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Characterization of the transit and transition times for a pathway unit of Michaelis–Menten mechanism

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Abstract

The transit time and a characteristic time constant for the transition time are formulated for a pathway unit of Michaelis–Menten mechanism in metabolic chains with mass-action-type dependence. The pathway unit in a chain is assigned to each metabolite and two consecutive Michaelis–Menten-type reactions associated with it. All the relevant functions such as control coefficients, elasticities and transit time for the pathway unit are expressed in terms of the flux J and other system parameters. The transition time is characterized by a time constant for a first-order system expressed as the derivative of the total concentration of the internal metabolite with respect to the flux J.

Keywords: Transit time; Transition time; Pathway unit; Michaelis–Menten mechanism; Metabolic control analysis

1. Introduction

Michaelis–Menten-type reactions are the most basic components in metabolic pathways to play a fundamental role in the cellular metabolism. The analysis of regulatory properties of this component reaction is essential for the analysis of the behavior of metabolic chains with "mass action" type of dependence [1]. In this study, formulation of two characteristic time constants, the transit time and the transition time, is performed for a pathway unit of Michaelis–Menten mechanism in the metabolic chains. The pathway unit in a chain is assigned to each metabolite and two consecutive Michaelis–Menten-type reactions associated with it. This formulation attained in use of the flux J, a key parameter in the metabolic control analysis, makes it possible that generation and analysis of the overall behavior for metabolic pathways may proceed in a straightforward manner of sequential connection of the procedure for the pathway units comprising a pathway. In fact, combination of the transit times of the consecutive pathway units leads to formulation of the transit time in a linear pathway of Michaelis–Menten-type reactions [2]. An approximation representation for the response to the flux change in consecutive Michaelis–Menten-type reactions is formed with successive combination of the expressions for the component reactions [3].

The transit time is a system variable suitable for characterization of steady-state behavior of the flux and the metabolites in a metabolic pathway. It represents the time required for the substrate entering the pathway to be transformed into the end-product through the enzymatic reactions and leave the pathway at a steady state [4]. While the transit time is relevant to steady-state properties of pathways, the transition time characterizes the dynamic properties of pathways in the vicinity of (and between) their stable steady states [5]. It would be clarified in this study of formulation of the both time constants that the transit time is distinguished from and related to the transition time.

In a metabolic chain of Michaelis–Menten-type reactions, a pathway unit is defined to each metabolite and two consecutive Michaelis–Menten-type reactions as follows:

$$S + E_1 \underset{k_{1r}}{\overset{k_{1f}}{\rightleftarrows}} X_1 \underset{k_{2f}}{\overset{k_{2r}}{\rightleftarrows}} E_1 + M \quad M + E_2 \underset{k_{3r}}{\overset{k_{3f}}{\rightleftarrows}} X_2 \underset{k_{4f}}{\overset{k_{4r}}{\rightleftarrows}} E_2 + P$$

where E_1 and E_2 are the free enzymes of the first and second reactions, respectively, and X_1 and X_2 are the respective

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enzyme–substrate complexes. The substrate of the first reaction, S, and the product of the second reaction, P, are the external metabolites, and M, the product of the first reaction and the substrate of the second reaction, is a single internal metabolite in the pathway unit, including the enzyme–substrate complex X_2. At a steady state the flux J is maintained in the pathway unit so that J is positive for the forward flow from S to P, and negative for the reverse flow from P to S. For the formulation in this study the flux J may be assumed to be positive in reference to M and X_2 since for negative J the same derivation with positive J is applicable to M and X_1 in the pathway unit from P to S.

