Quantitative Interpretation of the Randomness in Single Enzyme Turnover Times

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ABSTRACT   Fluctuating turnover times of a single enzyme become observable with the advent of modern cutting-edge, single enzyme experimental techniques. Although the conventional chemical kinetics and its modern generalizations could provide a good quantitative description for the mean of the enzymatic turnover times, to our knowledge there has not yet been a successful quantitative interpretation for the variance or the randomness of the enzymatic turnover times. In this review, we briefly review several theories in this field, and compare predictions of these theories to the randomness parameter data reported for β-galactosidase enzyme. We find the recently proposed kinetics for renewal reaction processes could provide an excellent quantitative interpretation of the randomness parameter data. From the analysis of the randomness parameter data of the single enzyme reaction, one can extract quantitative information about the mean lifetime of enzyme-substrate complex; the success or the failure probability of the catalytic reaction per each formation of ES complex; and the non-Poisson character of the reaction dynamics of the ES complex (which is beyond reach of the long-standing paradigm of the conventional chemical kinetics).

INTRODUCTION

Reactivity fluctuations of individual enzymes are ubiquitous, and their effects on a series of single enzyme turnover times become observable with advances in single molecule experimental techniques (1–8). Recently, English et al. (3) performed a state-of-the-art single-molecule measurement of catalytic turnover times for individual β-galactosidase enzymes catalyzing the hydrolysis of resorufin-β-D-galacto-pyranoside (RGP), and reported the statistical distribution of the catalytic turnover times of the individual enzyme at several different concentrations of substrate RGP. The authors found that the dependence of the mean enzymatic turnover time $\langle t \rangle$ on the substrate concentration can be well explained by the Michaelis-Menten relation, which provides the same information as that extracted from the conventional analysis for the macroscopic enzyme reaction system (9).

In the single enzyme experiment, fluctuation of the enzymatic turnover times is an important observable, carrying valuable information that cannot be obtained from conventional experimental analysis (10–17). In English et al. (3), the authors also reported the randomness parameter, or the relative variance of enzymatic turnover time fluctuations, for the single enzyme system for various substrate concentrations. However, to our knowledge, there has not yet been any quantitative interpretation of the randomness parameter data reported in English et al. (3). In this review, we present some of the previous theories given for a quantitative interpretation of the fluctuation of enzymatic turnover times (15–19), and compare predictions of these theories to the randomness parameter data reported in English et al. (3).

We find that, for a successful quantitative analysis of the randomness parameter data of the enzyme reaction, it is necessary to go beyond the conventional enzyme kinetics in which each and every elementary reaction process composing the Michaelis-Menten enzyme reaction scheme, $E + S \rightleftarrows ES \rightarrow E + P$ (with E, S, and P being the enzyme, the substrate, and the product molecule), is a simple rate process or a Poisson process. The randomness parameter data could not be explained with the assumption that the reaction processes of enzyme-substrate (ES) complex are Poisson processes (15,16). Previously, a generalization of the conventional enzyme kinetics was proposed for a quantitative description of the statistical distribution of the enzymatic turnover times, in which the catalytic reaction rate of the ES complex is assumed to be statically heterogeneous and distributed according to a certain probability distribution (3,17). However, we find that the behavior of the randomness parameter predicted by the latter approach is qualitatively different from that of the experimental randomness parameter data.

The randomness parameter data of the enzyme reaction could be quantitatively explained by the recently proposed kinetics for renewal reaction processes that are possibly non-Poisson stochastic processes (18,19). The generalization of the conventional chemical kinetics into the kinetics for renewal reaction processes is reminiscent of the generalization of the usual random walk model into the continuous-time random walk model for a general description of molecular transport in a dynamically heterogeneous environment (20,21). The mean enzymatic turnover time predicted by the renewal chemical kinetics is equivalent to that predicted by the conventional chemical kinetics (14,18). However, the prediction of the renewal chemical kinetics for the fluctuation of the single enzyme reaction times is

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much different from that of the conventional chemical kinetics whenever the stochastic properties of enzyme's reaction processes deviate from those of Poisson processes (19).

