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ORIGINAL ARTICLE

Determining important regulatory relations of amino acids from dynamic network analysis of plasma amino acids

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Abstract The changes in the concentrations of plasma amino acids do not always follow the flow-based metabolic pathway network. We have previously shown that there is a control-based network structure among plasma amino acids besides the metabolic pathway map. Based on this network structure, in this study, we performed dynamic analysis using time-course data of the plasma samples of rats fed single essential amino acid deficient diet. Using S-system model (conceptual mathematical model represented by power-law formalism), we inferred the dynamic network structure which reproduces the actual time-courses within the error allowance of 13.17%. By performing sensitivity analysis, three of the most dominant relations in this network were selected; the control paths from leucine to valine, from methionine to threonine, and from leucine to isoleucine. This result is in good agreement with the biological knowledge regarding branched-chain amino acids, and suggests the biological importance of the effect from methionine to threonine.

Keywords Plasma amino acids · Relation · Regulation · Network · Amino acid deficiency · Dynamic

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Introduction

In the field of metabonomics, the metabolite pattern of biological fluid obtained from healthy subjects and diseased subjects are compared in search of the biological and diagnostic markers for the disease (Brindle et al. 2002; Nicholson et al. 1999). This approach is based on the fact that the amount of the metabolites in biological fluids and tissues change in coordination with the physiological conditions of subjects. Multivariate analysis and pattern recognition studies have revealed that these metabolic profiles contain the phenotypic information which can be used as a signature for a physiological condition.

Amino acids are a group of metabolites which are important as substrates for protein synthesis as well as signaling molecules (Felig 1975). The plasma amino acid concentrations, like other metabolites, have distinctive features for various physiological conditions. There are many reports describing the disease-specific abnormalities in plasma amino acid profiles such as, liver failure (Holm et al. 1999; Soeters and Fischer 1976), renal failure(Hong et al. 1998), cancer (Watanabe et al. 1984; Weinlich et al. 2007), diabetes(Ohtsuka and Agishi 1992; Soltesz et al. 1978), cardiovascular disorders (Obeid 2005), etc. There are some trials which make use of plasma amino acid profiles to diagnosis and distinguish abnormal subjects from healthy subjects, or subtypes and stages of diseases (Noguchi et al. 2006). One of the traditional examples of using plasma amino acid profiles for diagnostic markers is the Fisher's ratio, which is a ratio of branched-chain amino acids to aromatic amino acids and is used for the diagnostic marker for liver fibrosis (Ferenci and Wewalka 1978; Sozeters and Fischer 1976). These studies clearly indicate that plasma amino acid profile itself can be a useful tool for monitoring the physiological state of an organism.

Although these previous studies discuss how to distinguish different physiological states using the plasma amino acid profile data, the control mechanism behind the change in the profile is not discussed in detail. The conventional approach to understand the changes in the amino acid profile focuses on the metabolic pathway map (Dahlquist et al. 2002; Ogata et al. 1999). However, the metabolic pathway maps are constructed from many pieces of biological findings each containing only one or a few enzymes and metabolites, and it only shows the topological relations between metabolites. It does not have the information of the metabolic equilibrium as a whole, nor the regulatory mechanism of the concentration of each metabolite. Therefore, the conventional investigations were not successful at figuring out why the plasma amino acid profiles change in accordance with the physiological state.

On the other hand, from the theoretical point of view, especially on the research of genetic network analysis, recent advances in the technology of bioinformatics have enabled gene expression to be comprehensive, while several approaches have been proposed to infer the corresponding genetic networks since the latter 1990s. Estimation of the interrelated mechanism among system components by using experimentally observed expression data is generally referred to as "inverse problem" and the expression data correspond to the restricted conditions for solving inverse problem.

These proposed network analysis methods are roughly divided into two modeling approaches depending on the variety of analytical data and mathematical model. One is a structural modeling approach, which treats the data representing binary relations of system components' expressions. These relations describe the effects of one component on the expression of others and are mainly provided by the changes of the state of component's expression patterns. And the other is a dynamic modeling approach, which relies on the analysis of temporal responses of component's expression patterns to perturbations or internal changes. The network analysis method in this modeling approach represents behavior of system components by differential equation.

In the structural modeling approach, there are a stationary network analysis method and a probabilistic network analysis method. The former method corresponds to a static Boolean network model, which mainly relies on the analysis of state changes of system component's expression patterns resulting from deletion or forcible expression of one component. Systematical analysis of the binary relations between pairs of components enables us to reconstruct a possible minimum architecture of the network which is consistent for all the data (Maki et al. 2001). The latter one corresponds to a Bayesian network model, which is a graph-based model that statically investigates the characteristics of dependence and conditional independence between the data sets of variables (Cooper and Herskovits 1992; Friedman et al. 2000; Imoto et al. 2002), and so on.

In the dynamic modeling approach, on the other hand, various network analysis models are proposed to estimate such interaction mechanisms among system components, and enable us to reconstruct genetic or reaction network architectures with the experimentally observed time-courses of the patterns of gene expression (Kikuchi et al. 2003; Maki et al. 2001; Savageau 1998; Somogyi and Sniegoski 1996).

The advantage of the understanding of the interaction between these system components by network analysis is to be able to infer the interrelated and directional network structure of system components without considering any prior topological information on metabolic pathways. This data driven analysis should lead us to the further understanding of the dynamics of the profiles of system components and gives us some clues for the factors affecting the interrelated network under various physiological conditions.