In this study the (steady-state) concentrations of every chemical species in the pathway unit are formulated as respective functions of the flux J, a key parameter for steady-state behavior, and a quadratic equation of the flux J is derived to represent its relationship with the concentrations of enzymes and external metabolites. The total concentration of the internal metabolite (σ) and the control coefficients for flux and concentration hence are expressed explicitly in terms of these parameters. The transit time τ defined by σ/J is represented as rectangular hyperbolic functions of the flux J and the metabolic control analysis is performed. The transition time is characterized by a time constant for a first-order system which is derived as $\partial\sigma/\partial J$. It follows that detailed analysis of correlation of the flux J and the characteristic time constants to the external metabolites and the kinetic parameters in the constituent pathway unit of Michaelis–Menten mechanism contributes to characterization of the flux behavior of linear pathways of Michaelis–Menten-type reactions.

2. Results

2.1. Steady-state relationships

For the first reaction in the pathway unit the following relationships are derived at a steady state with the flux J:

$$J - k_{1f}se_1 + k_{1r}x_1 = 0$$

$$J + k_{2f}me_1 - k_{2r}x_1 = 0$$

$$e_1 + x_1 = e_{1T}, \tag{1}$$

leading to the expressions with the flux J as

$$e_1 = \frac{J + k_{1r}e_{1T}}{k_{1r} + k_{1f}s} \quad x_1 = \frac{e_{1T}k_{1f}s - J}{k_{1r} + k_{1f}s}$$

$$m = \frac{e_{1T}k_{1f}k_{2r}s - J(k_{1r} + k_{2r} + k_{1f}s)}{k_{2f}(J + k_{1r}e_{1T})} \tag{2}$$

where the (steady-state) concentration is denoted by the italic lower case letter corresponding to the capital letter for a chemical species, and e_{1T} designates the total concentration of the enzyme E_1. Similarly, for the second reaction at the steady state, the equations,

$$J - k_{3f}me_2 + k_{3r}x_2 = 0$$

$$J + k_{4f}pe_2 - k_{4r}x_2 = 0$$

$$e_2 + x_2 = e_{2T}, \tag{3}$$

yield the following expressions:

$$e_2 = \frac{k_{4r}e_{2T} - J}{k_{4r} + k_{4f}p} \quad x_2 = \frac{J + k_{4f}pe_{2T}}{k_{4r} + k_{4f}p}$$

$$m = \frac{J(k_{3r} + k_{4r} + k_{4f}p) + e_{2T}k_{3r}k_{4f}p}{k_{3f}(k_{4r}e_{2T} - J)} \tag{4}$$

where e_{2T} is the total concentration of the enzyme E_2.

The internal metabolite M is common to the first and second reactions, so that the following equation from Eqs. (2) and (4),

$$\frac{e_{1T}k_{1f}k_{2r}s - J(k_{1r} + k_{2r} + k_{1f}s)}{k_{2f}(J + k_{1r}e_{1T})} = \frac{J(k_{3r} + k_{4r} + k_{4f}p) + e_{2T}k_{3r}k_{4f}p}{k_{3f}(k_{4r}e_{2T} - J)}, \tag{5}$$

leads to a quadratic equation for the flux J:

$$a_1J^2 - (b_1e_{1T} + b_2e_{2T})J + c_1e_{1T}e_{2T} = 0 \tag{6}$$

where

$$a_1 = k_{3f}(k_{1r} + k_{2r} + k_{1f}s) - k_{2f}(k_{3r} + k_{4r} + k_{4f}p)$$

$$b_1 = k_{1f}k_{2r}k_{3f}s + k_{1r}k_{2f}(k_{3r} + k_{4r} + k_{4f}p)$$

$$b_2 = k_{2f}k_{3r}k_{4f}p + k_{3f}k_{4r}(k_{1r} + k_{2r} + k_{1f}s)$$

$$c_1 = k_{1f}k_{2r}k_{3f}k_{4r}s - k_{1r}k_{2f}k_{3r}k_{4f}p, \tag{7}$$

indicating that J is determined by specifying the concentrations of the external metabolites S and P with given values of e_{1T}, e_{2T} and rate constants.