To begin, we present a review of the straightforward application of the conventional chemical kinetics for description of statistical distribution, $\psi(t)$, of enzymatic turnover times for the Michaelis-Menten scheme (15,16), which is then followed by reviews of a couple of modern theories that aim for quantitative description of the single enzyme turnover-time distribution (3,17–19). After the review of the theories, direct comparison is made between the predictions of these theories and the experimental data for the dependence of the randomness parameter on the substrate concentration.

CONVENTIONAL CHEMICAL KINETICS

A straightforward application of the conventional chemical kinetics to the single enzyme catalytic reaction,
$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\longrightarrow} E + P$, yields
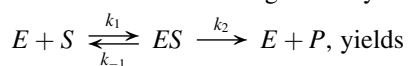
$$\frac{dP_{E+S}(t)}{dt} = -k_1[S]P_{E+S}(t) + k_{-1}P_{ES}(t), \qquad (1a)$$

$$\frac{dP_{ES}(t)}{dt} = k_1[S]P_{E+S}(t) - k_{-1}P_{ES}(t) - k_2P_{ES}(t), \qquad (1b)$$

$$\frac{dP_{E+P}(t)}{dt} = k_2P_{ES}(t), \qquad (1c)$$

where $P_J(t)$ denotes the probability that the single enzyme system is in chemical state J at time t (15,16,22). The initial condition associated with Eq. 1 is $P_{E+S}(0) = 1$, $P_{ES}(0) = P_{E+P}(0) = 0$. The values k_1, k_{-1}, and k_2, respectively, denote the rate constant for the association reaction $E + S \rightarrow ES$ of substrate molecules to the enzyme, that for the dissociation reaction $E + S \leftarrow ES$, and that for the catalytic reaction $ES \rightarrow E + P$ of ES complex. The value $[S]$ denotes the number density of substrate molecules in the system. We assume that the number of substrate molecules in the system is so large that $[S]$ does not change in time appreciably during the repeated measurements of fluctuating turnover times of a single enzyme. The solution of Eq. 1 can be easily obtained and is well known (23).

The statistical distribution $\psi(t)$ of the time elapsed for completion of a single enzyme catalytic reaction is related to $P_{E+P}(t)$ by $P_{E+P}(t) = \int_0^t d\tau\, \psi(\tau)$. Therefore, the enzymatic turnover-time distribution $\psi_C(t)$ in the conventional chemical kinetics is given by

$$\psi_C(t) = \frac{d}{dt}P_{E+P}(t) = k_2P_{ES}(t). \qquad (2)$$

Substituting the solution of Eq. 1 into Eq. 2, one gets the analytic expression for $\psi_C(t)$ as

$$\psi_C(t) = \frac{\alpha\beta}{\beta - \alpha}\left(e^{-\alpha t} - e^{-\beta t}\right). \qquad (3)$$

Here α and β are time-independent constants given by $\alpha = 2^{-1}(\lambda - \sqrt{\lambda^2 - 4\xi})$ and $\beta = 2^{-1}(\lambda + \sqrt{\lambda^2 - 4\xi})$, with λ and ξ being $\lambda = k_1[S] + k_{-1} + k_2$ and $\xi = k_2k_1[S]$, respectively.