In order to apply this data driven analysis method to the understanding of the regulatory mechanism of the plasma amino acids, we have applied stationary network analysis method to plasma amino acid concentration data and inferred the static regulatory network structure of the plasma amino acids in the previous study (Shikata et al. 2007). The plasma samples from rats fed on a single amino acid-deficient (minus-one) diet for several weeks were analyzed by the methods originally established to infer the genetic network structure (Maki et al. 2004). From the inferred network structure, we have shown that the network analysis methods are useful in finding the new relations between plasma amino acids and obtaining new insights into the biological functions of amino acids.

The static network analysis has revealed the topological regulatory relation between plasma amino acids, but this is not enough to understand the regulatory mechanism which controls the plasma amino acid concentrations under various physiological conditions. In order to determine which paths in the inferred network structure have important physiological role, the analysis on the dynamic property of the network is necessary. In this study, we performed the dynamic (temporal) network analysis by using the time-course data of the plasma amino acid concentrations under minus-one condition, and the analytical method based on the S-system model, which is one of the typical conceptual mathematical models represented by power-law formalism (Savageau 1976). Furthermore, we performed the sensitivity analysis for each interrelated control path in the candidate network model, and determined which paths or relations between amino acids are dominant in maintaining the network structure.

Materials and methods

Animals, experimental design and sample collection

Adult male Wistar rats were maintained on a 12:12-h light–dark cycle, with water provided freely. Starting from 9 weeks of age, the rats were randomly assigned ($n = 5$ or 6) and switched to control or one of the experimental diets (day 0). The composition of the diet is based on AIN93G standard diet, slightly modified by replacing dextrin and sucrose by corn starch, and casein by amino acid mixture shown in Table 1. Each experimental diet (minus-one diet) is completely depleted of either methionine or threonine and the total nitrogen content was corrected by adding glutamine. The rats were kept for 6 weeks under ad libitum feeding condition, and the blood samples were collected from tail vein once before the experiment, and on day 1, day 2, and once every week during the experimental period. To prevent coagulation of the blood samples, heparinized microcapillary tubes (cat. #22-362-566, Fisher Scientific) were used for collecting the blood samples. The plasma samples separated from the blood samples are mixed with two volumes of 5% (w/w) trichloroacetic acid, and centrifuged immediately at 4°C, 8,000×g for 20 min.

Table 1 Amino acid composition in minus-one diet

%, w/w	Control	-Met	-Thr
His	2.54	2.54	2.54
Ile	4.45	4.45	4.45
Leu	8.13	8.13	8.13
Lys·HCl	8.82	8.82	8.82
Met	2.43	0.00	2.43
Phe	4.50	4.50	4.50
Thr	3.81	3.81	0.00
Trp	1.08	1.08	1.08
Val	5.73	5.73	5.73
Ala	2.55	2.55	2.55
Arg	3.28	3.28	3.28
Asn·H_2O	3.60	3.60	3.60
Asp	3.16	3.16	3.16
(Cys)2	0.50	0.50	0.50
Gln	9.16	10.35	11.50
Glu	9.16	9.16	9.16
Gly	1.62	1.62	1.62
Pro	9.37	9.37	9.37
Ser	5.06	5.06	5.06
Tyr	4.85	4.85	4.85
AA-total	93.82	92.58	92.35
Starch	6.18	7.42	7.65
Total	100.00	100.00	100.00

(Cys)2 cystine

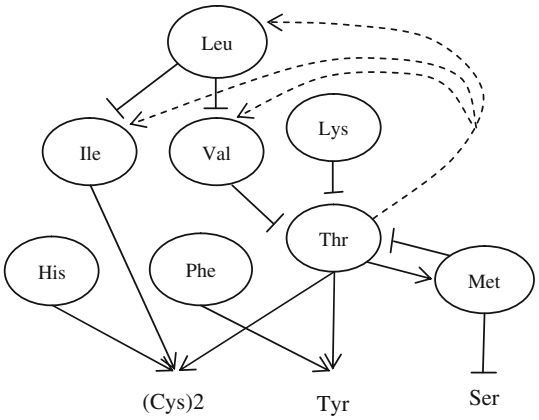

Fig. 1 Static regulatory network structure of plasma amino acids. The regulatory effects among plasma amino acids are connected to construct a network structure. The *arrows* indicate the positive effects and the *lines with bars* indicate the negative effects between the connected amino acids. *Dashed lines* indicate the relationship which is either positive or negative but could not be determined by the static analysis. *(Cys)2* represent cystine (Shikata et al. 2007)

The supernatant was filtrated with Ultrafree-MC filter (Cat. No. UFC3LGC00, Millipore), and the amino acid concentrations were measured by an automatic amino acid analyzer (L-8800; Hitachi, Tokyo, Japan).

Dynamic network analysis

Based on the static regulatory network structure inferred in the previous study (Fig. 1) (Shikata et al. 2007), we decided to analyze the dynamic property on the subnetwork structure including six essential amino acids located in the upstream of the network, namely, lysine, threonine, methionine, leucine, isoleucine, and valine. These amino acids constituted interrelated loop whose detailed network structure could not be determined by the static analysis.

In the dynamic network analysis, first, the interrelated coefficients between amino acids are determined, and then each path was subjected to sensitivity analysis for its dominance in maintaining the network structure.