The expressions of the S and P concentrations as functions of the flux J are derived from the rewritten form of Eq. (6),

$$f(J)s - g(J) - h(J)p = 0 \tag{8}$$

where

$$f(J) = k_{1f}k_{3f}(k_{2r}e_{1T} - J)(k_{4r}e_{2T} - J)$$

$$g(J) = k_{3f}J(k_{1r} + k_{2r})(k_{4r}e_{2T} - J) + k_{2f}J(k_{3r} + k_{4r})(J + k_{1r}e_{1T})$$

$$h(J) = k_{2f}k_{4f}(J + k_{1r}e_{1T})(J + k_{3r}e_{2T}) \tag{9}$$

It follows that for arbitrary values of p, s can always be determined, but determination of p is possible only if s is larger than a certain value.

The dependence of the flux J on the external metabolites (S and P) is shown by differentiation of Eq. (6) with respect to their concentrations (s and p):

$$\frac{\partial J}{\partial s} = \frac{f(J)}{-2a_1 J + b_1 e_{1T} + b_2 e_{2T}},$$

$$\frac{\partial J}{\partial p} = \frac{h(J)}{2a_1 J - b_1 e_{1T} - b_2 e_{2T}} \tag{10}$$

The flux J increases with s, and decreases with p, in general, since the denominator,

$$2a_1 J - b_1 e_{1T} - b_2 e_{2T} = \frac{\partial f}{\partial J} s - \frac{\partial g}{\partial J} - \frac{\partial h}{\partial J} p, \tag{11}$$

is negative for ordinary values in the metabolite and enzyme concentrations and the rate constants.

2.2. Control coefficients for the pathway unit

The flux control coefficients and the concentration control coefficients for the pathway unit are derived explicitly using the following derivatives:

$$\frac{\partial J}{\partial e_{1T}} = \frac{b_1 J - c e_{2T}}{2aJ - b_1 e_{1T} - b_2 e_{2T}},$$

$$\frac{\partial J}{\partial e_{2T}} = \frac{b_2 J - c e_{1T}}{2aJ - b_1 e_{1T} - b_2 e_{2T}}$$

$$\frac{\partial m}{\partial e_{1T}} = \frac{e_{2T}(k_{3r} + k_{4r})(k_{4r} + k_{4f} p)}{k_{3f}(k_{4r} e_{2T} - J)^2} \frac{\partial J}{\partial e_{1T}},$$

$$\frac{\partial m}{\partial e_{2T}} = -\frac{e_{1T}(k_{1r} + k_{2r})(k_{1r} + k_{1f} s)}{k_{2f}(J + k_{1r} e_{1T})^2} \frac{\partial J}{\partial e_{2T}} \tag{12}$$

It thus easily and directly is shown that the summation and connectivity theorems for the control coefficients [6] hold:

$$C_1^J + C_2^J = 1 \quad C_1^m + C_2^m = 0 \quad C_1^J \varepsilon_m^1 + C_2^J \varepsilon_m^2 = 0$$
$$C_1^m \varepsilon_m^1 + C_2^m \varepsilon_m^2 = -1 \tag{13}$$

where the common notations for the metabolic control analysis are used, and the elasticities with respect to m are derived as:

$$\varepsilon_m^1 = -\frac{k_{2f} m (J + k_{1r} e_{1T})^2}{J e_{1T}(k_{1r} + k_{2r})(k_{1r} + k_{1f} s)} = \frac{C_2^J}{C_2^m},$$

$$\varepsilon_m^2 = \frac{k_{3f} m (k_{4r} e_{2T} - J)^2}{J e_{2T}(k_{3r} + k_{4r})(k_{4r} + k_{4f} p)} = \frac{C_1^J}{C_1^m}. \tag{14}$$

2.3. Characterization of the transit time

The total concentration σ of the internal metabolite M is given by

$$\sigma = m + x_2 \tag{15}$$

The expressions in Eq. (4) yield

$$\sigma[s] = \frac{J(k_{3r} + k_{4r} + k_{4f} p) + e_{2T} k_{3r} k_{4f} p}{k_{3f}(k_{4r} e_{2T} - J)} + \frac{J + e_{2T} k_{4f} p}{k_{4r} + k_{4f} p} \tag{16}$$

when J varies due to steady-state concentration change in the external metabolite S, and $\sigma[s]$ designates dependence of σ on the change in s. A similar expression for $\sigma[p]$ is derived in Appendix A.