The mean, $\langle t \rangle_C (\equiv \int_0^\infty dt\, t\psi_C(t))$, of the enzymatic turnover-time distribution $\psi_C(t)$ in Eq. 3 is

$$\langle t \rangle_C = \langle t \rangle_{\min}\left(1 + \frac{K_M}{[S]}\right), \qquad (4)$$

where $\langle t \rangle_{\min}$ and K_M are the mean turnover time in the high substrate concentration limit and the Michaelis-Menten constant given by $\langle t \rangle_{\min} = k_2^{-1}$ and $K_M = k_{-1} + k_2/k_1$. The randomness parameter Q for an enzymatic turnover-time distribution $\psi(t)$ is defined by

$$Q = \frac{\langle t \rangle^2 - \langle t \rangle^2}{\langle t \rangle^2} - 1, \qquad (5)$$

where $\langle t^n \rangle$ is the nth moment of the enzymatic turnover-time distribution $\psi(t)$, i.e., $\langle t^n \rangle = \int_0^\infty t^n\psi(t)dt$. The expression for the randomness parameter for the enzymatic turnover-time distribution $\psi_C(t)$ given in Eq. 3 can be obtained as (15,17)

$$Q_C = -\frac{2p_2^0 x}{(1 + x)^2}, \qquad (6)$$

where p_2^0 and x are given by $p_2^0 = k_2/(k_{-1} + k_2)$ and $x = [S]/K_M$, respectively. Note that the randomness parameter predicted by the conventional chemical kinetics assumes a negative value for the whole range of substrate concentrations, which is inconsistent with the positive randomness parameter data for β-galactosidase reported in English et al. (3).

STATICALLY HETEROGENEOUS ENZYME REACTION KINETICS

A generalization of the conventional chemical kinetics that predicts a positive randomness parameter is made in English et al. (3) and Kou et al. (17) in which a probability distribution $w(k_2)$ of the rate coefficient k_2 of the catalytic reaction of the ES complex is assumed, and the following form of enzymatic turnover-time distribution is suggested:

$$\psi_{GC}(t) = \int dk_2 w(k_2)\psi_C(t). \qquad (7)$$

Noting that the nth moment $\langle t^n \rangle_{GC}(\equiv \int_0^\infty t^n\psi_{GC}(t)dt)$ of $\psi_{GC}(t)$ is given by $\langle t^n \rangle_{GC} = \int dk_2 w(k_2)\langle t^n \rangle_C$, with $\langle t^n \rangle_C$ being the nth moment of $\psi_C(t)$ given in Eq. 3, one can recover Eq. 4 for the mean $\langle t \rangle_{GC}$ of the enzymatic turnover

distribution $\psi_{GC}(t)$ with K_M in Eq. 4 being replaced by $K_M^{GC} = (k_{-1} + \langle k_2^{-1} \rangle^{-1}_w)/k_1$. Hereafter, $\langle k_2^{-n} \rangle_w$ is given by $\langle k_2^{-n} \rangle_w = \int dk_2 w(k_2) k_2^{-n}$. We obtain the expression of the randomness parameter Q_{GC} associated with $\psi_{GC}(t)$ as

$$Q_{GC} = 2 \left[\frac{\sigma^2_{k_2^{-1}}}{\langle k_2^{-1} \rangle^2} \frac{(1 + p_{-1}^{GC} x)^2}{(1+x)^2} - \frac{p_2^{GC} x}{(1+x)^2} \right]. \quad (8)$$

Here, $\sigma^2_{k_2^{-1}}$ and x, respectively, denote the variance of k_2^{-1} and the substrate concentration in unit of K_M^{GC}, i.e., $\sigma^2_{k_2^{-1}} = \langle k_2^{-2} \rangle - \langle k_2^{-1} \rangle^2$ and $x = [S]/K_M^{GC}$. p_{-1}^{GC} and p_2^{GC} in Eq. 8 are defined by $p_{-1}^{GC} \equiv k_{-1}/(k_{-1} + \langle k_2^{-1} \rangle^{-1})$ and $p_2^{GC} \equiv \langle k_2^{-1} \rangle^{-1}/(k_{-1} + \langle k_2^{-1} \rangle^{-1})$. The parameter Q_{GC} in Eq. 8 correctly reduces to Q_C in Eq. 6 in the absence of fluctuation of k_2, or in the small $\sigma^2_{k_2^{-1}}$ limit. However, for any distribution $w(k_2)$ of k_2 with a finite variance, Q_{GC} deviates from Q_C. Note that Q_{GC} can assume a positive value whereas Q_C cannot; in this sense, Q_{GC} provides a better description for the positive randomness parameter data of the β-galactosidase enzyme investigated in English et al. (3). However, the behavior of Q_{GC} given in Eq. 8 is qualitatively different from the experimental data, which will be discussed in more detail shortly.

