each substance in the complete methionine cycle.

4.2 Excess methionine intake results in multiple diseases

We simulate this situation by increasing the amount of metin. Finkelstein and Martin [11] demonstrated that the fraction of Hcy, which is the source for cystathionine synthesis, is a function of the concentration of AdoMet. Higher intake of dietary methionine results in higher concentrations of AdoMet. Increasing the concentration of AdoMet will elevate the transulfuration fraction, resulting in more cystathionine synthesis. We can observe a drastic increase in [AdoMet] and [Cystathionine] in Figure3. High levels of

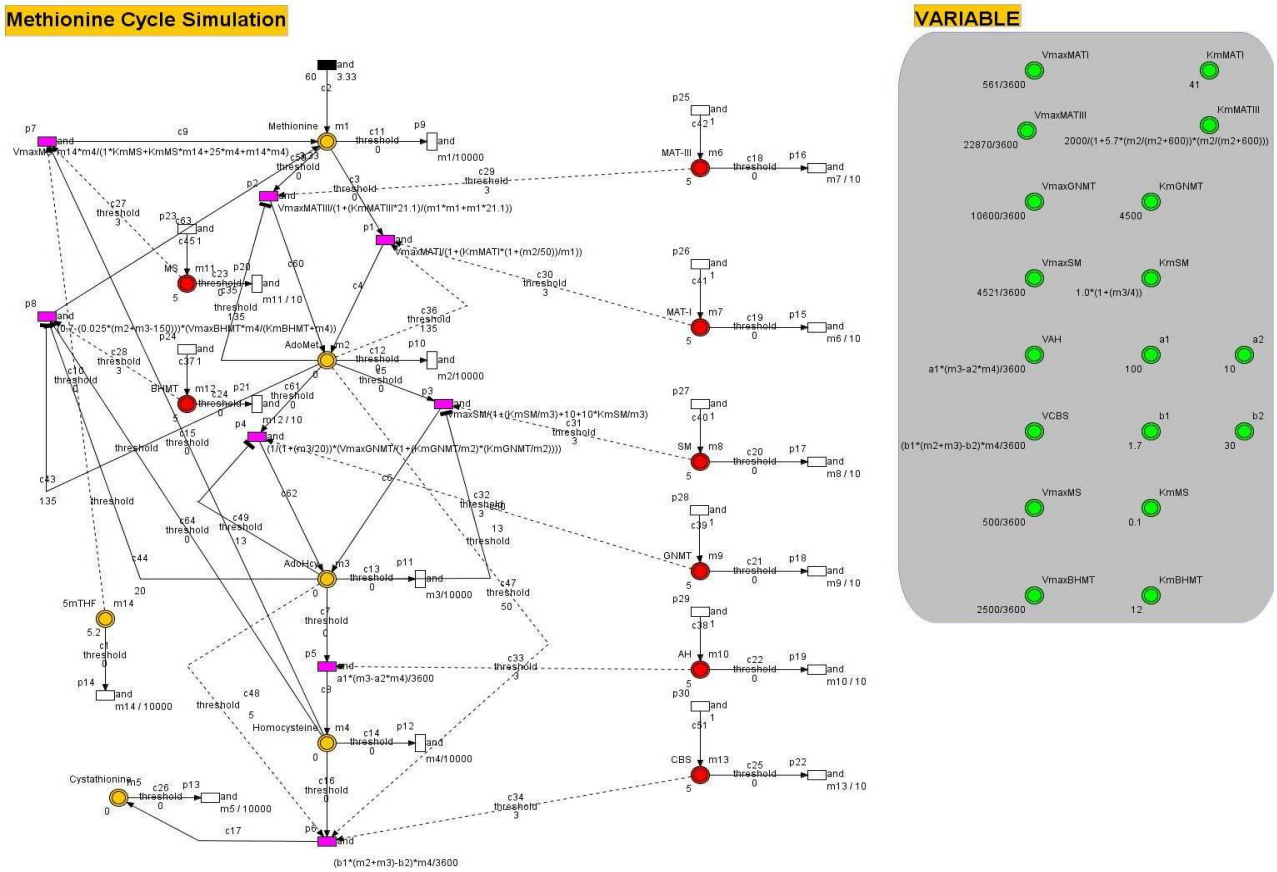

Figure 2 Simulation of methionine cycle and the variable are shown with all properties in each element.

cystathionine (cystathininuria) can lead to diseases such as Down Syndrome [1], hepatic cancer [6], and hepatic disease [4]. Methionine excess can also make [Hcy] increase over two times, leading to cardiovascular disease [3, 12].