The transit time τ for the pathway unit is defined as

$$\tau = \frac{\sigma}{J}, \tag{17}$$

and represented as sum of rectangular-hyperbolic functions of J:

$$\tau[s] = \frac{1}{k_{4r} + k_{4f} p} + \frac{k_{4f} p}{J}\left(\frac{e_{2T}}{k_{4r} + k_{4f} p} + \frac{k_{3r}}{k_{3f} k_{4r}}\right) + \frac{k_{3r} + k_{4r}}{k_{4r} e_{2T} - J} \frac{k_{4r} + k_{4f} p}{k_{3f} k_{4r}} \tag{18}$$

when J varies corresponding to change in s. A similar expression for $\tau[p]$ is derived in Appendix A.

The main conclusion for the control coefficients of the transit time is the summation theorem that the sum of them lies between 0 and -1 since the sum consists of the difference between the sum of control coefficients of the total concentration in internal metabolites and the sum of those of the flux [7]. The relationships for the control coefficients are verified by differentiation of τ with respect to e_{1T} and e_{2T}, so that

$$C_1^\tau + C_2^\tau = C_1^\sigma + C_2^\sigma - (C_1^J + C_2^J) = \frac{x_2}{\sigma} - 1 \text{ with } C_1^\sigma + C_2^\sigma = \frac{x_2}{\sigma} \tag{19}$$

The expression of $\tau[s]$ apparently indicates that the transit time has a minimum with respect to J, which, with the notation of $J[s]$, varies between null and the maximum velocity of the Michaelis–Menten-type reaction by change in s. The relationship between $\tau[s]$ and $J[s]$ is shown in Fig. 1 for some typical values in the parameters. The derivation of the minimal point for $\tau[s]$ is given in Appendix B.

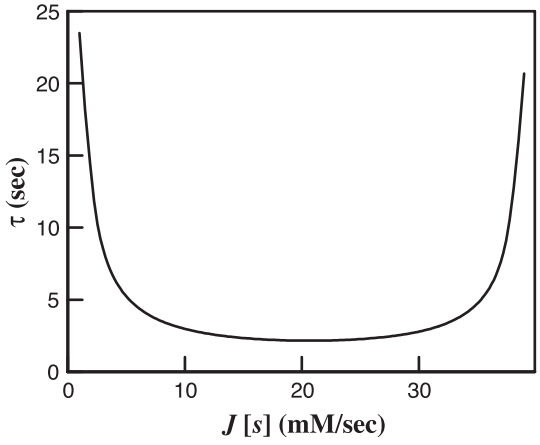

Fig. 1. Relationship between the transit time τ and the flux J with variation in the concentration of external metabolite S. $J[s]$ on the abscissa indicates the variation of J by changing the steady-state concentration of S. The following values in the kinetic parameters are used for the analysis: $k_{1f}=1000\ \mathrm{mM^{-1}\ s^{-1}}$, $k_{1r}=90\ \mathrm{s^{-1}}$, $k_{2f}=100\ \mathrm{mM^{-1}\ s^{-1}}$, $k_{2r}=10\ \mathrm{s^{-1}}$, $k_{3f}=800\ \mathrm{mM^{-1}\ s^{-1}}$, $k_{3r}=72\ \mathrm{s^{-1}}$, $k_{4f}=80\ \mathrm{mM^{-1}\ s^{-1}}$, $k_{4r}=8\ \mathrm{s^{-1}}$; $e_{1T}=e_{2T}=5$ mM; $p=20$ mM.