KINETICS FOR A RENEWAL REACTION PROCESS

Recently, a new type of chemical kinetics is developed for description of a single molecule reaction composed of possibly non-Poisson elementary reaction processes (18,19). In this approach, the turnover-time distribution $\psi(t)$ of the single enzyme reaction is represented in terms of the reaction time distributions, $\phi_1^0(t)$, $\phi_{-1}(t)$, and $\phi_2(t)$ for the three elementary reaction processes, $E + S \xrightarrow{\phi_1^0(t)} ES$, $E + S \xleftarrow{\phi_{-1}(t)} ES$, and $ES \xrightarrow{\phi_2(t)} E + P$. The reaction time distribution (RTD) for each of the elementary reactions represents the probability density of the time elapsed for a completion of the elementary reaction process. The precise definition of $\phi_1^0(t)dt$ is the probability that the enzyme-substrate association reaction, $E + S \rightarrow ES$, is completed in time interval $(t, t + dt)$, given that the reaction begins at time 0. Here, the superscript 0 in $\phi_1^0(t)$ signifies that the RTD of the enzyme-substrate encounter process is normalized, i.e., $\int_0^\infty d\tau \, \phi_1^0(\tau) = 1$.

On the other hand, $\phi_{-1(2)}(t)dt$ denotes the probability that the dissociation (catalytic) reaction of ES complex is completed in time interval $(t, t + dt)$, given that the ES complex is prepared at time 0. In contrast to $\phi_1^0(t)$, the terms $\phi_{-1}(t)$ or $\phi_2(t)$ for the dissociation or catalytic reaction of the ES complex do not satisfy the normalization condition; instead, $\int_0^\infty \phi_{-1}(t)dt$ and $\int_0^\infty dt\phi_2(t)$ are the probability p_{-1} of dissociation and the probability p_2 of catalytic reaction of the ES complex, respectively, so that their sum is normalized, i.e., $\int_0^\infty dt[\phi_{-1}(t) + \phi_2(t)] = 1$. The relation of the enzymatic turnover-time distribution ψ to the reaction time distributions, ϕ_1^0, ϕ_{-1}, and ϕ_2, of the elementary reaction processes is given in Laplace domain as

$$\widehat{\psi}(u) = \frac{\widehat{\phi}_1^0(u)\widehat{\phi}_2(u)}{1 - \widehat{\phi}_1^0(u)\widehat{\phi}_{-1}(u)}. \quad (9)$$

In Eq. 9, $\widehat{f}(u)$ denotes the Laplace transform of $f(t)$, defined by $\widehat{f}(u) = \int_0^\infty dt \exp(-ut)f(t)$.