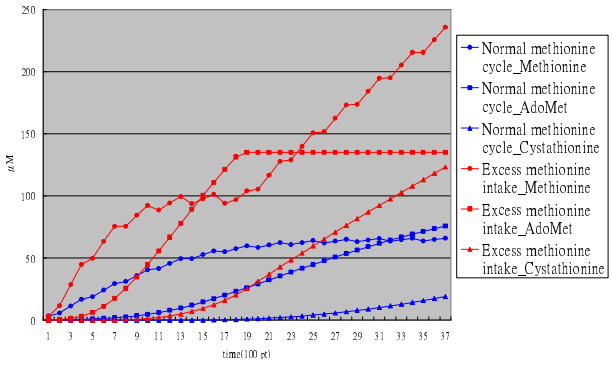

Figure 3 Effect of excess methionine intake

4.3 Methionine deficiency in diet

In contrast to an excess amount of methionine intake, a methionine deficient diet can lead to a different set of diseases. We simulate this phenomenon by reducing metin to 1.00 m/min. In Figure 4, we observed that both [AdoMet] and [Cystathionine] decrease dramatically when methionine intake declines. A decrease in [AdoMet] in vivo can cause diseases such as cirrhosis, intrahepatic cholestasis, fibrosis, NASH (non-alcoholic steatohepatitis), HCC (hepatocellular carcinoma), and steatosis [13]. A deficiency in [Cystathionine] leads to cardiovascular disease [3].

Methionine accumulation may lead to serious outcomes

It is also worth looking at the effect of low levels of MATI and MATIII. In our model, we assume that the initial concentration of both MATI and MATIII will be consumed within a very short time (~1 minute). In this situation, we can see that methionine accumulates quickly after running out of MATI and MATIII (MATI/III deficiency). All subsequent intermediate synthesis is thereafter completely blocked (Figure 9). This means that the whole cycle is non-functional and this can lead to various diseases. MATI/III deficiency results in hypermethioninemia [11] and a decline in AdoMet levels, resulting in cirrhosis [14].

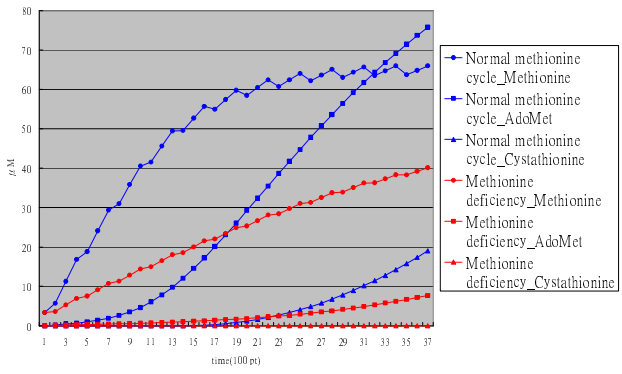

Figure 4 Effect of methionine deficiency

4.4 The effect of SM and GNMT deficiency

We set the initial concentrations of SM (S-adenosyl- methionine- dependent methyltransferases) and GNMT (glycine N-methyltransferase) at zero to mimic this condition. After running the simulation, we found that deficiency of GNMT did not significantly alter the methionine cycle. However, deficiencies in SM cause [AdoHcy] to drop, which is accompanied by dramatic decreases in [Homocysteine] and [Cystathionine] (Figure 5). The low concentration of cystathionine can lead to cardiovascular disease.