S-system analysis method

The S-system belongs to the type of power-law formalism in which the component processes are expressed by the differential equations;

$$\frac{dx_i}{dt} = \alpha_i \prod_{j=1}^{n} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n} X_j^{h_{ij}}, \quad (1)$$

where n is the total number of the state variables or network component (X_i), and i, j ($1 \le i, j \le n$) are suffixes of state variables. The terms g_{ij} and h_{ij} are interactive affectivity of X_j to X_i; g_{ij} (h_{ij}) represents the interrelated coefficient of X_j to the synthesis (degradation) of X_i. Thus,

the first term of the equation represents all influences that increase X_i, whereas the second term represents all influences that decrease X_i. In a network context, the non-negative parameters α_i and β_i are called relative inflow and outflow of network component X_i, and real-valued exponents, g_{ij} and h_{ij}, are referred to as the interrelated coefficients between components X_j and X_i.

To infer the network model, the parameters α_i, β_i, g_{ij} and h_{ij} must be optimized. In this optimization problem, each set of parameter values to be estimated is evaluated using the following procedure. Suppose that $X^{\mathrm{cal}}_{d,i,t}$ is the numerically calculated time-course of state variable X_i at time t in the d-th data set, and $X^{\mathrm{exp}}_{d,i,t}$ represents the experimentally observed time-course of X_i at time t in the d-th data set. We sum the relative error in $X^{\mathrm{cal}}_{d,i,t}$ to obtain the total error E;

$$E = \sum_{d=1}^{D} \sum_{i=1}^{N} \sum_{t=1}^{T} \left(\frac{X^{\mathrm{exp}}_{d,i,t} - X^{\mathrm{cal}}_{d,i,t}}{X^{\mathrm{exp}}_{d,i,t}} \right)^2, \qquad (2)$$

where, D is the total number of data sets experimentally observed under different experimental conditions, N is the total number of experimentally observable state variables, and T is the total number of sampling points over time in one experimental condition. The computational task is to determine a set of parameter values that minimizes the objective function E within a given error allowance.

For the optimization of the large number of real-value parameters, the computational technique based on Real-coded Genetic Algorithm (RCGA) (Nakatsui et al. 2008; Ono and Kobayashi 1997; Ueda et al. 2002) is introduced as a nonlinear numerical optimization method that is much less likely to be stranded in local minima. This technique is based on the combination of UNDX (Ono and Kobayashi 1997), with the alternation of the MGG model (Sato et al. 1997). The genome (design code) of each individual (each set of parameter values) is shown in Fig. 2. A genome

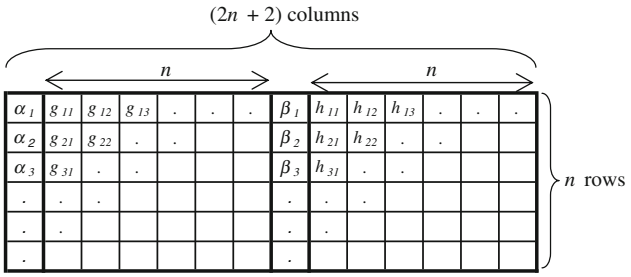

Fig. 2 Design code of an individual. A design code of an individual representing one S-system differential equation model is shown as a *matrix*. For each network component which is an amino acid (X_i), the non-negative parameters α_i and β_i determine relative inflow and outflow of X_i. The terms g_{ij} and h_{ij} determine the interactive affectivity of amino acid X_j to amino acid X_i; g_{ij} (h_{ij}) represents the interrelated coefficient of X_j to the synthesis (degradation) of X_i. These parameters form $n \times (2n + 2)$ matrix which represents one S-system model

(corresponding to one individual) contains a set of S-system parameters (n α_is and β_is and $n \times n$ g_{ij}s and h_{ij}s) which forms an $n \times (2n + 2)$ matrix. An individual represents one S-system model. Each small square in Fig. 2 corresponds to each parameter that has a real value.

Parameter optimization

Based on the stationary phase network structure inferred in the previous study (Fig. 1), the dynamic analysis was focused on six amino acids, namely, lysine, threonine, methionine, leucine, isoleucine, and valine. The interactions which were not detected in the stationary phase network structure were excluded from the parameter optimization. The experimental time-courses were obtained for control, methionine minus-one, and threonine minus-one conditions as described above.

In calculating the time-courses with each candidate parameter set and evaluating the average relative error from the experimental data, the measured time-courses of lysine were given for all three experimental conditions. This is because lysine is located at the highest layer in the investigating network model and no other amino acids can affect the concentration of lysine. Similarly, in calculation of methionine or threonine minus-one time-courses, the measured time-courses of methionine or threonine, respectively, were given, because the concentration of these amino acids are altered by the external (dietary) perturbation and not by other amino acids.

The calculation with genetic algorithm was performed with population of 300 in one generation, and one trial with 50,000 generations was repeated 50 times. From the last generation of each trial, the parameter set with the network structure consistent with the stationary phase and with the least average relative error between calculated value and experimental value per sampling point was selected as the candidate network model, and from 50 candidates, one parameter set with the least average error was selected as the final candidate network model. This final candidate was subjected to the next sensitivity analysis.

