Transition and Transit Time Distributions for Time Dependent Reactions with Application to Biochemical Networks

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Temporal aspects of the dynamic behavior of biochemical pathways in stationary states have been described by a *transition time* τ, which is the ratio of the sum of the pool concentrations of chemical intermediates to the flux for a given stationary state. In this paper, a related random variable is introduced, the *transit time* θ, which is defined as the age of (metabolic) intermediates at the time of leaving the system. The theory, based on a semi-stochastic approach, leads to calculations of the probability distributions of the ages of the intermediates, as functions of time. By assuming that the kinetics of the pathway is described by mass-action laws, a system of partial differential equations is derived for the distribution function of the transit time. By using the method of characteristics the solving of the evolution equations for the distribution function is reduced to the solving of the kinetic equations of the process. The method is applied to a simple enzyme−substrate reaction operated in two different regimes: (1) with a constant input of reagent and (2) with a periodically varying input. In the first case the transit time probability distributions in the steady state are calculated both analytically and numerically. The mean transit time, calculated as the first moment of the distribution, coincides with the transition time calculated in the literature. In addition, the presented approach provides information concerning the fluctuations of the transit time. For a periodic input, the distribution function of transit times can be evaluated semianalytically by using the technique of Green functions. We show that in this case the distribution oscillates in time, and both the distribution of the transit time and its different moments and cumulants oscillate.

I. Introduction

The present design of metabolic pathways is the result of evolution over long periods of time. The understanding of this design requires consideration of the different processes that have made possible the modeling of metabolism as we now see it. One such process is the evolution toward the optimization of metabolic functions; however, the targets of such optimizations are unclear. *Metabolic flux* is one of the properties that appears to be a candidate for optimization. Increased metabolite fluxes of some necessary substances may improve the competition of a given cell or organism. Rapid response to a given stimulus may also be a likely candidate for optimization. These two optimizations may interfere with each other. For example, if the system has large concentrations of intermediates the time to reach the stationary regime, or the time to change from one regime to another, may be long, and therefore large concentrations be a disadvantage in regard to maintaining short-time responses. So, in terms of optimization of metabolic networks, it seems necessary to have present not only adequate fluxes but also a sufficiently rapid response of the system in time. Hence the *time domain* of biochemical systems requires study.

Different terms have been used for the temporal analysis of metabolic systems (for example, see Meléndez-Hevia et al.[1]): *elapsed time*, *transit time*, *transition time*, *transient time*, *reaction time*, *response time*, *lag time*, and some others. A survey on the literature on the temporal response of multienzyme systems shows that generally the above concepts are evaluated in terms of the macroscopic description of the behavior of the system; not all of these terms describe the same concept and have the same significance.

The purpose of this article is to provide a firmer theoretical basis of several of these terms, and a clarification of their interrelations on the basis of deterministic and stochastic concepts.

The response of a given system in terms of the time needed to reach a stationary state, or to change from one stationary state to another, has been called with the accepted term of *transition time*, a name suggested by Easterby.[2−3] The transition time is the time needed to *fill up* a system to the stationary concentrations of intermediates, starting from a substrate and no intermediates.[4] Consider a given reaction sequence of n intermediate species (metabolites), I_1, ..., I_n,

$$S \rightarrow I_1 \rightleftarrows I_2 \rightleftarrows .... \rightleftarrows I_n \rightarrow P$$

in a stationary state with a constant flux, J^{st}. Let I_j^{st} be the concentration of jth species in the stationary state. Then the transition time of the jth species in the system τ_j is

$$\tau_j = I_j^{st}/J^{st}$$

The overall transition time is given by

$$\tau = \sum_{j=1}^{n} \tau_j = \sum_{j=1}^{n} I_j^{st}/J^{st} \qquad (1)$$

According to this equation, the transition time is the ratio of the total concentration of intermediates in the stationary state $\sum_{j=1}^{n} I_j^{st}$ divided by the flux J^{st} in this steady state. The validity of this definition is justified in the literature on the basis of mass conservation of the total flux of intermediates in the stationary regime. The definition of transition time can be easily extended to the case of enzyme reaction networks with non-negligible concentrations of enzyme intermediates and feedback

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controlled pathways.[5−6] The concept of transition time has been widely used to analyze the behavior of biochemical networks in terms of control of metabolic systems[1,7−10] and in the particular case of *metabolite channeling*.[3,12−13]

This definition of transition time has some problems that make it difficult to apply to some biochemical networks. First, the definition applies to asymptotically stable steady states (more precisely, a stable node), whereas many biological, and physiologically significant, systems operate under nonstationary regimes.[14−15] Even though there is not a clear definition of the transition time for a time dependent regime, for example, for a chemical oscillator, eq 1 is also applied for these systems, despite that in this case there is no theoretical justification for using it.

Another problem with the above definition of transition time is its applicability to systems with many different inputs and/or outputs, or to systems where the reaction mechanism involves the breaking up of the intermediates. In these cases it is not clear if the expression 1 is still valid, even for a stationary process.

One of the physical interpretations that has been suggested for the transition time[7] is in terms of the *mean time* that a given molecule spends inside the system during the stationary regime (we refer to this time as the *transit time*). This interpretation is based on the intuition that, to a given extent, these two variables are related: the longer the transition time (taken as filling-up time) the longer the residence time of a molecule in the system; however, this conjecture has never been proven. Moreover, according with the kinetic descriptions of chemical chain reactions, the *mean lifetime* of a given species (or intermediate) is calculated as the ratio of the number of the reactive species (or the concentration of the intermediate) to its disappearance rate.[16,17] In the stationary regime this time coincides *precisely* with Easterby's definition of transition time.[3]

In this paper we provide evidence that the Easterby's definition 1 of the transition time is equivalent to the average value of the lifetime of the pool of intermediates only in the particular case of a stationary regime. For time-dependent processes we give a new, more general definition of the transition time; this new definition includes Easterby's expression 1 as a particular case. The outline of the article is the following. In section II we present a general approach for computing the distribution functions of the residence time of the pool of intermediates in a complex biochemical system and suggest analytic and numerical approaches for solving the corresponding evolution equations. In section III the theory is applied to two different operating regimes for a simple enzyme−substrate reaction with an externally controlled input rate. Finally, in section IV, we discuss some general implications of our approach.

II. Theory

A. Formulation of the Model. The kinetics of a system can be described either deterministically or stochastically. Easterby's definition of transition time is based on macroscopic and deterministic kinetics, whereas a proper definition of *mean* lifetimes requires a stochastic description. Here we propose a mixed approach to this problem and use some statistical properties of time distributions to calculate macroscopically observable quantities. We assume that the numbers of molecules making up the system are large enough that the concentration fluctuations can be neglected and the dynamics of the process can be described in terms of the deterministic form of the mass-action law. However, we also assume that the different molecules leaving the system are characterized by their residence times and that these are random variables characterized by statistical laws. Our main supposition, which allows us to evaluate the statistical distributions of the residence times is the assumption of local equilibrium, which is commonly used in nonequilibrium thermodynamics. We consider that the nonreactive collisions occurring in the system maintain the (local) energy distributions for the reaction intermediates and thus the process can be described in terms of total concentrations rather than in terms of energy densities. Under these circumstances the mass-action law gives a reasonable description of the process. An important consequence of the mass-action law approximation is that all molecules of a given intermediate have precisely the same probability of undergoing a chemical transformation, irrespective of their individual lifetimes. This consequence is used in the following for building a statistical description for the residence time distribution of the active intermediates in the system.