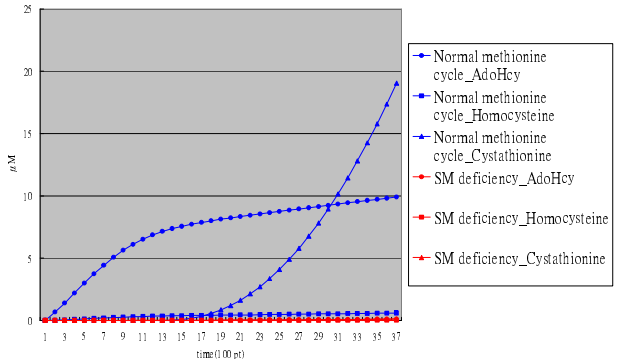

Figure 5 Effect of SM deficiency

4.5 The effect of AH deficiency

In Figure 6, a simulation of AH deficiency is presented. We observed that if there is no functional AH in vivo, [Homocycteine] will decrease directly and then cause a decline in the concentrations of cystathionine and methionine. This also may increase the risk of cardiovascular disease.

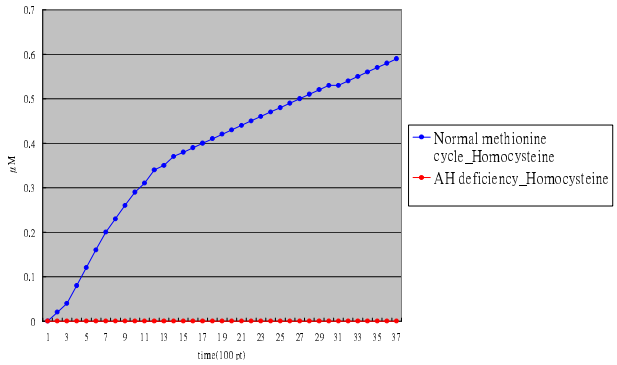

Figure 6 Effect of AH deficiency

4.6 The effect of folate deficiency

It has been shown that high blood levels of homocysteine can cause cardiovascular disease. By decreasing plasma total homocysteine using nutritional cofactors (such as folate), one can reduce the risk of cardiovascular events. In our model, after removing the folate supply, we can see that both [Methionine] and [AdoMet] decrease slightly, while [Homocysteine] and [Cystathionine] increase (Figure 7 (a) and (b)). This explains the findings described in literature [15, 16] that mildly elevated circulating homocysteine concentrations are associated with increased risk of vascular occlusion.

In our model, we have shown that a lack of folate will elevate levels of the endothelial cell toxin, homocysteine, which can cause colorectal cancer [16, 7] and neural tube defects (NTDs) [5].