Sensitivity analysis method

In order to analyze the importance of each interrelated control path in the estimated network, we evaluated the sensitivity of each parameter in the network candidate. If the sensitivity of a certain parameter is high, the corresponding path must be essential or rigid for realizing the experimentally observed time-courses. The scheme of the analysis is shown in Fig. 3.

We added 10% perturbation to each interrelated coefficient, g_{ij} or h_{ij}, in the network candidate, and the relative squared error between the calculated time-course data

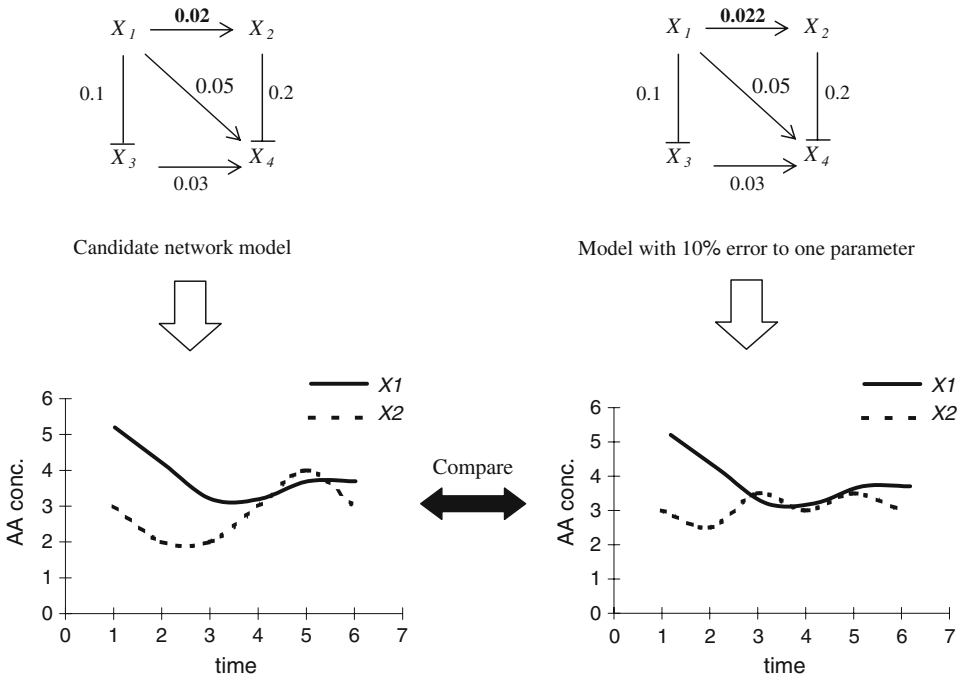

Fig. 3 Scheme of sensitivity analysis. Suppose there is a network model indicated with the drawing in the *top left*. In this drawing, the *arrows* indicate the positive effects and the *lines with bars* indicate the negative effects. Add 10% error to one parameter in the network model and create a new network model as indicated in the *top right*. Calculate the time-course of all the amino acids in both network models, and evaluate the difference generated by the 10% error. This procedure is repeated for all the parameters in the network

before adding perturbation and those after adding perturbation was evaluated. Here, we defined the evaluated value of sensitivity $S_{g_{ij}}$ ($S_{h_{ij}}$) for interaction coefficient g_{ij} (h_{ij}) as follows:

$$S_{g_{ij}(h_{ij})} = \sum_{d=1}^{D} \sum_{i=1}^{N} \sum_{t=1}^{T} \left(\frac{\mathrm{PER}_{d,i,t} - \mathrm{CAL}_{d,i,t}}{\mathrm{CAL}_{d,i,t}} \right)^2, \quad (3)$$

where, $\mathrm{CAL}_{d,i,t}$ shows the numerically calculated time-course before adding 10% perturbation at time t of state variable X_i in the d-th data set, and $\mathrm{PER}_{d,i,t}$ shows those after adding 10% perturbation at time t of state variable X_i in the d-th data set. D is the number of time-course data, N is the number of variables, and T is the number of sampling points in each time-course. For the convenience of comparison, we performed scaling so that the highest evaluated value on the network candidate is 100 and other scores are expressed as the ratio to the highest value.

Result

Time-course data under Met minus-one and Thr minus-one condition

The time-course of plasma amino acids are shown with the radar graph in the Fig. 4. Under methionine minus-one condition (Fig. 4b), the concentration of methionine gradually decreased throughout the experimental period. By day 42, it reached down to 24.40% of the concentration measured before the experiment. The increase in the concentration of serine was observed from day 7, and the increase in the concentration of threonine was observed from day 14. After day 14, the pattern of the change in the amino acid profile indicated by the shape of the radar graph was fixed, and only the degree of the imbalance escalated. At the end of the 42-day-period, the concentrations of serine and threonine increased up to almost 400%.

Under threonine minus-one condition (Fig. 4c), the concentration of threonine dropped drastically to 26.30% on day 1 and stayed low through the whole experimental period. The final concentration of threonine was 13.78% of pre-experimental measurement. The concentration of serine started to increase on day 7 as observed in methionine minus-one condition, and all the other amino acids started to show a slight decrease on day 14. Similar to the methionine minus-one condition, the pattern of the change in the amino acid profile was determined by day 14 and after that, only the degree of the change escalated. At the end of the 42-day-period, the concentration of serine increased to 200% and those of other amino acids were slightly decreased.