We consider that the system studied is made up of two types of reaction intermediates: (a) main intermediates, denoted by X_j, $j = 1, ..., n$, which, according to Easterby's classification, can be either free intermediates I_u, $u = 1, 2, ...$ or bound intermediates I_uE_v, $u, v = 1, 2, ...$, where E_v, $v = 1, 2, ...$ are different enzymes. (b) Other chemicals, denoted by A_j, $j = 1, 2, ...$, this second group includes the free enzymes E_v, $v = 1, 2, ...$, as well as other chemicals which may be present in the system. We assume that the reactions involving the main intermediates X_j, $j = 1, ..., n$ have the following structure:

$$... + X_j \underset{k_{j'j}(\mathbf{A})}{\overset{k_{jj'}(\mathbf{A})}{\rightleftharpoons}} X_{j'} +, ..., n \geq j' \geq j \geq 1 \qquad (2)$$

where (... + and ... +) denotes the contribution of the other chemicals A_j, $j = 1, 2, ...$ to the process and $k_{jj'}(\mathbf{A})$, $k_{j'j}(\mathbf{A})$ are effective rate coefficients depending on the composition vector $\mathbf{A}$ of the concentrations $A_1, A_2, ...$ of the other chemicals ($\mathbf{A} = (A_1, A_2, ...)$). In addition to the processes 2, the intermediates X_j, $j = 1, ..., n$ are involved in two other types of processes. (a) They are generated with different rates $J_1(t), ..., J_n(t)$, which are generally time-dependent:

$$... \xrightarrow{J_j(t)} X_j, \quad j = 1, ..., n \qquad (3)$$

(b) They interact with the other chemicals present in the system and generate different final products $P_1, ..., P_n$

$$... + X_j \xrightarrow{k_j^*(\mathbf{A})} P_j, \quad j = 1, ..., n \qquad (4)$$

where $k_j^*(\mathbf{A})$, $j = 1, ..., n$ are effective rate coefficients. Thus all reactions involving each intermediate are linear in that intermediate. However, the overall kinetics of the process is nonlinear.

B. Evolution Equations. The kinetic equations corresponding to these chemical processes are

$$\frac{\mathrm{d}}{\mathrm{d}t}X_j = J_j(t) + \sum_{j' \neq j} k_{j'j}(\mathbf{A})X_{j'} - X_j \sum_{j' \neq j} k_{jj'}(\mathbf{A}) - X_j k_j^*(\mathbf{A}) \qquad (5)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}P_j = X_j k_j^* \qquad (6)$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathbf{A} = \mathbf{g}(\mathbf{A}, X_1, ..., X_n) \qquad (7)$$

where eq 7 describes the dynamic behavior of the composition vector $\mathbf{A}$ and $\mathbf{g}(\mathbf{A}, X_1, ..., X_n)$ is a nonlinear vectorial function of $\mathbf{A}$ and $X_1, ..., X_n$. If we assume that initially there are no

main intermediates in the system, then the initial conditions for X_j, $j = 1, .., n$ are

$$X_j(t = 0) = 0, \quad j = 1, ..., n \tag{8}$$

The model expressed by eqs 5−7 is a generalization of similar models presented in the biochemical literature.[2−6,10,12−13] A common characteristic of these models is the assumption that one or more products are generated by certain irreversible reaction steps. These products either accumulate or are used by other biochemical processes. The same feature exists in our model. The products P_j, $j = 1, ..., n$ are generated by the irreversible reaction step 4. Further on, these products accumulate according to kinetic eq 6 or are used in other processes as reagents, in which case we should add a loss term in eq 6. However, since we focus mainly on the evaluation of transit and transient time, rather than on the evaluations of the concentrations of the different chemicals, we limit our calculations to the case of eq 6. Since the reaction steps 4 are assumed to be irreversible the expressions for the distribution of transit time are identical in both cases, with or without consumption of P_j, $j = 1, ..., n$.

If the effective rate coefficients $k_{jj'}(\mathbf{A})$, $k_{j'j}(\mathbf{A})$ and $k_j^*(\mathbf{A})$, $j, j' = 1, ..., n$, the function $\mathbf{g}(\mathbf{A}, X_1, ..., X_n)$ and the input fluxes $J_1(t), ..., J_n(t)$ are known, then eqs 5−7, together with the initial conditions (eq 8) for the main intermediates and with suitable initial conditions for the other chemicals, determine completely the time evolution of all concentrations $X_1, X_2, ..., A_1, A_2, ...$ and $P_1, P_2, ...$ Since eqs 5−7 are nonlinear, they can only be solved numerically, in general. The knowledge of the solution of these equations, however, is not enough for evaluating the statistical properties of the residence time associated to the different molecules passing through the system. We assume that all intermediates X_j, $j = 1, ..., n$, as well as the reaction products $P_1, ..., P_n$, share the same common molecular *skeleton* which is maintained unchanged during the chemical transformations represented by the reactions 2−4. Thus, the intermediates must be traceable and identified without ambiguity during the process. The concept of skeleton is illustrated with an example taken from the well known biochemical pathway of anaerobic glycolysis. In Figure 1 are represented the different transformations of carbohydrates from glycogen to the input to the Krebs cycle. In this example the pathway is assumed to start from the hydrolysis of glycogen and finish with the output of pyruvate to other parts of the metabolism. Each transformation is catalyzed by an enzyme and most of them involve other metabolites. This pathway has two differentiated parts, the first one involving transformations of sugars of six carbons (hexoses) and the second with sugars of three carbons (trioses). The same subskeleton of carbon−carbon bonds is present in either of these two parts. The differences among the intermediates concern only the transformations that occur to this carbon chain, that can be identified with the skeleton of the molecule. Notice that the theory presented in this paper also applies to the critical step of the division of fructose-1,6-bisphosphate in two different trioses, which includes a futile cycle in the reaction pathway. In this transition, as well as in the rest of the pathway, a given molecule can be identified unambiguously tracking one of the carbons, for example the first carbon (α-carbon). The age or lifetime of each molecule can be calculated following the trajectory of the corresponding α-carbon.

A simple example is given in section III, eq 43. In the following we assume that the residence time θ is the time interval necessary for such a molecular skeleton to pass through the system, from the entrance in any of the fluxes $J_1(t), ..., J_n(t)$ up to the exit in the form of any of the products $P_1, ..., P_n$. From the entrance to the exit any molecular skeleton can undergo any, complicated or not, succession of transformations of the type

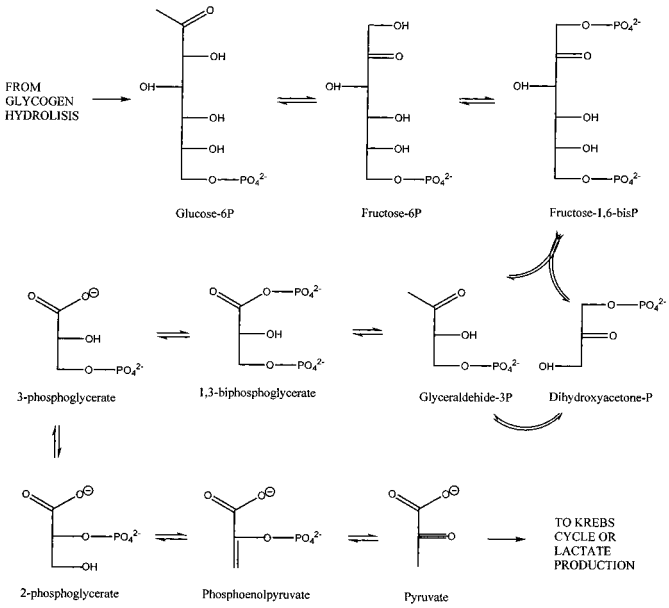

Figure 1. Schematic representation of the anaerobic part of the glycolytic pathway. For simplicity the carbon atoms, their bonds, and the corresponding hydrogen atoms are not explictly represented. Also, the different enzymes and other intermediates, as ATP or NADH, have not been included. The representation is not sterical but maintains the chirality of the molecules when necessary.

$$I_{j_1} \rightarrow I_{j_2} \rightarrow I_{j_3} \rightarrow ... \tag{9}$$

where all intermediate states $j_1, j_2, j_3, ...$ can take any values from $j = 1$ to $j = n$. In order to evaluate the statistical properties of the transit time θ, we should take into account the contributions of all transformations of type 9 and evaluate their probabilities of occurrence. At least in principle, this can be done by using an approach from nonequilibrium statistical physics, for instance, the method of *continuous time random walks*[18] or of other diagrammatic techniques.[22] We do not intend to use these techniques here; instead, we shall make use of an alternative approach, the method of *age dependent balance equations*. This technique has been used in population dynamics for over 3 decades.[19] More recently, it has been also introduced to nonequilibrium statistical physics and in chemical kinetics.[17,20,21]

For each of the intermediates X_j, $j = 1, ..., n$, we introduce a residence time density function:

$$\eta_j(\theta;t)\, d\theta \qquad \text{with} \qquad \int_0^t \eta_j(\theta;t)\, d\theta = X_j \qquad j = 1, ..., n \tag{10}$$

$\eta_j(\theta;t)\, d\theta$ is the concentration of a main intermediate of the type X_j with a transit time between θ and $\theta + d\theta$. According to the initial condition 8, initially there are no main intermediates in the system and then the transit time θ is at most equal to the clock time t. It follows that the integral of the density function $\eta_j(\theta;t)\, d\theta$ over θ from $\theta = 0$ to $\theta = t$ equals the total concentration X_j of the intermediate.