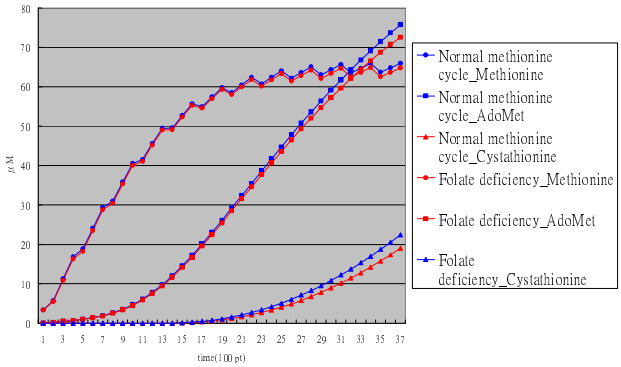

Figure 7 (a) Effect of folate deficiency on homocysteine

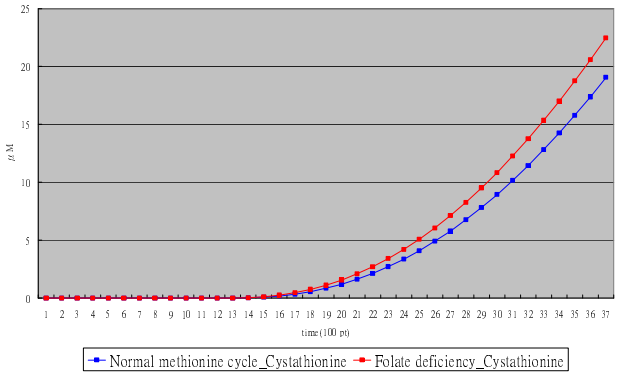

Figure 7 (b) Effect of folate deficiency on cystathionine

4.7 The effect of betaine and methionine synthase (MS) deficiency

Betaine is a chemical that activates the enzyme BHMT. In order to simulate the effect of betaine and MS deficiency, we set [BHMT] and [MS] equal to zero. The result is that [Cystathionine] and [Homocysteine] increase (Figure 8 (a) (b), 9 (a) (b)). In humans, this translates into diseases such as megaloblastic anemia and neurological abnormalities (e.g. NTDs) [7, 8].

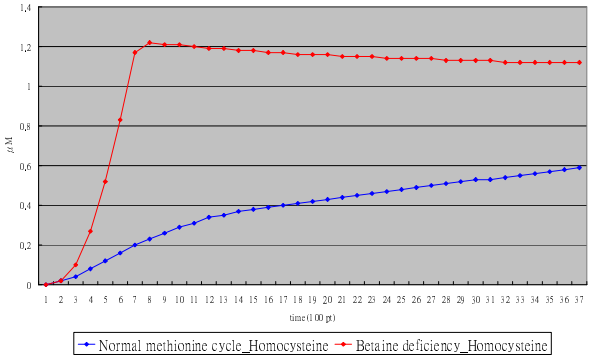

Figure 8 (a) Effect of betaine deficiency on homocysteine

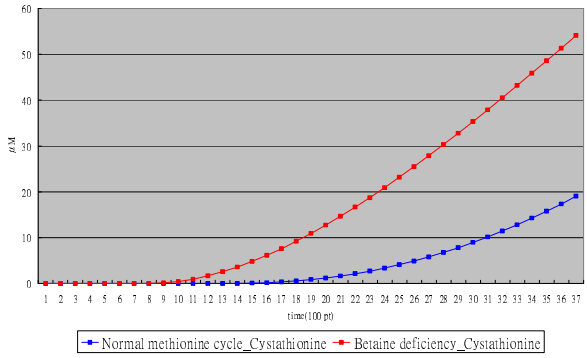

Figure 8 (b) Effect of betaine deficiency on cystathionine

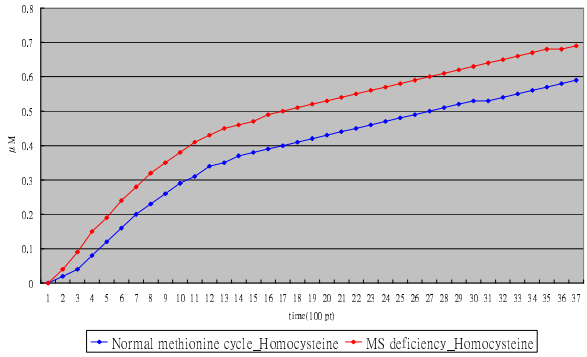

Figure 9 (a) Effect of methionine synthase deficiency on homocysteine

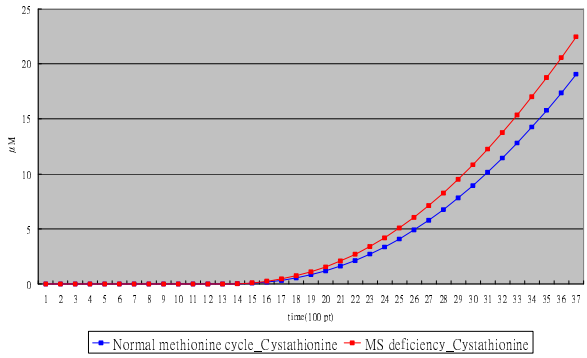

Figure 9 (b) Effect of methionine synthase deficiency on cystathionine

5: CONCLUSION

By adding protein self-degradation, feedback activation and inhibition into our integrated methionine cycle, the simulation results are quite intriguing. Our study has demonstrated the utility of using a program based model to predict various effects on the methionine cycle.