Optimization of the parameter set

Using the two time-course data described above and one obtained from the control group, the dynamic network structure was investigated using S-system analysis method. The S-system is one of the best formalism to estimate interaction mechanisms among system components, and enables us to reconstruct the network architectures with the experimentally observed time-courses of the quantity of the network components. The inference of the dynamic

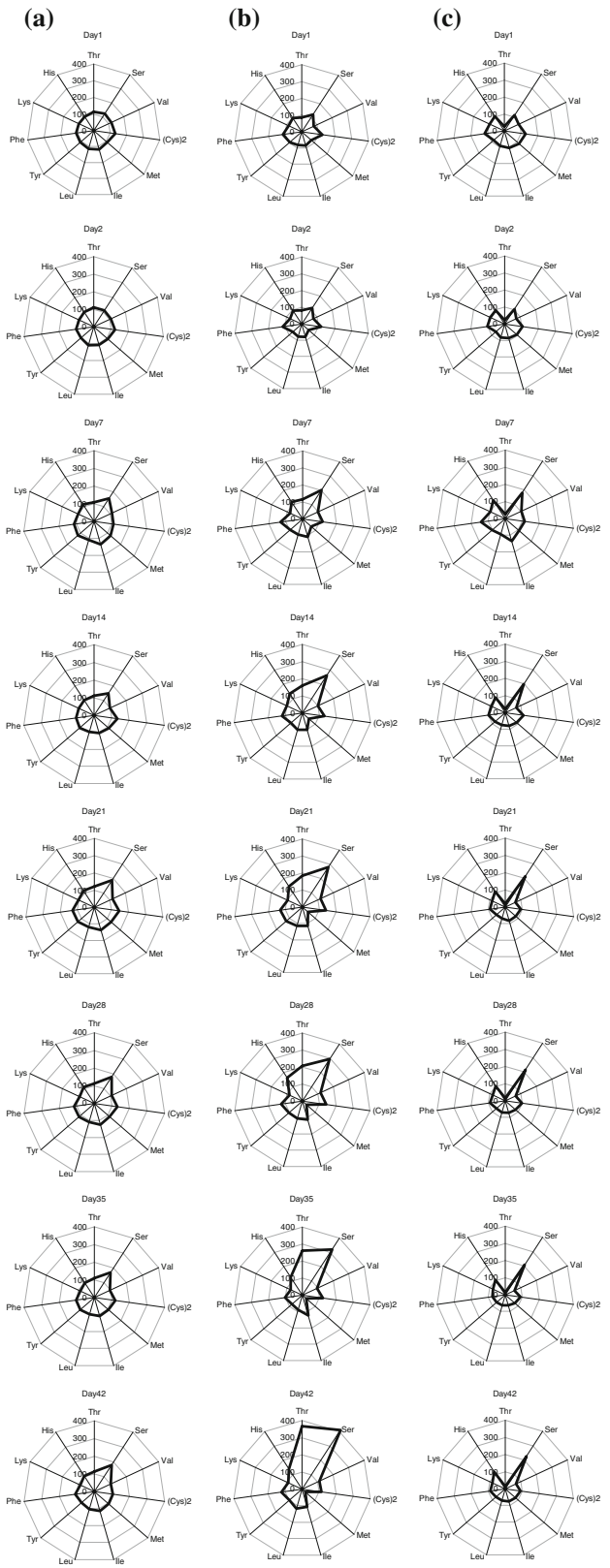

Fig. 4 Time-course of plasma amino acid concentrations under minus-one conditions. The concentrations of each plasma amino acid are shown as percent ratio to the plasma concentrations at pre-experimental period. *(Cys)2* represent cystine. **a** Control, **b** Met minus-one, and **c** Thr minus-one

network structure is achieved by optimizing the S-system parameter set. For the evaluation of the network structure defined by each parameter set, the average relative error between calculated value and experimental value per sampling point was computed, and the network model which gave the least average error was evaluated highly.

After 50 trials of optimization by genetic algorithm described in the "Materials and methods", one network model was selected as the final candidate network model, which has the structure consistent with the stationary phase network model and which reproduced the experimentally obtained time-courses with the least average relative error. The obtained parameter set of this model is shown in Table 2, in which the interrelated coefficient of each path in the network is indicated as a matrix, where the amino acid giving the influence (j) is indicated at the top row and the amino acid receiving the effect (i) is indicated at the left column. The same candidate network model is visualized as a network figure in Fig. 5. This network model reproduced the actual time-course with the average error of 13.17% (Fig. 6).

Sensitivity analysis

For the parameter set of the candidate network model shown in Table 2, we performed the sensitivity analysis. The sensitivity of each parameter is evaluated by adding 10% error to estimated value of parameter and quantifying the difference it generates to the time-course. The larger the difference, the more strictly regulated the parameter is, and thus, the parameters which cause large differences are dominant in determining the network structure. For the convenience of comparison, the sensitivity of each parameter is scaled to relative values with the highest value as 100.

The relative sensitivity of each parameter in the candidate network model is shown in the Fig. 7. The three of the most dominant interactions were negative effect of leucine to valine, negative effect of methionine to threonine, and negative effect of leucine to isoleucine.