The density functions $\eta_j(\theta;t)\, d\theta$ obey a system of balance equations similar to the kinetic eqs 5 for the total concentrations X_j of the main intermediates:

$$\left(\frac{\partial}{\partial t}+\frac{\partial}{\partial \theta}\right)\eta_j(\theta;t)=\sum_{j'\neq j}k_{j'j}(\mathbf{A})\eta_{j'}(\theta;t)-\eta_j(\theta;t)[\sum_{j'\neq j}k_{jj'}(\mathbf{A})+k_j^*(\mathbf{A})] \quad (11)$$

with the initial and boundary conditions:

$$\eta_j(\theta;0)=0; \quad j=1, ..., n \quad (12)$$

$$\eta_j(\theta=0;t)=J_j(t) \quad j=1, ..., n \quad (13)$$

Equations 11 express the process of aging of a given molecular skeleton undergoing a succession of transformations of type 9, whereas the boundary conditions 13 express the "birth" of a skeleton of a given type. The boundary condition 13 also expresses the equality between the input fluxes and the residence time density functions for $\theta = 0$. We notice that both $J_j(t)$ and $\eta_j(\theta = 0;t)$ have the physical dimension [concentration/time]. Although not identical, eqs 11−13 have a structure similar to the balance equations for the lifetime densities of active intermediates in chemical kinetics.[17] The lifetime of an intermediate is the time interval that elapses from the moment a molecule of that intermediate is generated up to its disappearance. The analysis of the statistical properties of the lifetime is much simpler than the analysis of the statistical properties of the transit time considered in this paper. The value of the lifetime of an intermediate is characterized by only two random events, the generation of an intermediate and its disappearance, whereas the value of the transit time is the result of a possibly large number of individual transition events of the type 9; this is the reason that the evolution eqs 5−7 for the statistics of the transit time are more complicated than the corresponding evolution equations for the statistics of the lifetime presented in the literature.

C. Statistics of the Transit Time. In terms of the density functions $\eta_j(\theta;t)\,d\theta$, , $j = 1, ..., n$ we can introduce three different frequency distributions for the transit time: (a) The distribution function of the jth state and of the transit time θ of the intermediates present in the system at time t:

$$\psi_j(\theta;t)\,d\theta=\frac{\eta_j(\theta;t)\,d\theta}{\sum_j\int_0^t\eta_j(\theta;t)\,d\theta} \quad \text{with}$$

$$\sum_j\int_0^t\psi_j(\theta;t)\,d\theta=1 \quad (14)$$

(b) The distribution function of the jth state and of the transit time θ of the intermediates leaving the system at time t:

$$\phi_j(\theta;t)\,d\theta=\frac{k_j^*(\mathbf{A})\eta_j(\theta;t)\,d\theta}{\sum_j\int_0^t k_j^*(\mathbf{A})\eta_j(\theta;t)\,d\theta} \quad \text{with}$$

$$\sum_j\int_0^t\phi_j(\theta;t)\,d\theta=1 \quad (15)$$

(c) The total distribution function of the transit time θ of the main intermediates leaving the system, irrespective of their states

$$\phi(\theta;t)\,d\theta=\frac{\sum_j k_j^*(\mathbf{A})\eta_j(\theta;t)\,d\theta}{\sum_j\int_0^t k_j^*(\mathbf{A})\eta_j(\theta;t)\,d\theta}=\sum_j\phi_j(\theta;t)\,d\theta \quad \text{with}$$

$$\int_0^t\phi(\theta;t)\,d\theta=1 \quad (16)$$

We assume that the size of the system is large enough so that the concentration fluctuations can be neglected and, as a consequence of this assumption, the above introduced distribution function may also be interpreted as probability densities of the transit time of the reaction intermediates present in the system at time t or leaving the system at the same time, respectively. The most important distribution is the one given by eq 16 because it describes the statistical properties of the reaction intermediates leaving the system, irrespective of the states of the different intermediates. It seems plausible that the average transit time corresponding to the distribution of eq 16

$$\langle\theta(t)\rangle=\int_0^t\theta\phi(\theta;t)\,d\theta \quad (17)$$

may make possible the generalization of the transient description for nonstationary processes. At first sight it seems that the average transit time given by eq 17 is different from eq 1 for the transient time because in general the average value in eq 17 is time dependent, i.e., it depends on the moment at which it is evaluated, whereas the transition time given by eq 1 is not time dependent. This contradiction is only apparent because Easterby's expression for the transition time is restricted to a steady state. If the process evolves toward a steady state, the average value given by eq 17 also tends toward a time independent value. In the following section dealing with the application of the present approach, we shall show that, subject to the condition of stationarity, the expression 17 and Easterby's relationship 1 lead to the same results; in other words, that transit and transition time coincide for systems in a stationary regime.

An advantage of the present approach is that, in addition to the expression 17 for the average transit time, it also provides information concerning the fluctuations of the transit time. We can compute the different moments of the transit time:

$$\langle\theta^m(t)\rangle=\int_0^t\theta^m\phi(\theta;t)\,d\theta, \; m=1, 2, ... \quad (18)$$

in terms of which we can compute the absolute typical fluctuations of different order expressed by the cumulants $\langle\langle\theta^m(t)\rangle\rangle$, $m = 1, 2, ...$ of the distribution 16. In particular, the cumulant of the second order $\langle\langle\theta^2(t)\rangle\rangle$ is equal to the dispersion of the distribution:

$$\langle\langle\theta^2(t)\rangle\rangle=\int_0^t(\theta-\langle\theta(t)\rangle)^2\phi(\theta;t)\,d\theta=\langle\theta^2(t)\rangle-\langle\theta(t)\rangle^2 \quad (19)$$

In terms of $\langle\langle\theta^2(t)\rangle\rangle$ we can also evaluate the relative fluctuation of second order of the transit time:

$$\rho_2(t)=\sqrt{\langle\langle\theta^2(t)\rangle\rangle/\langle\theta(t)\rangle} \quad (20)$$

The behavior of the relative fluctuation of second order in the limit of large times provides information concerning the nature of fluctuations of the transit time. If this function tends toward a value different from zero in the limit of infinite time, the fluctuations are intermittent; otherwise, if the relative fluctuation $\rho_2(t)$ tends to zero as $t \rightarrow \infty$, then the fluctuations are nonintermittent.

D. Integration of the Evolution Equations. For computing all these properties of the transit time we need to know the density functions $\eta_j(\theta;t)\,d\theta$. If the solution of the kinetic equations is known, eqs 5−7, then the evaluation of the density functions $\eta_j(\theta;t)\,d\theta$ reduces to solving the partial differential eqs 11 with the initial and boundary conditions 12−13. The integration can be carried out analytically by applying a general technique from the theory of partial differential equations, the method of characteristics. The main steps of the computation

are outlined in Appendix A. The density functions $\eta_j(\theta;t)\,\mathrm{d}\theta$ can be expressed as

$$\eta_j(\theta;t) = \sum_{j'=1}^{n} J_{j'}(t-\theta)G_{j'j}(t|t-\theta) \tag{21}$$

where $G_{j'j}(t|t')$ are Green functions attached to the partial differential eqs 11. These Green functions are the solutions of a system of ordinary linear differential equations with time-dependent coefficients:

$$\frac{\mathrm{d}}{\mathrm{d}t}G_{j'j}(t|t') = \sum_{u=1}^{n} G_{j'u}(t|t')\epsilon_{uj}(t), \quad j', j = 1, ..., n \tag{22}$$

with the initial conditions

$$G_{j'j}(t=t'|t') = \delta_{j'j}, \quad j', j = 1, ..., n \tag{23}$$

where the coefficients $\epsilon_{uj}(t)$ are functions of time, depending on the solutions of the deterministic kinetic eqs 5−7,

$$\epsilon_{uj}(t) = (1-\delta_{uj})k_{uj}[\mathbf{A}(t)] + \delta_{uj}\{k_j^*[\mathbf{A}(t)] + \sum_{j'\neq j} k_{jj'}[\mathbf{A}(t)]\}, \quad u, j = 1, ..., n \tag{24}$$

