J Math Chem (2011) 49:1949–1960
DOI 10.1007/s10910-011-9868-6

ORIGINAL PAPER

Enzyme kinetics with a twist

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Received: 19 May 2011 / Accepted: 13 June 2011 / Published online: 26 June 2011
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Abstract A different approach to enzyme kinetics stressing the cyclic nature of the catalytic process is presented. The time-dependence of the substrate concentration is derived in a simple way not invoking the quasi-steady-state approximation. According to this approach the turnover rate can be written as the ratio of two parameters with a direct meaning: enzyme efficiency and average cycle duration. Real kinetic data for two enzyme-substrate pairs is used to show that the enzyme kinetic efficiency is best measured by the turnover rate.

Keywords Enzyme kinetics · Quasi-steady-state approximation · Lambert function

1 Introduction

Enzyme E and substrate S (usually in large excess) associate by a fast bimolecular reaction ($k_a \approx 10^7$ to $10^{10}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$) to form the enzyme-substrate complex ES. The substrate is then transformed into product P and the complex finally dissociates releasing the product and regenerating the enzyme. However, the reaction is in general reversible, and the complex can also dissociate back to give free substrate and free enzyme. All these elementary steps are condensed in the Henri-Michaelis-Menten (HMM) kinetic scheme [1,2], which is the simplest description of enzyme action:

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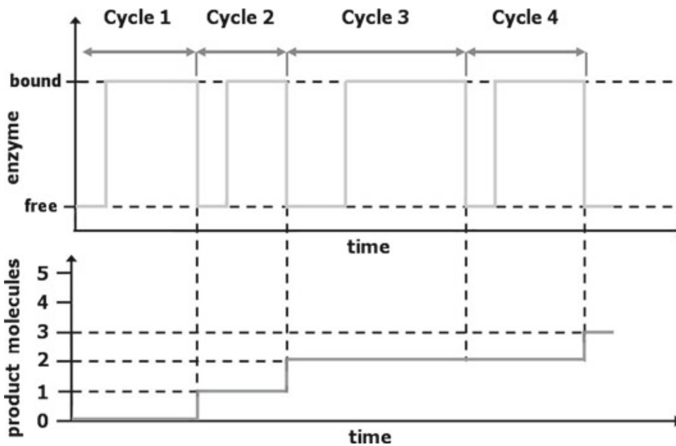
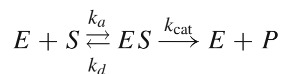


Fig. 1 A single enzyme molecule alternates between free (E) and bound (ES) states. At the end of each cycle, a product molecule may or may not be generated. In this example the third cycle is unsuccessful as the complex dissociates back into the reactants. The process is inherently stochastic, and only the probability of generating a product molecule after each cyle is known for each enzyme-substrate pair under specific conditions. Note that the durations of the free and bound states, and hence of each cycle, are also random variables



$$E + S \underset{k_d}{\overset{k_a}{\rightleftarrows}} ES \xrightarrow{k_{\text{cat}}} E + P$$

In this scheme, reversibility of the second step is neglected, and also the necessary existence of a second complex EP is not accounted for explicitly. If attention is focused on a single enzyme molecule, the process is inherently stochastic and the elementary kinetic steps can depicted as shown in Fig. 1.

An important quantity defining enzyme activity is the turnover rate r_t [1,2],

$$r_t = \frac{1}{[E]_0}\frac{d[P]}{dt}, \tag{1}$$

where $[E]_0 = [E] + [ES]$. The turnover rate is the number of product molecules generated per unit time and per enzyme molecule present. This quantity is especially meaningful when the enzyme concentration is much lower than that of substrate, which is a common situation.

In this work, a different approach to enzyme kinetics stressing the cyclic nature of the catalytic process is developed. The time-dependence of the substrate concentration is derived in a simple way not invoking the quasi-steady-state approximation. It is also shown that the enzyme kinetic efficiency is best measured by the turnover rate.