Discussions

With the time-course data of the plasma amino acid concentrations obtained under methionine or threonine minus-one condition, the dynamic network model was inferred in the form of S-system differential equation model. The candidate network model could reproduce the experimentally obtained time-courses of the plasma amino acids with the average error of 13.17% (Fig. 6). The size of the biological experimental error is estimated with the coefficient of variation (Cv), which is a ratio of standard deviation to

Table 2 The parameter set defining the candidate network model

		g_{ij}							h_{ij}					
		j							j					
i	α	Thr	Met	Lys	Val	Ile	Leu	β	Thr	Met	Lys	Val	Ile	Leu
Thr	9.26	0	−0.64	−0.01	−0.01	0	0	2.11	0.02	0	0	0	0	0
Met	7.13	0.07	0	0	0	0	0	2.83	0	0.62	0	0	0	0
Lys	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	7.76	0.11	0	0	0	0	−0.24	1.15	0	0	0	0.52	0	0
Ile	9.19	0.12	0	0	0	0	−0.38	1.09	0	0	0	0	0.68	0
Leu	6.83	0.09	0	0	0	0	0	1.28	0	0	0	0	0	0.73

Interrelated coefficient of each path in the candidate network model is indicated as a matrix, where the amino acid giving the influence (j) is indicated at the top row and the amino acid receiving the effect (i) is indicated at the left column. g_{ij} indicate the effects between each pair of amino acids and h_{ij} indicate the speed of degradation for each amino acid. The parameters with value 0 indicate that there is no relation between the two amino acids

average calculated for each amino acid at every sampling point. The overall average Cv of all sampling points of all amino acids was 19.83% in this experiment. Since the average error for the candidate network model is smaller than the biological error, we presumed that the candidate model reproduced the experimental time-course within the permissible error range.

By sensitivity analysis, the parameters in the network were ranked by its dominance in determining the network structure. The three of the most dominant interactions were negative effect of leucine to valine, negative effect of methionine to threonine, and negative effect of leucine to isoleucine (Fig. 7). This result is in good agreement with some biological knowledge and demonstrates the advantage of applying system analysis to plasma amino acid data in finding new biological insights.

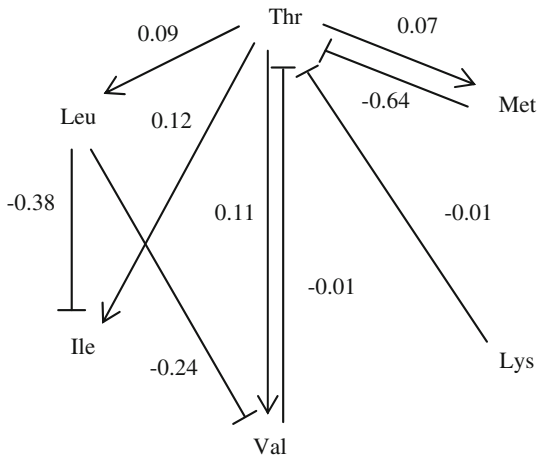

Fig. 5 Candidate network model defined by the parameter set in Table 2. The *arrows* indicate the positive effects and the *lines with bars* indicate the negative effects. The *numbers beside the lines* indicate the interrelated coefficient of each path

Negative effect of leucine to isoleucine and valine

The negative effect of leucine to isoleucine and valine can be explained by the activity of branched-chain α-ketoacid dehydrogenase (BCKD). BCKD is one of the rate limiting catalytic enzyme shared by all three branched-chain amino acids (Brosnan and Brosnan 2006; Harris et al. 2004). Its activity is regulated by its phosphorylation state. When it is phosphorylated by BDK (BCKD kinase), it is inactivated, whereas when it is dephosphorylated by BDP (BCKD phosphatase), its activity is restored.

It is reported that the α-isocaproate, which is a catabolite of leucine, inhibits BDK activity and activates BCKD indirectly (Parker and Randle 1978). So when the concentration of leucine increases, so does the concentration of the α-isocaproate, which activates BCKD and accelerates the catabolism of all three branched-chain amino acids. Thus the concentration of leucine has a negative effect on concentrations of isoleucine and valine.

Negative effect of methionine to threonine

The negative effect of methionine to threonine was the second dominant interaction in the candidate network model. At present, this relation does not have a reasonable explanation supported by biological knowledge. However, there are some observations in agreement with this relation. One report from Katz and Baker (1975) shows when chicks are fed methionine excess diet, the plasma concentration of threonine decreases. They explain that the metabolism of threonine is accelerated by the excess methionine intake and this causes the decrease in plasma threonine concentration, but the detailed mechanism has not been revealed yet.