From the results presented above it turns out that the density functions $\eta_j(\theta;t)\,\mathrm{d}\theta$ can be evaluated by integrating numerically the nonlinear kinetic eqs 5−7 followed by the determination of the Green functions $G_{j'j}(t|t')$ by means of the repeated numerical integration of eqs 22−23 for different initial times $t' = t'_1, t'_2, t'_3, ...$ For an accurate evaluation of the statistics of the transit time, it is necessary that the dependence of the Green functions $G_{j'j}(t|t')$ on the initial times $t' = t'_1, t'_2, t'_3, ...$ is known in great detail. This can be achieved by repeating the integration of eqs 22−23 for a large number of different initial values $t' = t'_1, t'_2, t'_3, ...$ For a large system, such a procedure is time consuming and requires a workstation for numerical computations. For a small number of reactions, however, reasonable accuracy can be achieved by using a personal computer. The results of such numerical computations are presented later in this article.

If the system is operated in a stationary regime, then the evaluation of the statistical properties of the transit time reduces to solving a system of linear differential equations with time independent coefficients; in this case the computations can be carried out analytically, for example, by using the Laplace transform technique. For the occurrence of a stationary regime it is necessary that the input fluxes $J_1(t), .., J_n(t)$ be time independent

$$J_j(t) = J_j^{\mathrm{st}} \qquad \text{independent of} \qquad t, j = 1, ..., n \tag{25}$$

If eqs 25 are valid, then the deterministic kinetic equations can have one or more stationary solutions corresponding to the solutions of the equations:

$$J_j^{\mathrm{st}} + \sum_{j'\neq j} k_{j'j}(\mathbf{A}^{\mathrm{st}})X_j^{\mathrm{st}} - X_j^{\mathrm{st}}\sum_{j'\neq j} k_{jj'}(\mathbf{A}^{\mathrm{st}}) - X_j^{\mathrm{st}}k_j^*(\mathbf{A}^{\mathrm{st}}) = 0 \tag{26}$$

$$\mathbf{g}(\mathbf{A}^{\mathrm{st}}, X_1^{\mathrm{st}}, ..., X_n^{\mathrm{st}}) = 0 \tag{27}$$

We assume that there is at least one physically significant stationary solution $(\mathbf{X}^{\mathrm{st}}, \mathbf{A}^{\mathrm{st}}) = (X_1^{\mathrm{st}}, ..., X_n^{\mathrm{st}}, A_1^{\mathrm{st}}, A_2^{\mathrm{st}}, ...)$ of the kinetic equations which is stable and try to evaluate the corresponding density functions $\eta_j^{\mathrm{st}}(\theta)\,\mathrm{d}\theta$. For a steady-state characterized by the composition vector $(\mathbf{X}^{\mathrm{st}}, \mathbf{A}^{\mathrm{st}}) = (X_1^{\mathrm{st}}, ..., X_n^{\mathrm{st}}, A_1^{\mathrm{st}}, A_2^{\mathrm{st}}, ...)$ the matrix elements $\epsilon_{uj}(t)$, $u, j = 1, ..., n$, and the effective exit rate coefficients $k_j^*(\mathbf{A})$, $j = 1, ..., n$ are time independent and are given by

$$\epsilon_{uj}^{\mathrm{st}} = (1-\delta_{uj})k_{uj}[\mathbf{A}^{\mathrm{st}}] + \delta_{uj}\{k_j^*[\mathbf{A}^{\mathrm{st}}] + \sum_{j'\neq j} k_{jj'}[\mathbf{A}^{\mathrm{st}}]\}, \quad u, j = 1, ..., n \tag{28}$$

$$k_j^{*\mathrm{st}} = k_j^*[\mathbf{A}^{\mathrm{st}}], \quad j = 1, ..., n \tag{29}$$

From eqs 22 we notice that for a steady state the Green functions $G_{j'j}(t|t')$ depend only on the time difference $t - t'$ which is actually the transit time θ (see also Appendix A). We have

$$\frac{\mathrm{d}}{\mathrm{d}\theta}G_{j'j}^{\mathrm{st}}(\theta) = \sum_{u=1}^{n} G_{j'u}^{\mathrm{st}}(\theta)\epsilon_{uj}^{\mathrm{st}}, \quad j', j = 1, ..., n \tag{30}$$

with the initial condition

$$G_{j'j}^{\mathrm{st}}(\theta = 0) = \delta_{j'j}, \quad j', j = 1, ..., n \tag{31}$$

where $G_{j'j}^{\mathrm{st}}(\theta) = G_{j'j}^{\mathrm{st}}(t-t') = G_{j'j}^{\mathrm{st}}(t|t')$ are the stationary values of the Green functions. We introduce the matrix notations

$$\mathbf{G}^{\mathrm{st}}(\theta) = [G_{j'j}^{\mathrm{st}}(\theta)], \quad \epsilon^{\mathrm{st}} = [\epsilon_{uj}^{\mathrm{st}}] \tag{32}$$

and the Laplace transform

$$\bar{\mathbf{G}}^{\mathrm{st}}(s) = \int_0^\infty \exp(-s\theta)\mathbf{G}^{\mathrm{st}}(\theta)\,\mathrm{d}\theta \tag{33}$$

where the overbar denotes the Laplace transformation and s is the Laplace variable conjugate to the transit time θ. By taking the Laplace transform of eqs 30 and using eqs 32−33 we come, after some algebraic manipulations, to the following expression for the Laplace transform of the matrix of the Green functions:

$$\bar{\mathbf{G}}^{\mathrm{st}}(s) = [\mathbf{I}s - \epsilon^{\mathrm{st}}]^{-1} \tag{34}$$

where $\mathbf{I}$ is the unity matrix. The poles of the matrix function $\bar{\mathbf{G}}^{\mathrm{st}}(s)$ are the eigenvalues of the secular equation

$$\det|\mathbf{I}s - \epsilon^{\mathrm{st}}| = 0 \tag{35}$$

If the secular eq 35 has the roots $s_1, s_2, s_3, ...$ with the multiplicities $m_1, m_2, m_3, ...$, respectively, then the stationary Green functions are given by linear combinations of exponential terms of the form $\exp(s_\beta\theta)$, $\beta = 1, 2, 3, ...$ modulated by polynomials in the transit time. For computing the explicit expression for the matrix of stationary Green functions $\mathbf{G}^{\mathrm{st}}(\theta)$ we must compute the matrix on the right hand side of eq 34 and evaluate the inverse Laplace transform of the resulting matrix. The calculations, based on Heaviside's second expansion theorem, are lengthy but standard. To save space we give here only the final results. A similar computation, performed in a different context, is presented in Vlad and Pop (1989, Appendix E). The expression of $\mathbf{G}^{\mathrm{st}}(\theta)$ is

$$\mathbf{G}^{\mathrm{st}}(\theta) = \sum_{\beta}\sum_{b=1}^{m_\beta} \frac{\theta^{b-1}\exp(s_\beta\theta)}{(b-1)!(m_\beta - b)!} \tag{36}$$

where $\mathbf{G}_{\beta b}$ are constant matrices determined by the relationship

$$\mathbf{G}_{\beta b} = \frac{d^{m_\beta - b}}{ds^{m_\beta - b}}\left\{\left[\frac{\mathrm{Adj}(\mathbf{I}s - \epsilon^{\mathrm{st}})}{\det|\mathbf{I}s - \epsilon^{\mathrm{st}}|}\right](s - s_\beta)^{m_\beta}\right\}|_{s=s_\beta} \tag{37}$$