On the other hand, when the flux J varies by change in p, the transit time $\tau[p]$ decreases with J because

$$\frac{\partial\tau[p]}{\partial J[p]} = -\frac{k_{1f}k_{2r}s}{J^2}\left(\frac{1}{k_{1r}k_{2f}}+\frac{k_{3f}e_{2T}}{b_3}\right) - (k_{1r}+k_{2r})(k_{1r}+k_{1f}s)\left\{\frac{1}{k_{1r}k_{2f}(J+k_{1r}e_{1T})^2} + \frac{k_{2f}k_{3f}(b_3e_{1T}+k_{3r}b_4e_{2T})}{b_3(b_3e_{1T}-b_4J)^2}\right\} < 0. \quad (20)$$

It thus is found that the transit time for a pathway unit of Michaelis–Menten mechanism has a minimum at a certain value of the flux J with variation in the concentration of S. The relationship between the flux J and the concentration of S is again dependent on the concentration of the other external metabolite P. Suitable selection of the S and P concentrations would lead the pathway unit to run with an optimal value of the transit time. Further minimization of the transit time may be attained by examining how the total enzyme concentrations and the rate constants affect the relationship between the transit time τ and the concentration of S.

2.4. Characteristic time constant for the transition time

Responding to a concentration change in the external metabolite S, the flux J is assumed to increase or decrease stepwisely from J_1 to J_2 at $t = 0$, i.e.,

$$J(t) = J_1 + aU(t) \quad (21)$$

where a ($=\Delta J=J_2-J_1$), J_1 and J_2 ($=J_1+a$) are constants, and $U(t)$ is a step function defined as

$$U(t) = 0 \quad t \le 0$$
$$U(t) = 1 \quad t > 0 \quad (22)$$

The total concentration of the internal metabolite M in the pathway unit at $t=T$ is represented by $\sigma(T)$, whose increment during a time interval $[0,T]$ is obtained by

$$\sigma(T)-\sigma(0) = \int_0^T \frac{\mathrm{d}\sigma}{\mathrm{d}t}\mathrm{d}t = \int_0^T [J_2 - y(t)]\mathrm{d}t, \quad (23)$$

where $y(t)$ expresses the production rate of the external metabolite P, and the pathway unit is assumed to be at a steady state until $t=0$, i.e., $J(0)=y(0)=J_1$ (that is, equal flux and production rates at a steady state) and $\sigma(0)=\sigma[J_1]$ which denotes the steady-state value of σ corresponding to a flux J_1. The pathway unit responds to a stepwise change from J_1 to J_2, eventually reaching a new steady state with $\sigma[J_2]$ so that the increment $\{\sigma(T)-\sigma(0)\}$ becomes equivalent to $\{\sigma[J_2]-\sigma[J_1]\}$.

The characteristic time constant for the transition time results from approximation of $y(t)$ by $z(t)$ such that

$$z(t) = J_1 + a(1-e^{-ct}) \quad (24)$$

where a positive constant c is the relaxation constant of first-order system, which quantitatively represents the quickness of the response of the pathway unit in the valid range of the approximation. This relaxation constant c is defined so as for $z(t)$ to give the equal change in σ resulting from Eq. (23) as follows. Substitution of $z(t)$ into $y(t)$ in Eq. (23) yields

$$\sigma(T)-\sigma(0) = \int_0^T [J_2 - z(t)]\mathrm{d}t = \frac{a}{c}(1-e^{-cT}) \quad (25)$$

and by setting $T=\infty$, the time constant t_r is obtained by

$$t_r = \frac{1}{c} = \frac{\sigma[J_2]-\sigma[J_1]}{a} \quad (26)$$

It is neither general nor practical, however, that the relaxation constant c has to be evaluated for every value of a, which is unknown in the actual progress of reactions in the metabolic pathways. The constant c independent of a may be defined as

$$t_r = \frac{1}{c} = \lim_{a\to 0}\frac{\sigma[J_2]-\sigma[J_1]}{a} = \frac{\partial\sigma}{\partial J} \quad (27)$$

The characteristic time constant for the transition time in the pathway unit thus is formulated as:

$$t_r[s] = \frac{\partial \sigma[s]}{\partial J[s]} = \frac{k_{4r}(k_{3r} + k_{4r} + k_{4f}p) + k_{3r}k_{4f}p}{k_{3f}(k_{4r}e_{2T} - J_1)^2} e_{2T} + \frac{1}{k_{4r} + k_{4f}p} \quad (28)$$

for the flux change due to steady-state concentration change in the external metabolite S. A similar expression for $t_r[p]$ is derived in Appendix C.