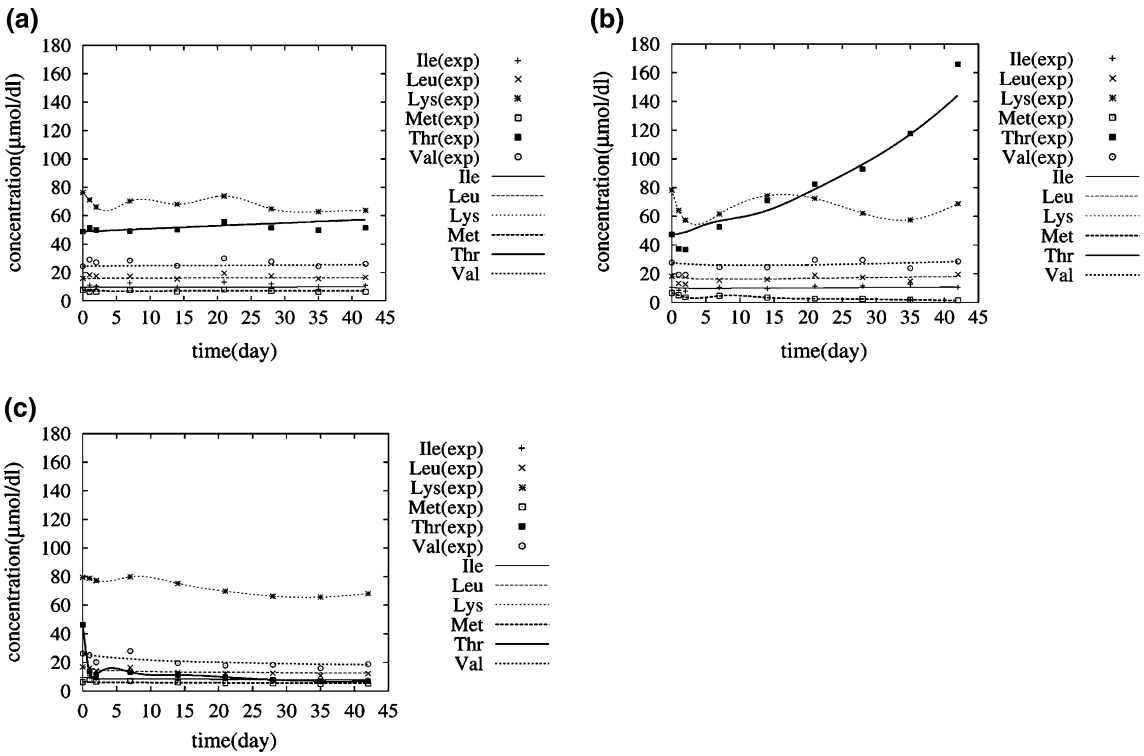

Fig. 6 Plasma amino acid time-course calculated using the candidate parameter set in Table 2. The experimentally measured concentrations of each amino acids are indicated with *signs* and the calculated time-courses are indicated with *lines*. **a** Control time-course given measured time-course of lysine, **b** methionine minus-one time-course given measured time-courses of lysine and methionine, and **c** threonine minus-one time-course given measured time-courses of lysine and threonine

Advantages of applying system analysis to biological data

Although our analysis did not consider any topological information on amino acid metabolism and was completely data driven, two of the top three interactions determined as dominant by dynamic analysis and sensitivity analysis were well known regulatory relations between the three branched-chain amino acids. This fact indicates that our analytical methods are useful in finding the important relations between amino acids without any prior knowledge on the amino acid metabolism. It also indicates that the negative effect of methionine on threonine, which was selected with the same analytical procedure, may have some physiologically important function which we have not yet discovered.

In this study, we demonstrated that applying network analysis to plasma amino acid data was useful in bringing up new insights into the relations between amino acids, which could not have attracted our attention from a metabolic pathway map. This could be a powerful strategy to construct a new hypothesis in investigating the changes in regulation of amino acid profile in accordance with the physiological state of an organism, and would lead us to further understanding of the causes of the diseases in the future.

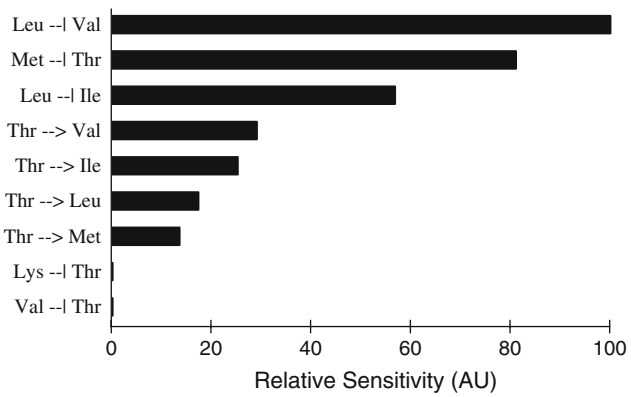

Fig. 7 Relative sensitivity of each parameters in the candidate network model. The *arrows* indicate the positive effects from amino acids on the *left* to the one on the *right*, and the *bars* indicate the negative effects from amino acids on the *left* to the one on the *right*

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References

Brindle JT, Antti H, Holmes E, Tranter G, Nicholson JK, Bethell HW, Clarke S, Schofield PM, McKilligin E, Mosedale DE, Grainger DJ (2002) Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. Nat Med 8:1439–1444. doi:10.1038/nm802

Brosnan JT, Brosnan ME (2006) Branched-chain amino acids: enzyme and substrate regulation. J Nutr 136:207S–211S

Cooper GF, Herskovits E (1992) A Bayesian method for the induction of probabilistic networks from data. Mach Learn 9:309

Dahlquist KD, Salomonis N, Vranizan K, Lawlor SC, Conklin BR (2002) GenMAPP, a new tool for viewing and analyzing microarray data on biological pathways. Nat Genet 31:19–20. doi:10.1038/ng0502-19

Felig P (1975) Amino acid metabolism in man. Annu Rev Biochem 44:933–955. doi:10.1146/annurev.bi.44.070175.004441

Ferenci P, Wewalka F (1978) Plasma amino acids in hepatic encephalopathy. J Neural Transm Suppl 14:87–94