For a stationary regime the total distribution function of the transit time is also time-independent

$$\phi(\theta;t) = \phi^{st}(\theta) \tag{38}$$

For evaluating $\phi^{st}(\theta)$ we sum in eqs 26 over the label j, which results in

$$\sum_{j=1}^{n} J_j^{st} = \sum_{j=1}^{n} k_j^*(\mathbf{A}^{st}) X_j^{st} \tag{39}$$

eq 39 expresses the conservation of the sum of the input fluxes, which for a stationary regime should equal the sum of the output fluxes, expressed by the total rate of formation of the final products P_1, ..., P_n. To show that this is the case, we notice that for a steady state the rates of formation of the final products are constant and that their amounts increase linearly in time

$$[\mathrm{d}P_j(t)/\mathrm{d}t]^{st} = k_j^*(\mathbf{A}^{st}) X_j^{st}, \quad \text{i.e.,}$$
$$P_j(t) = P_j(t') + (t - t') k_j^*(\mathbf{A}^{st}) X_j^{st} \tag{40}$$

and thus eq 39 can be rewritten as

$$\sum_{j=1}^{n} J_j^{st} = \sum_{j=1}^{n} [\mathrm{d}P_j(t)/\mathrm{d}t]^{st} \tag{41}$$

The conservation law eq 39 allows us to express the distribution function $\phi^{st}(\theta)$ terms of the input fluxes entering the system. By applying eqs 10, 16, 21, and 39 we obtain

$$\phi^{st}(\theta) = \frac{\sum_{j=1}^{n} \eta_j^{st}(\theta) k_j^*(\mathbf{A}^{st})}{\sum_{j=1}^{n} X_j^{st} k_j^*(\mathbf{A}^{st})} = \frac{\sum_{j=1}^{n}\sum_{j'=1}^{n} J_{j'}^{st} G_{j'j}^{st}(\theta) k_j^*(\mathbf{A}^{st})}{\sum_{j=1}^{n} J_j^{st}} \tag{42}$$

III. Examples

We apply the theory developed in the preceding section to a simple enzyme−substrate reaction. We assume that an intermediate I is introduced in the system with an externally controlled input rate $J(t)$, interacts with an enzyme E and is transformed into a reaction product P. The process can be represented by the chemical reactions

$$\ldots \xrightarrow{J(t)} \mathrm{I}; \quad \mathrm{E} + \mathrm{I} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{EI} \xrightarrow{k_{+2}} \mathrm{E} + \mathrm{P} \tag{43}$$

The reaction mechanism is a particular case of the process investigated in the preceding section, corresponding to a single input flux, $J(t)$, to two different reaction intermediates, $X_1 \equiv I$ and $X_2 = EI$ (italics denotes concentrations), to a single other chemical, the free enzyme E ($\mathbf{A} = (E)$), and to a single reaction product, P. For this system the deterministic kinetic equations are given by

$$\mathrm{d}X_1/\mathrm{d}t = -k_{+1}X_1(E_0 - X_2) + k_{-1}X_2 + J(t); \quad X_1(t=0) = 0 \tag{44}$$

$$\mathrm{d}X_2/\mathrm{d}t = k_{+1}X_1(E_0 - X_2) - (k_{-1} + k_{+2})X_2; \quad X_2(t=0) = 0 \tag{45}$$

where E_0, the total concentration of enzyme in the system, free and bound, is constant. The total distribution of transit time at time t, $\phi(\theta;t)\,\mathrm{d}\theta$, can be computed from eqs 10, 16, and 21:

$$\phi(\theta;t) = [J(t-\theta)G_{12}(t|t-\theta)]/X_2(t) \tag{46}$$

where the Green function $G_{12}(t|t')$ can be evaluated by the repeated numerical integration of the following differential equations for different initial times:

$$\frac{\mathrm{d}}{\mathrm{d}t}G_{11}(t|t') = -k_{+1}(E_0 - X_2(t))G_{11}(t|t') + k_{-1}G_{12}(t|t');$$
$$G_{11}(t = t'|t') = 1 \tag{47}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}G_{12}(t|t') = k_{+1}(E_0 - X_2(t))G_{11}(t|t') - (k_{-1} + k_{+2})G_{12}(t|t');$$
$$G_{12}(t = t'|t') = 0 \tag{48}$$

A. Analytical Calculation of the Distributions in the Steady State. In particular, if the input flux is constant, $J(t) = J^{st}$ is independent of t, then the system can be operated in a steady state characterized by

$$X_1^{st} = \frac{(k_{-1} + k_{+2})J^{st}}{k_{+1}(k_{+2}E_0 - J^{st})}; \quad X_2^{st} = \frac{J^{st}}{k_{+2}};$$
$$E^{st} = E_0 - X_2^{st} = \frac{k_{+2}E_0 - J^{st}}{k_{+2}} \tag{49}$$

which is physically significant and stable provided that

$$k_{+2}E_0 - J^{st} > 0 \tag{49a}$$

If the constraint (eq 49a) is fulfilled then the stationary distribution of the transit time can be evaluated by applying eqs 40−42. After lengthy calculations we come to

$$\phi^{st}(\theta) = \frac{2k_{+1}E^{st}k_{+2}}{[(k_{-1} + k_{+2} + k_{+1}E^{st})^2 - 4k_{+1}E^{st}k_{+2}]^{1/2}} \exp\left[-\frac{\theta}{2}(k_{-1} + k_{+2} + k_{+1}E^{st})\right] \sinh\left[\frac{\theta}{2}[(k_{-1} + k_{+2} + k_{+1}E^{st})^2 - 4k_{+1}E^{st}k_{+2}]^{1/2}\right] \tag{50}$$

The positive moments corresponding to the distribution 50 are given by

$$\langle\theta^m\rangle^{st} = \int_0^\infty \theta^m \phi^{st}(\theta)\,\mathrm{d}\theta =$$
$$\frac{m!(k_{+1}E^{st}k_{+2})^{-m}}{2^{m+1}[(k_{-1} + k_{+2} + k_{+1}E^{st})^2 - 4k_{+1}E^{st}k_{+2}]^{1/2}} \{[[(k_{-1} + k_{+2} + k_{+1}E^{st})^2 - 4k_{+1}E^{st}k_{+2}]^{1/2} + (k_{-1} + k_{+2} + k_{+1}E^{st})]^{m+1} - [[(k_{-1} + k_{+2} + k_{+1}E^{st})^2 - 4k_{+1}E^{st}k_{+2}]^{1/2} - (k_{-1} + k_{+2} + k_{+1}E^{st})]^{m+1}\} \tag{51}$$

The cumulants $\langle\langle\theta^m\rangle\rangle^{st}$, $m = 1, 2, \ldots$ of the transit time corresponding to the distribution 50 can be also computed analytically, resulting in (see Appendix B)

$$\langle\langle\theta^m\rangle\rangle^{st} = (m-1)!(2k_{+1}E^{st}k_{+2})^{-m}\{[[(k_{-1} + k_{+2} + k_{+1}E^{st})^2 - 4k_{+1}E^{st}k_{+2}]^{1/2} + (k_{-1} + k_{+2} + k_{+1}E^{st})]^m + (-1)^m[[(k_{-1} + k_{+2} + k_{+1}E^{st})^2 - 4k_{+1}E^{st}k_{+2}]^{1/2} - (k_{-1} + k_{+2} + k_{+1}E^{st})]^m\} \tag{52}$$

By applying the general relationships 51−52 for $m = 1$ and 2 we obtain

$$\langle\theta\rangle^{st} = \frac{k_{-1} + k_{+2} + k_{+1}E^{st}}{k_{+1}E^{st}k_{+2}} \tag{53}$$

$$\langle\theta^2\rangle^{st} = 2\left[\frac{k_{-1} + k_{+2} + k_{+1}E^{st}}{k_{+1}E^{st}k_{+2}}\right]^2 - \frac{2}{k_{+1}E^{st}k_{+2}} \tag{54}$$

$$\langle\langle\theta^2\rangle\rangle^{st} = \langle\Delta\theta^2\rangle^{st} = \left[\frac{k_{-1} + k_{+2} + k_{+1}E^{st}}{k_{+1}E^{st}k_{+2}}\right]^2 - \frac{2}{k_{+1}E^{st}k_{+2}} \tag{55}$$

$$\rho^{st} = \sqrt{\langle\langle\theta^2\rangle\rangle^{st}}/\langle\theta\rangle^{st} = \frac{\sqrt{(k_{-1})^2 + (k_{+1}E^{st})^2 + (k_{+2})^2 + 2k_{-1}(k_{+2} + k_{+1}E^{st})}}{k_{-1} + k_{+2} + k_{+1}E^{st}} > 0 \tag{56}$$

In order to clarify the relationship between transient time τ given by eq 1 and the average transition time at a steady state given by eq 53, we evaluate τ for the substrate−enzyme reactions 43 operated in the steady state corresponding to the stationary point (eq 49). By combining eqs 1, 49, and 53 we get

$$\tau = \frac{k_{-1} + k_{+2} + k_{+1}E^{st}}{k_{+1}E^{st}k_{+2}} = \langle\theta\rangle^{st} \tag{57}$$

that is, the transition time τ is the same as average stationary value of the transit time $\langle\theta\rangle^{st}$ given by eq 53. It follows that, for a stationary regime, our theory is consistent with Easterby's approach.