The validity of the approximation of $y(t)$ by $z(t)$ with this time constant is ensured as seen in Figs. 2 and 3, employing the similar procedure described previously [3] and the simulation with *Mathematica*. The approximation can represent the response of the pathway unit to the transition within an allowable error. The relationship between D and a is found to be affected by the flux level of the pathway unit such that a higher flux level causes a larger D, that is, reduction in the validity. The transition time may thus be characterized with t_r in Eq. (27), that is, the derivative of the total concentration of the internal metabolite in the pathway unit with respect to the flux J.

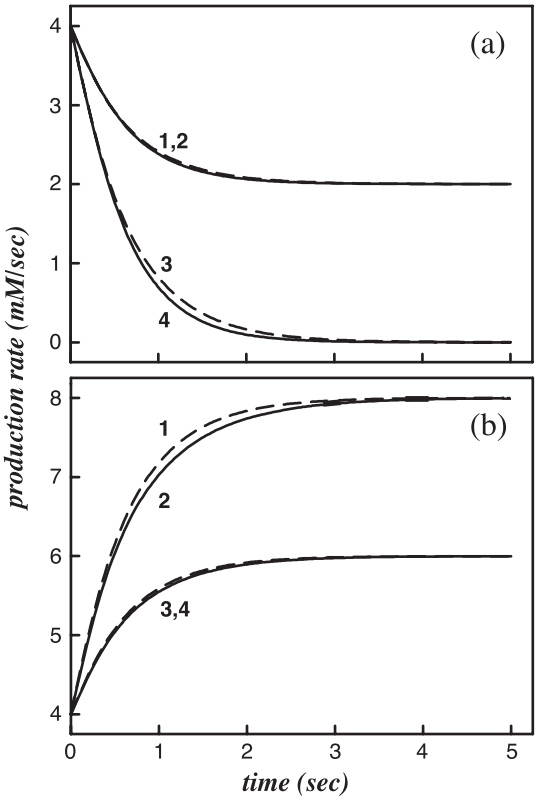

Fig. 2. Approximation by $z(t)$ for time courses in production rate of external metabolite P. The production rate is examined for the case $J=4$, where the dashed lines (1, 3) corresponds to $z(t)$ and the full lines (2, 4) to $y(t)$, respectively. (a) The value of a is set to -2 (curves 1, 2), and to -4 (curves 3, 4). (b) The value of a is set to 4 (curves 1, 2), and to 2 (curves 3, 4). The kinetic parameters used are the same as in Fig. 1 except for k_{2f}, the value of which is set to be 0 to avoid a reverse flow for the input to the pathway unit.

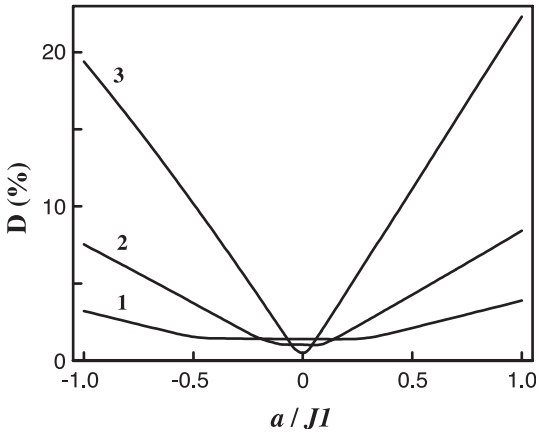

Fig. 3. Effects of the flux level on the validity of the characterization of the transition time. For a transition of the flux $J[s]$ by a from J_1 in the pathway unit, D on the ordinate is a relative error defined by $D = \sup_t |(y(t) - z(t))/a| \cdot (a/J_1)$ on the abscissa indicates the transition of the flux relative to J_1. The numbers on the curves correspond to J_1/V_{max} as follows: (1) 0.1, (2) 0.2, and (3) 0.4. The kinetic parameters used are the same as in Fig. 2.