When the elementary reaction processes composing the enzyme reaction are Poisson processes, $\psi(t)$, the Laplace transform of which is given in Eq. 9, reduces to $\psi_C(t)$. The RTD, $\phi_1^0(t)$, of the Poisson enzyme-substrate encounter process $(E + S \xrightarrow{k_1} ES)$ is given by $\phi_1^0(t) = k_1[S]\exp(-k_1[S]t)$. The RTD, $\phi_{-1}(t)$ and $\phi_2(t)$ for the dissociation $(E + S \leftarrow ES)$ and the catalytic reaction $(ES \rightarrow E + P)$ of the ES complex, are given by $\phi_{-1}(t) = \phi_{-1}^0(t) \int_t^\infty d\tau \phi_2^0(\tau)$ and $\phi_2(t) = \phi_2^0(t) \int_t^\infty d\tau \phi_{-1}^0(\tau)$, respectively (21), where $\phi_{-1/(2)}^0(t)$ denotes the normalized one-channel reaction-time distribution for the dissociation (catalytic reaction) of the ES complex in the absence of the competing catalytic reaction (dissociation). When both the dissociation and the catalytic reaction of the ES complex are Poisson processes, $\phi_{-1}^0(t)$ and $\phi_2^0(t)$ are given by $k_{-1}\exp(-k_{-1}t)$ and $k_2 \exp(-k_2t)$, respectively; therefore, $\phi_{-1}(t)$ and $\phi_2(t)$ become $\phi_{-1}(t) = k_{-1}\exp[-(k_{-1} + k_2)t]$ and $\phi_2(t) = k_2 \exp[-(k_{-1} + k_2)t]$. Substituting $\widehat{\phi}_1^0(u) = k_1[S]/(u + k_1[S])$, $\widehat{\phi}_{-1}(u) = k_{-1}/(u + k_{-1} + k_2)$, and $\widehat{\phi}_2(u) = k_2/(u + k_{-1} + k_2)$ into Eq. 9, one gets

$$\widehat{\psi}(u) = \frac{\xi}{u^2 + \lambda u + \xi}, \quad (10)$$

where λ and ξ are given by $\lambda = k_1[S] + k_{-1} + k_2$ and $\xi = k_2 k_1[S]$. The inverse Laplace transform of $\widehat{\psi}(u)$ given in Eq. 10, $\psi(t)$, is equal to $\psi_C(t)$ predicted by the conventional chemical kinetics.

In the single enzyme reaction, the enzyme-substrate encounter process may be approximated as a simple Poisson process in the steady state. However, the dissociation or the catalytic reaction of the ES complex may not be a Poisson process, as the reactivity of the ES complex is dynamically fluctuating in line with the conformational dynamics of the ES complex (24). For a given conformation of the ES complex, the reaction of the ES complex can still be a non-Poisson process when the substrate or product escape process out of the enzyme molecule is a complex one involving a number of different intermediate states and multiple reaction channels (19). Depending on the microscopic reaction dynamics of the ES complex, the functional form for $\phi_{-1}(t)$ and $\phi_2(t)$ can be various. However, it is possible to obtain the expression for the mean $\langle t \rangle$ and the randomness parameter Q of enzymatic turnover-time distribution $\psi(t)$ without assuming a particular functional form

for $\phi_{-1}(t)$ and $\phi_2(t)$. The expression for the mean of enzymatic turnover-time distribution $\psi(t)$ conforms to the conventional MM equation, Eq. 4, which is given by (18,19)

$$\langle t \rangle = \frac{\overline{n}+1}{k_1[S]} + \overline{n}\langle t_{-1} \rangle + \langle t_2 \rangle. \quad (11)$$

Here $\overline{n}$ is the average number of dissociation event per each enzymatic turnover given by $\overline{n} = p_{-1}/p_2$ with $p_{-1(2)}$ being the reaction probability, $\int_0^\infty dt\phi_{-1(2)}(t)$, of ES complex for the dissociation (catalytic) reaction, $ES \rightarrow E + S(P)$. In Eq. 11, $\langle t_{-1(2)} \rangle$ denotes the mean dissociation (catalytic) reaction time of the ES complex in the presence of the competing catalytic (dissociation) reaction, defined by $\int_0^\infty dt\, t\phi_{-1(2)}(t)/p_{-1(2)}$. From the comparison between Eqs. 4 and 11, K_M and $\langle t \rangle_{\min}$ can be identified as $K_M = (k_1\langle t_{ES} \rangle)^{-1}$ and $\langle t \rangle_{\min} = \langle t_{ES} \rangle/p_2$, where $\langle t_{ES} \rangle$ is the mean lifetime of the ES complex, defined by $\langle t_{ES} \rangle = p_{-1}\langle t_{-1} \rangle + p_2\langle t_2 \rangle$. In comparison, the expression for the randomness parameter Q calculated from Eq. 9 is much different from Q_C or Q_{GC} (19):