From eq 56 we notice that, the for a stationary regime, the relative fluctuation of the transit time is positive; it follows that the fluctuations of the transit time are intermittent.

B. Calculation of the Evolution of Transit Time Distributions. In the simple reaction mechanism 43 it is also feasible to calculate the evolution of the probability distribution of transit time (eq 46) from any given initial condition, toward the stationary regime. We assume that the system starts empty of product and intermediate, so the system fulfills the conditions given by (eqs 47 and 48). In the first example we assume that the input flux $J(t)$ increases exponentially toward the approximate constant threshold value J_0. Such a dependence has been suggested by Easterby.[6]

$$J(t) = J_0(1 - e^{-\lambda t}) \tag{58}$$

The solution of the probability distribution 46 requires the solution of the Green function $G_{12}(t|t - \theta)$, the input flux $J(t - \theta)$, and the free intermediate $X_2(t)$. These may be obtained by numerical integration of the system of differential eqs 47 and 48 for a collection of different initial times t'. Since this system depends on $X_2(t)$, the simultaneous integration of systems 45 and 46 and eqs 47 and 48 is required. In Figure 2 we present the evolution of the probability distribution for a given set of system parameters. After an initial transient period, (at about $t = 30$ in Figure 2) the distribution reaches a stationary regime as predicted by the analysis presented in the previous section. The accuracy of the distribution depends on the time interval for sampling different initial values of time, t'. In this case, reasonable accuracy has been obtained with a value of 0.5 (arbitrary time units). The time evolution of the different moments

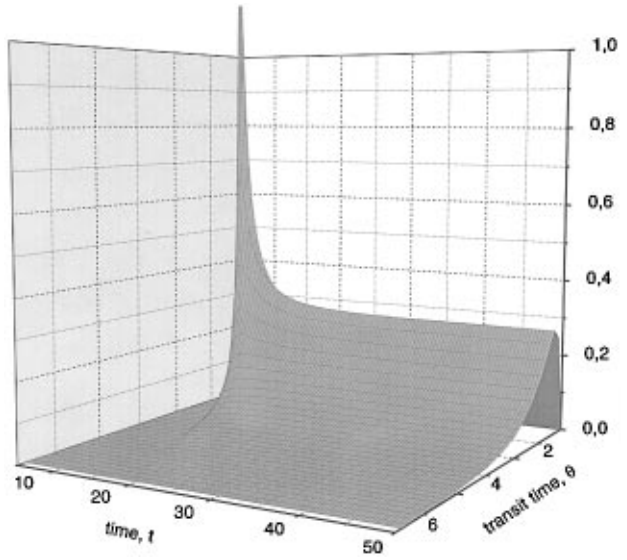

Figure 2. Probability distribution of transit time θ as a function of time t for the system 43 under the input flux given by eq 58. The values of the parameters are: k_{+1}, k_{-1}, $k_{+2} = 1$; $E_0 = 2$; $J_0 = 1$; $\lambda = 1$. For small times the transit time distribution is very narrow, very close to a delta function; this is due to the fact that initially the system is empty. For large times the distribution of transit times spreads out and tends toward a stationary form described by eq 50.

$$\langle\theta^m(t)\rangle = \int \theta^m \Phi(\theta,t)\, d\theta, \quad m = 1, 2, ... \tag{59}$$

and cumulants can be calculated from this probability distribution at each given time (Figure 3). The accuracy of the computations has been tested by checking the conservation of the normalization condition

$$\langle\theta^0(t)\rangle = 1 = \int \Phi(\theta,t)\, d\theta \tag{60}$$

which is represented in Figure 3 as the integral for each time. With the exception of the transient initial moments, the integral rapidly converges to 1. The results for the first moment (the average, $m = 1$), the second cumulant (the dispersion), and the relative fluctuation are also shown in Figure 3. As expected, the asymptotic value, but only that, of the average of the transit time, $\langle\theta\rangle^{st} = 2.998$, is a good approximation of the transition time calculated from the deterministic description (eq 1, Easterby[6]), which for the same parameter values is $\tau = 3$. In addition, the value of $\langle\theta\rangle^{st}$ calculated from the expression 53 is also 3. This result shows, once again, that for a stationary regime Easterby's approach is consistent with our more general method.

C. Evolution of Transit Time Distribution under Periodic Forcing. One of the main advantages of the present theory is the possibility of the calculation of transit time under nonstationary condition. To check this, we have evaluated the transit time distribution in the system 43 under periodic input of substrate of the form

$$J(t) = J_0(1 + \sigma \sin(\omega_1 t)) \tag{61}$$

where ω_1 represents the frequency of the periodic input and J_0 the amplitude.

The system of differential equations of the Green functions 47 and 48, together with the system (eqs 44 and 45), is solved in the same way as described in the previous section, giving the probability distribution presented in Figure 4. In this case, the transit time distribution oscillates with the same frequency as that of the input flux. Accordingly the moments and the

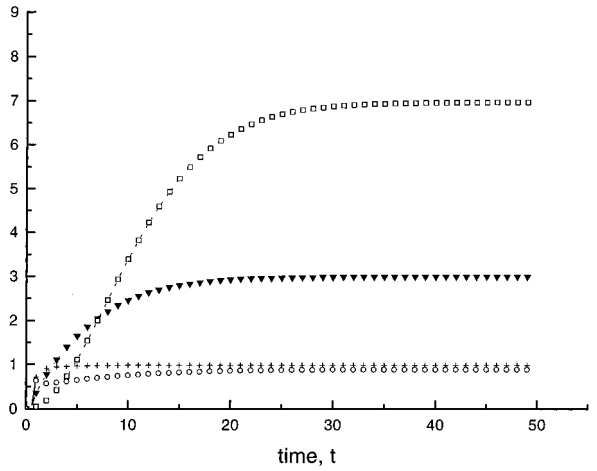

Figure 3. Time evolution of integral (+), the average ($\tilde{n}$), the dispersion (□), and the relative fluctuation (○) attached to the distribution of transit time of Figure 2 (from numerical simulation as well as from eqs 53−57). We notice a satisfactory agreement with the normalization condition 60.

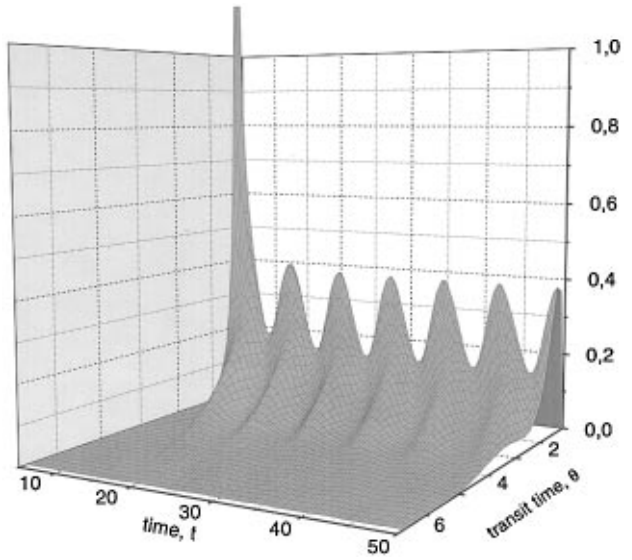

Figure 4. Probability distribution of transit time θ as a function of time t for the system 43 under the input flux given by eq 59. The values of the parameters are: k_{+1}, k_{-1}, $k_{+2} = 1$; $E_0 = 2$; $J_0 = 1$; $\sigma = 0.5$; $\omega_1 = 5$.