Friedman N, Linial M, Nachman I, Pe'er D (2000) Using Bayesian networks to analyze expression data. J Comput Biol 7:601–620. doi:10.1089/106652700750050961

Harris RA, Joshi M, Jeoung NH (2004) Mechanisms responsible for regulation of branched-chain amino acid catabolism. Biochem Biophys Res Commun 313:391–396. doi:10.1016/j.bbrc.2003.11.007

Holm E, Sedlaczek O, Grips E (1999) Amino acid metabolism in liver disease. Curr Opin Clin Nutr Metab Care 2:47–53. doi:10.1097/00075197-199901000-00009

Hong SY, Yang DH, Chang SK (1998) The relationship between plasma homocysteine and amino acid concentrations in patients with end-stage renal disease. J Ren Nutr 8:34–39. doi:10.1016/S1051-2276(98)90035-8

Imoto S, Goto T, Miyano S (2002) Estimation of genetic networks and functional structures between genes by using Bayesian networks and nonparametric regression. Pac Symp Biocomput, pp 175–186

Katz RS, Baker DH (1975) Methionine toxicity in the chick: nutritional and metabolic implications. J Nutr 105:1168–1175

Kikuchi S, Tominaga D, Arita M, Takahashi K, Tomita M (2003) Dynamic modeling of genetic networks using genetic algorithm and S-system. Bioinformatics 19:643–650. doi:10.1093/bioinformatics/btg027

Maki Y, Tominaga D, Okamoto M, Watanabe S, Eguchi Y (2001) Development of a system for the inference of large scale genetic networks. Pac Symp Biocomput, pp 446–458

Maki Y, Takahashi Y, Arikawa Y, Watanabe S, Aoshima K, Eguchi Y, Ueda T, Aburatani S, Kuhara S, Okamoto M (2004) An integrated comprehensive workbench for inferring genetic networks: VoyaGene. J Bioinform Comput Biol 2:533–550. doi:10.1142/S0219720004000727

Nakatsui M, Ueda T, Maki Y, Ono I, Okamoto M (2008) Method for inferring and extracting reliable genetic interactions from time-series profile of gene expression. Math Biosci 215:105–114. doi:10.1016/j.mbs.2008.06.007

Nicholson JK, Lindon JC, Holmes E (1999) 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 29:1181–1189. doi:10.1080/004982599238047

Noguchi Y, Zhang QW, Sugimoto T, Furuhata Y, Sakai R, Mori M, Takahashi M, Kimura T (2006) Network analysis of plasma and tissue amino acids and the generation of an amino index for potential diagnostic use. Am J Clin Nutr 83:513S–519S

Obeid OA (2005) Plasma amino acid concentrations in patients with coronary heart disease: a comparison between U.K. Indian Asian and Caucasian men. Int J Vitam Nutr Res 75:267–273. doi:10.1024/0300-9831.75.4.267

Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M (1999) KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 27:29–34

Ohtsuka Y, Agishi Y (1992) Abnormal amino acid metabolism in diabetes mellitus. Nippon Rinsho 50:1631–1636

Ono I, Kobayashi S (1997) A real-coded genetic algorithm for function optimization using unimodal distribution crossover. 7th ICGA pp 249–253

Parker PJ, Randle PJ (1978) Inactivation of rat heart branched-chain 2-oxoacid dehydrogenase complex by adenosine triphosphate. FEBS Lett 95:153–156. doi:10.1016/0014-5793(78)80072-6

Sato H, Ono I, Kobayashi S (1997) A new generation alternation model of genetic algorithm and its assessment. J. Jpn Soc Artif Intell 12:734–744

Savageau MA (1976) Biochemical systems analysis: a study of function and design in molecular biology. Addison-Wesley, Reading

Savageau MA (1998) Rules for the evolution of gene circuitry. Pac Symp Biocomput, pp 54–65

Shikata N, Maki Y, Noguchi Y, Mori M, Hanai T, Takahashi M, Okamoto M (2007) Multi-layered network structure of amino acid (AA) metabolism characterized by each essential AA-deficient condition. Amino Acids 33:113–121. doi:10.1007/s00726-006-0412-0

Soeters PB, Fischer JE (1976) Insulin, glucagon, aminoacid imbalance, and hepatic encephalopathy. Lancet 2:880–882. doi:10.1016/S0140-6736(76)90541-9

Soltesz G, Schultz K, Mestyan J, Horvath I (1978) Blood glucose and plasma amino acid concentrations in infants of diabetic mothers. Pediatrics 61:77–82

Somogyi R, Sniegoski CA (1996) Modeling the complexity of genetic networks: understanding multigenetic and pleiotropic regulation. Complexity 1:45–63

Ueda T, Koga N, Ono I, Okamoto M (2002) Efficient numerical optimization technique based on real-coded genetic altorithm for inverse problem. 7th international symposium on artificial life and robotics (AROB 7th '02), 290–293

Watanabe A, Higashi T, Sakata T, Nagashima H (1984) Serum amino acid levels in patients with hepatocellular carcinoma. Cancer 54:1875–1882. doi:10.1002/1097-0142(19841101)54:9<1875::AID-CNCR2820540918>3.0.CO;2-O

Weinlich G, Murr C, Richardsen L, Winkler C, Fuchs D (2007) Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients. Dermatology 214:8–14. doi:10.1159/000096906