$$Q = Q_\infty \frac{x(x-\eta)}{(x+1)^2}. \quad (12)$$

Here Q_∞, η, and x are given by $Q_\infty = p_2 q_{ES}/\langle t_{ES} \rangle^2$, $\eta = 2p_2\langle t_2 \rangle/(Q_\infty\langle t_{ES} \rangle)$, and $x = [S]/K_M$ with q_{ES} being defined by $q_{ES} = p_2(\langle t_2^2 \rangle) - 2\langle t_2 \rangle^2) + p_{-1}(\langle t_{-1}^2 \rangle - 2\langle t_{-1} \rangle\langle t_2 \rangle)$. The value q_{ES} is the parameter representing the stochastic property of the reactions of the ES complex. When the dissociation and the catalytic reaction processes of the ES complex are Poisson processes, q_{ES} vanishes and Q in Eq. 12 reduces to Q_C in Eq. 6. It is known that q_{ES} appearing in Q_∞ of Eq. 12 assumes a positive value if the reaction processes of ES complex is a generalized Poisson process of which rate coefficient, $k_{-1}(\mathbf{r})$ or $k_2(\mathbf{r})$, is dependent on microscopic configuration $\mathbf{r}$ of the ES complex. On the other hand, q_{ES} assumes a negative value when the reaction of ES complex is a multistep reaction composed of consecutive Poisson reaction processes, $ES \rightleftharpoons I_1 \rightleftharpoons \cdots \rightleftharpoons I_n \rightarrow E + S(P)$, with I_k being the kth intermediate state during the reaction of ES complex (19).

Equations 11 and 12 for the mean turnover time and the randomness parameter hold regardless of whether the normalized reaction time distribution $\phi_{-1}(t)/p_{-1}$ for the dissociation reaction of ES complex is the same as $\phi_2(t)/p_2$ for the catalytic dissociation reaction. That is to say, in the analysis of the mean turnover time and the randomness parameter, one can map the model considered above into the simpler model in which $\phi_{-1}(t)/p_{-1}$ is the same as $\phi_2(t)/p_2$. For the latter model, the physical interpretation of q_{ES} and ηQ_∞ in Eq. 12 becomes simpler; q_{ES} is the variance $\langle t_{ES}^2 \rangle - \langle t_{ES} \rangle^2$ of the conformation-dependent mean lifetime of the ES complex and ηQ_∞ is twice the success probability p_2 of the catalytic reaction of the ES complex. From the analysis of the experimental randomness parameter data along

with the mean turnover time data with use of the latter model, one can separately extract values of the physical parameters p_2, $\langle t_{ES} \rangle$, $\langle t_{ES}^2 \rangle - \langle t_{ES} \rangle^2$, and k_1 of the enzyme reaction system.