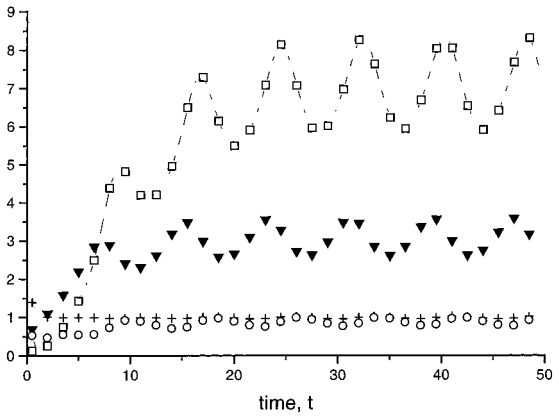

Figure 5. Time evolution of integral (+), the average ($\tilde{n}$), the dispersion (□), and the relative fluctuation (○) attached to the distribution of transit time of Figure 4.

cumulants also oscillate as shown in Figure 5, and attain a sustained periodic regime. A close observation of the surface of Figure 4 reveals an influence of the maximum peaks in the tail of the next minimum: after a maximum in the transit time, in the next semiperiod the molecules tend to stay longer in the system.

IV. Discussion

In this paper we have adopted the term transit time θ to refer the mean time that a given molecule takes in "crossing" the reaction pathway of the system, for example, from I to P in eq 43. In our approach the transit time may be defined as a function of the *age distribution* of the molecules at the moment when they are leaving the system. If there is more than one intermediate, our theory remains valid provided that each intermediate is traceable in the reaction network. Thus, if a given intermediate reacts and is transformed into another intermediate, then a significant part (a skeleton) of the first intermediate is conserved in the following intermediates. In our approach this condition has been included in the linear terms of eqs 5 and 6. With this condition the theory permits the calculation of the distribution of transit time. This distribution takes the form of a surface over the plane of time and transit time (see Figures 2 and 4). From this, it is possible to calculate the different moments (average and variance) and the cumulants, at each time. The first moment of the distribution is the transit time as a function of time. For a given initial time, the intersection of the distribution surface with the plane $t =$ constant gives the age distribution of a molecule leaving the system at that time. Experimentally it is possible to construct these age lines with the technique of isotopic tracers. For example, this may be done introducing an "instantaneous" pulse of labeled molecules (for example, radioactive isotopes), without affecting the parameters of the system, including the net input flux, and tracking their concentration as a function of time.

The application of the theory to a simple enzymatic mechanisms, with only one enzyme reaction with Michaelis−Menten kinetics, shows that, under condition of stationary state, both transition and transit times coincide. Thus we conclude that the mean age of the molecules leaving the system (transit time) is equal to the sum of all intermediate concentration divided by the flux (i.e., lifetime and transition time) in the stationary regime. That is, the mean time that an average molecule lasts in crossing the reaction network in the stationary regime (transit time) is the same as the time necessary for the system to reach the stationary state from initial conditions of zero concentration of intermediate (i.e., transient time and transition time). This relation, initially suggested by Torres *et al.*[7] has been proven here.

For the case of the asymptotic behavior of oscillatory processes, the definition of transition time given by eq 1 does not apply. For those systems we have provided a way to calculate the distribution of transit times as a function of time. As seen in Figure 5 in those cases the mean transit time also oscillates. In this case a new variable can be introduced, that is, the time average of the (ensemble) mean transit time over one period of oscillation T,

$$\overline{\langle\theta\rangle} = \frac{1}{T}\int_0^T \langle\theta(t)\rangle \, dt \tag{62}$$

which is a measure of the "overall" transit time of the system during oscillations. The temporal average $\overline{\langle q\rangle}$ of the transit time may serve as a basis for establishing a connection between our approach and the theory of control analysis of periodic phenomena in biological systems.[23] A key factor for establishing a connection between these two theories is Landau's description of periodic motion in a rapidly fluctuating force field.[24] Work on this problem is in progress.

From the point of view of mathematical statistics the model developed in this article is of the 'frontier' type;[25] that is, although it is essentially deterministic, it displays certain statistic and stochastic features. We emphasize that our approach neglects both the intrinsic (molecular) concentration fluctuations as well as the possible external (environmental fluctuations). Our evolution equations do not contain any random concentration sources nor any random transition rates. Due to this omission of any stochastic factors the numbers of molecules of the different chemicals in the system are deterministic functions of time. On the other hand, at a microscopic level each individual molecule has a different life time and this life time is a statistically distributed variable. Our model deals with two different levels of description: (a) a macroscopic one, for which the state of the system is described in terms of the concentrations of the different molecular species and (b) a microscopic level described in terms of the life times of the different molecules. We consider large systems, for which the thermodynamic limit is a very good approximation and thus at the macroscopic level a deterministic description of the process in terms of rate equations is appropriate. However, even in the thermodynamic limit, if we are interested in the evolution of an individual molecule, its age is a statistical quantity and its statistical properties can also be described in terms of the deterministic kinetic equations. Although at first sight this feature may seem surprising, it is commonly encountered in physical chemistry and population biology. For example the nonequilibrium behavior of a macroscopic mass of gas is given in terms of a one-particle Boltzmann equation which is a deterministic equation for the density $n(\mathbf{v},\mathbf{r};t)$ of particles at time t, with a position vector between $\mathbf{r}$ and $\mathbf{r} + \mathrm{d}\mathbf{r}$ and a velocity vector between $\mathbf{v}$ and $\mathbf{v} + \mathrm{d}\mathbf{v}$. The integral of the density $n(\mathbf{v},\mathbf{r};t)$ over the position and velocity vectors $\mathbf{r}$ and $\mathbf{v}$ gives the total number N of molecules in the system:

$$N = \int\int n(\mathbf{v},\mathbf{r};t)\, \mathrm{d}\mathbf{v}\, \mathrm{d}\mathbf{r} \tag{63}$$

Although, in the thermodynamic limit, the density $n(\mathbf{v},\mathbf{r};t)$ is a deterministic function of position and velocity vectors, the position and speed of an individual molecule is random. Despite being deterministic, the Boltzmann equation provides useful information about the statistical properties of $\mathbf{r}$ and $\mathbf{v}$. In the thermodynamic limit the relative frequency

$$f(\mathbf{v},\mathbf{r},t)\, \mathrm{d}\mathbf{v}\, \mathrm{d}\mathbf{r} = n(\mathbf{v},\mathbf{r};t)\, \mathrm{d}\mathbf{v}\, \mathrm{d}\mathbf{r}/N \qquad \text{with} \qquad \int\int f(\mathbf{v},\mathbf{r};t)\, \mathrm{d}\mathbf{v}\, \mathrm{d}\mathbf{r} = 1 \tag{64}$$

is a good estimate of the probability distribution of the position and velocity vector of an individual molecule. Although deterministic, the Boltzmann equation contains some useful information concerning the statistical behavior of an individual molecule. The Boltzmann equation corresponds to a model of the frontier type:[25] depending on the context it can be viewed both as a deterministic macroscopic equation describing the evolution of the density function $n(\mathbf{v},\mathbf{r};t)$ or as a microscopic equation describing the evolution of the distribution $f(\mathbf{v},\mathbf{r};t)\, \mathrm{d}\mathbf{v}\, \mathrm{d}\mathbf{r}$ of the position and velocity vectors of an individual molecule. Similarly our evolution eqs 11−13, based on the mass action law, can be viewed both as deterministic equations describing the macroscopic properties of the system in terms of the density functions $\eta_j(\theta;t)\, \mathrm{d}\theta$ or as microscopic equations describing the properties of the distributions $\psi_j(\theta;t)\, \mathrm{d}\theta$, $\phi_j(\theta;t)\, \mathrm{d}\theta$ and $\phi(\theta;t)\, \mathrm{d}\theta$.

The above discussion about the interconnection between the deterministic and statistical aspects of our model outlines the usefulness of our approach. By using a simple deterministic description on the basis of the mass-action law, it is possible to extract useful information about the statistical properties of the transit time of a molecular fragment in a biochemical system. The transit time may be used for examining the kinetic data characteristic of a biochemical network from a different perspective and for developing new methods of experimental analysis. Traditionally a biochemical process is studied in a passive way; the scientists simply follow the time evolution of a process evolving under controlled conditions. On the other hand, the transition time may be experimentally accessible only by developing "active" methods of investigation (i.e., by perturbing the system and recording its response to the perturbation). We are working on a proposal for an "active" experiment of this type, based on the use of radioactive tracers.