3. Discussion

Sequential application of the procedure formulated in this study for the pathway unit of Michaelis–Menten mechanism (associated with one substrate, or a sole internal metabolite, and its enzyme–substrate complex) works as a general procedure for representation of linear pathways comprised of a sequence of the pathway units. For a single pathway unit the steady-state concentrations of every chemical species are expressed as respective functions of the flux J, a key parameter for steady-state behavior. A quadratic equation of the flux J is generated to represent its relationship with the external metabolite and total enzyme concentrations and the rate constants. This formulation may thus lead to the complete metabolic control analysis of the pathway unit. As described in this study, the flux and concentration control coefficients and the elasticities are derived explicitly in terms of the system parameters. The transit time τ is formulated as a sum of rectangular-hyperbolic functions of the flux J and a constant term. Through the flux J the relationship of the transit time τ with the steady-state concentration of the respective external metabolites may be expressed in practical forms.

It follows that this process of representation for the pathway unit leads us to generate and analyze the overall behavior for any linear pathways in a straightforward manner of sequential connection of the expressions of the constituent pathway units. As described previously [2], the characterization of the transit time τ with respect to the flux J for a linear pathway consisting of two pathway units can

be performed with the connection of the expressions for the first and the second pathway units in the pathway. The cubic equation relating the flux J to the other system parameters may be obtained by substitution of the corresponding expression of p as the internal metabolite in the second pathway unit into the term p in a quadratic equation of the flux J for the first pathway unit. The expression for the transit time is derived from sum of the respective transit times of the two pathway units. Furthermore, every control coefficient for the pathway unit are now expressed as the explicit functions of the system parameters and their behavior may be analyzed precisely against variation in the individual system parameters, yielding more detailed characterization for the metabolic control analysis.

The transition time is characterized by analysis of the dynamic response of the pathway unit to flux change between its stable steady states. A characteristic time constant for a first-order system is derived as $\partial\sigma/\partial J$ for the first approximation of the transition time, and the validity of this characterization is ensured. This formulation connotes not only that the transition time may be clearly distinguished from the transit time (σ/J) which is relevant to steady-state properties of the pathway unit, but also that the two characteristic time constants are closely related with respect to the formulation. It should be noted that the characteristic time constant for the transition time corresponds to an experimentally measurable parameter. As shown for a Michaelis–Menten-type reaction in closed system [8], the validity of the first-order approximation is now demonstrated for the pathway unit (Michaelis–Menten-type reaction in open system).

Detailed analysis of correlation of the flux J and the transit and transition times to the external metabolite concentrations and the kinetic parameters in the constituent pathway unit of Michaelis–Menten mechanism contributes to characterization of the flux behavior of linear pathways of Michaelis–Menten-type reactions. The similar formulation is applicable for constituent allosteric enzymes, so that the metabolic control analysis in this direction for a linear pathway with allosteric feedback regulation will be described elsewhere. The detailed formulation for representation of the pathway unit as provided in this study is required for proper application of the characterization procedure to various metabolic systems.