COMPARISON TO EXPERIMENTAL RANDOMNESS PARAMETER DATA

In Fig. 1, we make a direct comparison between the predictions of the above-mentioned theories for the randomness parameter and the experimental randomness parameter data reported in English et al. (3). The parameter Q_C given in Eq. 6 yields a negative value for any substrate concentration, inconsistent with the experimental randomness parameter data. In comparison, Q_{GC} in Eq. 8 yields a positive value for the randomness parameter. However, as shown in Fig. 1, the dependence of Q_{GC} on substrate concentration appears much different from the experimental randomness parameter data. The randomness parameter Q_{GC} calculated from $\psi_{GC}(t)$ looks nearly constant at all substrate concentrations investigated in English et al. (3), whereas the experimental randomness parameter data exhibit a strongly nonlinear behavior. Particularly, the behavior of the randomness parameter Q_{GC} predicted by $\psi_{GC}(t)$ is qualitatively different from the experimental data in the low substrate concentration regime. The parameter Q_{GC} yields the following
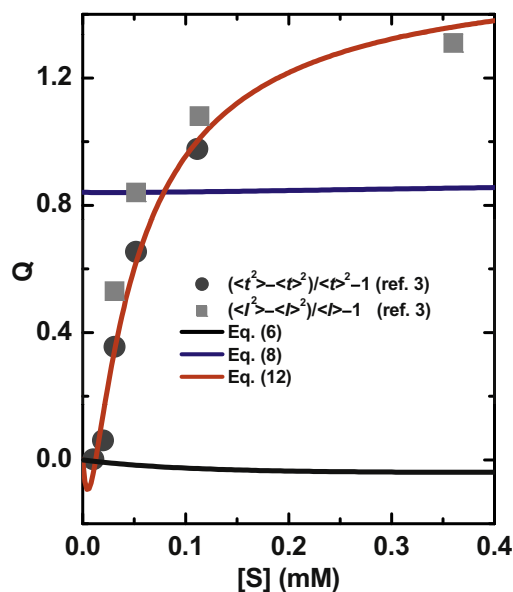

FIGURE 1 Comparison between the results of the previously reported theories and the experimental data for the randomness parameter data of enzymatic turnover times. (*Solid circles* and *solid squares*) Randomness parameter data reported in English et al. (3) for β-galactosidase catalyzing the hydrolysis of RGP. (*Black line*) The result, Eq. 6, of the conventional chemical kinetics, which is always negative (15,16). (*Blue line*) The result, Eq. 8, obtained from the enzymatic turnover-time distribution proposed in English et al. (3) and Kou et al. (17). (*Red line*) The result, Eq. 12, of the single molecule kinetics for non-Poisson renewal reaction processes (18,19).

expression for the randomness parameter Q_0 in the low substrate concentration limit,

$$Q_0 \left(\equiv \lim_{[S] \to 0} \frac{\langle t^2 \rangle - \langle t \rangle^2}{\langle t \rangle^2} - 1 \right) = \frac{2\left(\langle k_2^{-2} \rangle_w - \langle k_2^{-1} \rangle_w^2 \right)}{\langle k_2^{-1} \rangle_w^2}, \quad (13)$$

which could not vanish for any probability density function $w(k_2)$ with a finite variance. This fact indicates that $\psi_{GC}(t)$ cannot be the correct enzymatic turnover-time distribution of the β-galactosidase enzyme investigated in English et al. (3), for any choice of $w(k_2)$. In producing the curves for Q_{GC} in Fig. 1, the values of the adjustable parameters and the functional form of $w(k_2)$ are chosen as given in English et al. (3).

As a matter of fact, $\psi_{GC}(t)$ is the exact enzymatic turnover-time distribution for such statically heterogeneous enzymes in which each enzyme has constant values for rate constants, k_1, k_{-1}, and k_2 throughout the experiment but the value of k_2 is different from enzyme to enzyme, distributed over the enzymes according to $w(k_2)$. In the latter system, each enzyme has a turnover-time distribution $\psi_C(t)$ with different values of k_2 from each other, and the average of the turnover-time distribution over the enzymes with equal weight for every enzyme results in $\psi_{GC}(t)$. However, the behavior of the randomness parameter data reported in English et al. (3) is inconsistent with that of the statically heterogeneous enzyme model.