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Appendix A

To solve the partial differential eqs 11 with the initial and boundary conditions 12−13, we express the right hand sides of these equations in terms of the time-dependent matrix elements $\epsilon_{uj}(t)$ defined by eqs 24,

$$\left(\frac{\partial}{\partial t} + \frac{\partial}{\partial \theta}\right)\eta_j(\theta;t) = \sum_{u=1}^{n} \eta_u(\theta;t)\epsilon_{uj}(t) \tag{A.1}$$

The characteristic curves in the (t,θ) plane attached to these equations fulfill the differential equation:

$$\mathrm{d}\theta/\mathrm{d}t = 1, \quad \text{that is,} \quad \theta = t - t' \tag{A.2}$$

where t' is an arbitrary integration constant. By following a standard procedure for solving partial differential equations of first order, we try to solve eqs A.1 along the characteristics. For this we make use of the new integration variables

$$\mathrm{d}\theta/\mathrm{d}t = 1, \quad \text{that is,} \quad \theta = t - t' \tag{A.3}$$

and introduce the transformed functions

$$\tilde{\eta}_j(t';t)|\mathrm{d}t'| = \eta(\theta;t)\, \mathrm{d}\theta \qquad (\text{i.e., } \tilde{\eta}_j(t';t) = \eta(\theta = t - t';t)) \tag{A.4}$$

With the use of these new integration variables and functions, the partial differential eqs A.1 can be transformed into a system of ordinary differential equations

$$\frac{\mathrm{d}}{\mathrm{d}t}\tilde{\eta}_j(t';t) = \sum_{u=1}^{n} \tilde{\eta}_u(t';t)\epsilon_{uj}(t) \tag{A.5}$$

and eqs 13 become

$$\tilde{\eta}_j(t' = t;t) = J_j(t) \tag{A.6}$$

Eqs A.6 can be rewritten in the equivalent form:

$$\tilde{\eta}_j(t',t = t') = J_j(t') \tag{A.7}$$

that is, in the new representation given by eqs A.3−A.4, the

boundary conditions 13 are transformed into a set of initial conditions for the ordinary differential eqs A.5.

Although linear, the evolution eqs A.5 cannot be solved analytically because the coefficients of the unknown functions are time-dependent. However, the solutions of these equations can be represented in a formal analytical form by using the method of time ordering operators from quantum field theory (Parisi[22]). The solution corresponding to the initial conditions A.7 can be written as

$$\tilde{\eta}_j(t';t) = \sum_{u=1}^{n} J_u(t')[\hat{\mathbf{T}} \exp[\int_{t'}^{t} \epsilon(t'')\, \mathrm{d}t'']]_{uj} \qquad (A.8)$$

where $\hat{\mathbf{T}}$ is the time-ordering chronological operator. The time-ordered exponential in eqs A.8 is actually a matrix of Green functions

$$G(t|t') = \hat{\mathbf{T}} \exp[\int_{t'}^{t} \epsilon(t'')\, \mathrm{d}t''] \qquad (A.9)$$

By combining eqs A.8−A.9 and coming back to the functions $\eta_j(\theta;t)$ obtain eqs 21. By direct differentiation of eq A.9 with respect to the time variable t it is easy to check that the matrix elements $G_{j'j}(t|t')$ fulfill the differential eqs 22 with the initial conditions 23.

Appendix B

Since the transit time θ is a nonnegative random variable, the characteristic function of the stationary probability density $\phi^{st}(\theta)$ can be defined as a Laplace transform

$$\bar{\phi}^{st}(s) = \int_0^{\infty} \exp(-s\theta)\phi^{st}(\theta)\, \mathrm{d}\theta \qquad (B.1)$$

According to their definition the cumulants $\langle\langle\theta^m\rangle\rangle^{st}$, $m = 1, 2, \ldots$ of the distribution $\phi^{st}(\theta)$ can be evaluated from the coefficients of a Taylor series expansion of $\ln \bar{\phi}^{st}(s)$ in s:

$$\ln \bar{\phi}^{st}(s) = \sum_{m=1}^{\infty} \frac{(-s)^m}{m!} \langle\langle\theta^m\rangle\rangle^{st} \qquad (B.2)$$

that is

$$\langle\langle\theta^m\rangle\rangle^{st} = (-1)^m[\mathrm{d}^m \ln \bar{\phi}^{st}(s=0)/\mathrm{d}s^m] \qquad (B.3)$$

The Laplace transform of the distribution 50 is given by

$$\bar{\phi}^{st}(s) = \frac{k_{+1}E^{st}k_{+2}}{s_2 + s(k_{-1} + k_{+2} + k_{+1}E^{st}) + k_{+1}E^{st}k_{+2}} = \frac{s_1 s_2}{(s - s_1)(s - s_2)} \qquad (B.4)$$

where

$$s_{1,2} = {}^1/_2[-(k_{-1} + k_{+2} + k_{+1}E^{st}) \pm [(k_{-1} + k_{+2} + k_{+1}E^{st})^2 - 4k_{+1}E^{st}k_{+2}]^{1/2}] \qquad (B.5)$$

By combining eqs B.3−B.5 after some calculations we obtain eqs 52.

References and Notes

(1) Meléndez-Hevia, E.; Torres, N.; Sicilia, J.; Kacser, H. *Biochem J.* **1990**, *265*, 195−202.
(2) Easterby, J. S. *Biochem. J.* **1981**, *199*, 155−161.
(3) Welch, G. R.; Easterby, J. S. *TIBS* **1994**, May 19, 193−197.
(4) Easterby, J. S. *Biochem. Biophys. Acta* **1973**, *293*, 552−558.
(5) Easterby, J. S. *Biochem. J.* **1984**, *219*, 843−847.
(6) Easterby, J. S. *Biochem. J.* **1986**, *233*, 871−875.
(7) Torres, N.; Souto, R.; Meléndez-Hevia, E. *Biochem. J.* **1989**, *260*, 763−769.
(8) Acerenza, L.; Sauro, H. M.; Kacser, H. *J. Theor. Biol.* **1989**, *137*, 423−444.
(9) Acerenza, L. In *Control of Metabolic Processes*; Cornish-Bowden, A., Cárdenas, M. L., Eds.; Plenum Press: New York, 1990; pp 297−302.
(10) Easterby, J. S. *Biochem. J.* **1990**, *269*, 255−259.
(11) Torres, N.; Meléndez-Hevia, E. *Mol. Cell. Biochem.* **1992**, *112*, 109−115.
(12) Easterby, J. S. *Biochem. J.* **1989**, *264*, 605−607.
(13) Easterby, J. S. *J. Mol. Recognit.* **1993**, *6*, 179−185.
(14) Rapp, P. *J. Exp. Biol.* **1979**, *81*, 281−306.
(15) Goldbeter, A. *Biochemical Oscillations and Cellular Rhythms*; Cambridge University Press: Cambridge, UK, 1996.
(16) Sochet, L. R. *La cinétique des reactions en chaînes*; Dunod: Paris, 1971; p 22.
(17) Vlad, M. O.; Popa, V. T. *Chem. Phys.* **1987**, *112*, 307−318.
(18) Haus, J. W.; Kehr, K. *Phys. Rep.* **1987**, *150*, 263.
(19) Smith, P. D.; Keyfitz, N. *Mathematical Demography*; Springer-Verlag: Berlin, 1977.
(20) Vlad, M. O.; Pop, A. *Physica* **1989**, *A155*, 276−310.
(21) Vlad, M. O.; Ross, J. *J. Chem. Phys.* **1994**, *100*, 7268−7278.
(22) Parisi, G. *Statistical Field Theory*; Addison-Wesley: Reading, MA, 1988.
(23) Kholodenko, B.; Demin, O. V.; Westerhoff, H. V. *J. Phys. Chem.* **1997**, *101*, 2070−2081.
(24) Landau, L.; Lifchits, E. M. *Mechanics*; Pergamon Press: Oxford, 1960.
(25) Iosifescu, M. *Stochastic Processes and Applications in Biology and Medicine*; Springer: Berlin, 1973; Vols 1−2.