Although these simulation results are quite intriguing, metabolic pathways are not closed and independent systems. Other amino acids and intermediates may also interact with the methionine cycle, which we did not take into account in this paper. Future studies will encompass more of these variables and hopefully further elucidate these metabolic pathways and the diseases attributed to their dysregulation. KEGG provides many metabolic pathways that include metabolites that interact within the methionine cycle. The full-size simulation is a challenging endeavor and we intend to add them in our model in the near future.

ACKNOWLEDGES

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REFERENCES

[1]. L, Miller A: The methionine-homocysteine cycle and its effects on cognitive diseases. Altern Med Rev 2003, 8: 7-19.
[2]. Pogribna, M., Melnyk, S., Pogrybny, I., Chango, A., Yi, P., James, J: Homocysteine metabolism in children with Down Syndrome:in vitro modulation. Am. J. Hum. Genet 2001, 69: 88–95.
[3]. Refsum, H., Ueland, P.M., Nygard, O., Vollset, S.E: Homocysteine and cardiovascular disease. Ann. Rev. Med 1998, 49: 31–62.
[4]. Finkelstein, J: Methionine metabolism in liver disease. Am. J. Clin. Nutr 2003, 77: 1094–1095.
[5]. Eskes, T.K.A.B: Neural tube defects, vitamins and homocysteine. Eur J Pediatr 1998, 157: 139–141
[6]. L, Helson, Peterson R.H., Schwartz M.K: Cystathionine excess in children with hepatic cancer. Cancer Res 1973, 33: 1570-3.
[7]. Potter, J.D: Colorectal cancer: molecules and populations. J. Nat. Cancer Inst 1999, 91: 916–932.
[8]. Martinov, M., Vitvitsky, V., Mosharov, E., Banerjee, R., Ataullakhanov, F: A substrate switch: a new mode of regulation in the methionine metabolic pathway. J. Theor. Biol 2000, 204: 521–532.
[9]. Reed, Michael C., H. Frederik Nijhout, Rachel Sparks, Cornelia M. Ulrich: A mathematical model of the methionine cycle. Journal of Theoretical Biology 2004, 226: 33–43.
[10]. Doi, A., Nagasaki, M., Fujita, S., Matsuno, H., Miyano, S: Genomic Object Net:II. Modelling biopathways by hybrid functional Petri net with extension. Applied Bioinformatics 3004, 2: 185–188.
[11]. Finkelstein, J: Methionine metabolism in mammals. J. Nutr. Biochem 1990, 1: 228–237.
[12]. Troen, Aron M., Esther Lutgens, Donald E. Smith, Irwin H. Rosenberg, and Jacob Selhub: The atherogenic effect of excess methionine intake. PNAS 2003, 100 (25): 15089-94.
[13]. Mato, Jose M., Fernando J. Corrales, Shelly C. Lu, Matias A. Avila: S-Adenosylmethionine: a control switch that regulates liver function. FASEB J. 2002, 16: 15-26.
[14]. Martínez-Chantar, M. Luz, Elena R. García-Trevijano, M. Ujue Latasa, Isabel Pérez-Mato, Manuel M. Sánchez del Pino, Fernando J. Corrales, Matias A. Avila, and José M. Mato: Importance of a deficiency in S-adenosyl-L-methionine synthesis in the pathogenesis of liver injury. Am. J. Clin. Nutr. 2002, 76 (suppl): 1177S-82S.
[15]. Brattström, Lars, David EL Wilcken: Homocysteine and cardiovascular disease: cause or effect? Am J Clin Nutr 2000, 72: 315–23
[16]. Duthie, S.J: Folic acid deficiency and cancer: mechanisms of DNA instability. Brit. Med. Bull 1999, 55: 578–592.