We find that Q given in Eq. 12 provides an excellent quantitative description of the randomness parameter data unless we set the value of the MM constant, K_M, to be the same as that reported in English et al. (3). (Note also that the value of K_M reported in English et al. (3) is 380 mM. With use of the latter value of K_M, Eq. 12 could not provide a good quantitative description of the randomness parameter data. See Fig. 5 of Jung et al. (19).) The values of the extracted parameters are given by $Q_\infty = 1.57$ and $\eta = 0.624$, and $K_M \cong 20\,\mu M$, which yield $p_2 \cong 0.49$, and $(\langle t^2_{ES} \rangle - \langle t_{ES} \rangle^2)/\langle t_{ES} \rangle^2 \cong 3.2$. Using the value of $1/\langle t \rangle_{min}$ as $730\,s^{-1}$, extracted from the mean turnover-time analysis in English et al. (3), we can determine the value of the mean lifetime $\langle t_{ES} \rangle$ of the ES complex: $\langle t_{ES} \rangle = p_2 \langle t_{min} \rangle = 0.67$ ms. In addition, the value of the bimolecular rate coefficient k_1 associated with the enzyme-substrate encounter reaction can be obtained from the MM constant by $k_1 = 1/(K_M \langle t_{ES} \rangle) \cong 7.46 \times 10^7\,M^{-1}\,s^{-1}$.

We finish by noting that the single enzyme reaction is not really a renewal process, as the turnover-time distribution of an enzyme would change in time in line with thermal fluctuations of the enzyme's conformation with a wide range of timescales. For this reason, the enzymatic turnover times are correlated, and the renewal kinetics would not be enough for a quantitative description of the turnover-events' counting statistics or the probability $P_m(T)$ where we observe m enzymatic turnover reactions in observation time T (11,12,14). (For a renewal process, the randomness parameter Q in this review is the same as Mandel's Q parameter, which has been analyzed in the context of photon statistics; see Barkai et al. (25). The randomness parameter Q can be different from Mandel's Q parameter for nonrenewal processes such as a single enzyme reaction.) However, when the observation time is much longer than the conformational relaxation time of the ES complex, the distribution of single enzymatic turnover times can be described by Eq. 9 (19). For a particular realization of a single enzymatic turnover, the enzymatic turnover time t is given by $t = t_1 + n(t_{-1} + t_1) + t_2$, where t_1, t_{-1}, and t_2, respectively, denote the reaction times associated with $E + S \to ES$, $E + S \leftarrow ES$, and $ES \to E + P$, and n denotes the number of dissociation-association cycles realized during the single enzymatic turnover.

As long as the probability density functions of reaction times, t_{-1} and t_2, of the ES complex are independent of the number, n, of dissociation-association cycles in the single enzymatic turnover, the joint probability $\psi_n(t)dt$ that the single enzymatic turnover time lies between t and $t + dt$ and n cycles of dissociation-association reactions occur during the single enzymatic turnover can be represented as $\widehat{\psi}_n(u) = \widehat{\phi}_1^0(u)[\widehat{\phi}_{-1}(u)\widehat{\phi}_1^0(u)]^n\widehat{\phi}_2(u)$ in the Laplace domain for any value of n. Note that $\int_0^\infty dt\psi_n(t) = \widehat{\psi}_n(0) = (1 - p_2)^n p_2$ is nothing but the probability that the ES complex suffers n cycles of dissociation-association reactions during a single enzymatic turnover. Note, in addition, that $\sum_{n=0}^\infty \psi_n(t)$ yields the single enzymatic turnover-time distribution for which the Laplace transform is given in Eq. 9.

This result indicates that the only assumption involved in Eq. 9 is that probability density functions of reaction times, t_{-1} and t_2, of the ES complex are independent of the number, n, of dissociation-association cycles suffered by the ES complex in a single enzymatic turnover. The significant assumption in the derivation of Eq. 12 from Eq. 9 is that the substrate-enzyme association reaction has a constant steady-state reaction rate, which is widely accepted and also assumed in English et al. (3). Nevertheless, when the substrate concentration is low enough, the latter assumption may not hold and the substrate-enzyme association may not be a Poisson process. Effects of the non-Poisson substrate-enzyme association reaction on the mean and the randomness parameter of single enzyme turnover times will be discussed elsewhere shortly.

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