Appendix A. Derivation of $\tau[p]$

By use of Eqs. (2) and (8),

$$\sigma[p]=\frac{e_{1T}k_{1f}k_{2r}s-J(k_{1r}+k_{2r}+k_{1f}s)}{k_{2f}(J+k_{1r}e_{1T})}+\frac{e_{2T}k_{3f}\{e_{1T}k_{1f}k_{2r}s-J(k_{1r}+k_{2r}+k_{1f}s)\}-k_{2f}J(J+k_{1r}e_{1T})}{k_{3f}\{e_{1T}k_{1f}k_{2r}s-J(k_{1r}+k_{2r}+k_{1f}s)\}-k_{2f}k_{3r}(J+k_{1r}e_{1T})} \tag{A1}$$

is obtained when J varies corresponding to steady-state concentration change in the external metabolite P, leading to

$$\tau[p]=-\frac{k_{1r}k_{2f}}{b_3}+\frac{k_{1f}k_{2r}s}{J}\left(\frac{1}{k_{1r}k_{2f}}+\frac{k_{3f}e_{2T}}{b_3}\right)-(k_{1r}+k_{2r})(k_{1r}+k_{1f}s)\left\{\frac{1}{k_{1r}k_{2f}(J+k_{1r}e_{1T})}+\frac{k_{2f}k_{3f}(J+k_{3r}e_{2T})}{b_3(b_3e_{1T}-b_4J)}\right\} \tag{A2}$$

where

$$b_3=k_{1f}k_{2r}k_{3f}s+k_{1r}k_{2f}k_{3r}$$

$$b_4=k_{3f}(k_{1r}+k_{2r}+k_{1f}s)-k_{2f}k_{3r} \tag{A3}$$

Appendix B. Derivation of the minimal point for $\tau[s]$

It is derived from

$$\frac{\partial\tau[s]}{\partial J[s]}=-\frac{k_{4f}p}{J^2}\left(\frac{e_{2T}}{k_{4r}+k_{4f}p}+\frac{k_{3r}}{k_{3f}k_{4r}}\right)+\frac{k_{3r}+k_{4r}}{k_{3f}k_{4r}}\frac{k_{4r}+k_{4f}p}{(k_{4r}e_{2T}-J)^2}=0 \tag{A4}$$

that the transit time $\tau[s]$ has a minimum at

$$J=e_{2T}\frac{-k_{4f}p\{k_{3f}k_{4r}e_{2T}+k_{3r}(k_{4r}+k_{4f}p)\}+(k_{4r}+k_{4f}p)\sqrt{k_{4f}p(k_{3r}+k_{4r})\{k_{3f}k_{4r}e_{2T}+k_{3r}(k_{4r}+k_{4f}p)\}}}{(k_{3r}+k_{4r}+k_{4f}p)(k_{4r}+k_{4f}p)-e_{2T}k_{3f}k_{4f}p}$$

$$x_2=e_{2T}\frac{k_{4f}p(k_{4r}+k_{4f}p-k_{3f}e_{2T})+\sqrt{k_{4f}p(k_{3r}+k_{4r})\{k_{3f}k_{4r}e_{2T}+k_{3r}(k_{4r}+k_{4f}p)\}}}{(k_{3r}+k_{4r}+k_{4f}p)(k_{4r}+k_{4f}p)-e_{2T}k_{3f}k_{4f}p}. \tag{A5}$$

Appendix C. Derivation of $t_r[p]$

For the flux change due to steady-state concentration change in the external metabolite P, the characteristic time constant for the transition time in the pathway unit is formulated as:

$$t_r[p] = \frac{\partial \sigma[p]}{\partial J[p]} = e_{1T}(k_{1r} + k_{2r})(k_{1r} + k_{1f}s) \times \left\{ \frac{k_{2f}k_{3f}(k_{3r}e_{2T} - J_1)}{[k(J_1)]^2} - \frac{1}{k_{2f}(J_1 + k_{1r}e_{1T})^2} \right\} - \frac{k_{2f}(J_1 + k_{1r}e_{1T})}{k(J_1)} \quad \text{(A6)}$$

where

$$k(J) = k_{3f}\{e_{1T}k_{2r}k_{1f}s - J(k_{1r} + k_{2r} + k_{1f}s)\} - k_{2f}k_{3r}(J + k_{1r}e_{1T}). \quad \text{(A7)}$$

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