2 The basic equations

The differential equations of the HMM mechanism relating the concentrations of the four species present in a batch reactor are:

$$\frac{d[S]}{dt} = -k_a [E][S] + k_d [ES], \tag{2}$$
$$\frac{d[ES]}{dt} = k_a [E][S] - (k_{\text{cat}} + k_d)[ES], \tag{3}$$
$$\frac{d[E]}{dt} = -k_a [E][S] + (k_{\text{cat}} + k_d)[ES], \tag{4}$$
$$\frac{d[P]}{dt} = k_{\text{cat}} [ES]. \tag{5}$$

These equations embody the two mass conservation equations

$$[E]_0 = [E] + [ES], \tag{6}$$
$$[S]_0 = [S] + [ES] + [P]. \tag{7}$$

The state of the system is thus defined by the concentrations of any two species. If the mass conservation equations are used, only two differential equations are needed. Suitable additional assumptions for a batch reactor are $[ES]_0 = 0$ and $[P]_0 = 0$. The kinetics is thus controlled by five parameters: Two initial concentrations, $[S]_0$, $[E]_0$, and three rate constants, k_a, k_d, and k_{cat}.

No analytical solution is known for the HMM kinetics, that is, the system of two first-order differential equations has not been solved to yield expressions for the concentrations of all species in closed form, i.e., in terms of known functions. Recently, a general solution was nevertheless obtained in terms of power series expansions [3]. The system of differential equations can also be readily integrated numerically [3], but at the expense of providing numerical values for all parameters (initial concentrations and rate constants) and thus losing in part the general view of the functional dependence on the parameters. For this reason approximate analytical equations are of considerable interest.

3 Quasi-steady-state approximation

The traditional approach to HMM kinetics, pioneered by Briggs and Haldane [1,2], has been to reduce the system of two first-order differential equations to a single first-order differential equation by using the quasi-steady-state approximation (QSSA), according to which one of the differential equations, Eq. 3 or 4, is converted into an algebraic equation by equating the time derivative to zero. In this treatment, based on observations corresponding to an experimentally meaningful but limited region of parameter space, it is assumed that after a short period (transient kinetics) the concentration of enzyme-substrate complex *ES* is approximately constant, and that this in turn implies a time derivative of [*ES*] that is precisely zero. This reasoning is clearly questionable without further discussion (an obvious counter-example is a fast oscillating concentration not sensibly departing from a constant average value).

The QSSA converts Eqs. 3 and 4 in a simple algebraic equation, and the turnover rate becomes [1,2]

$$r_t = \frac{k_a k_{\text{cat}}[S]}{k_d + k_{\text{cat}} + k_a[S]} = \frac{k_{\text{cat}}[S]}{K_m + [S]}, \tag{8}$$

where K_m is the Michaelis constant,

$$K_m = \frac{k_d + k_{\text{cat}}}{k_a}. \tag{9}$$

Within the QSSA approximation, the Michaelis constant defines the fraction of bound enzyme for a given substrate concentration

$$\frac{[ES]}{[E] + [ES]} = \frac{[S]}{K_m + [S]}. \tag{10}$$

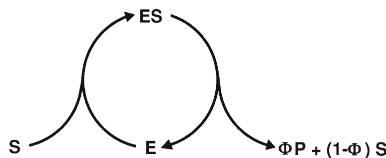
If $[S] \gg K_m$, then practically all enzyme exists in bound form, saturation is reached, and the turnover rate attains its maximum value, k_{cat}, also called the turnover number [1,2]. For $[S] = K_m$ the turnover rate is half of the maximal value. Typical turnover numbers are 100 to $1,000\,\text{s}^{-1}$, with maximum values of about $10^6\,\text{s}^{-1}$. Note that $\frac{k_{\text{cat}}}{K_m} = \frac{k_a}{1+\frac{k_d}{k_{\text{cat}}}} < k_a$ and therefore this ratio gives a minimum value estimate for k_a.

The concentration of enzyme-substrate complex is in fact never constant, although it may change very slowly. The QSSA only holds exactly at a precise time, when the *ES* concentration attains its maximum value, and the initial arguments invoked for its use were qualitative. Only relatively recently were the limits of applicability of the QSSA to HMM kinetics thoroughly investigated, see e.g. [3–7]. In the following, a simple approach leading to the same results, but without the need to invoke an approximation based on a questionable argument, is presented.

4 A different approach to enzyme kinetics

4.1 Catalytic cycles

Enzyme activity can be advantageously depicted as a simple catalytic cycle,



Reversibility is incorporated in the diagram by assigning an effective stoichiometric coefficient Φ to the product. This coefficient, which takes values between 0 and 1, is the so-called enzyme efficiency, i.e., the product yield per cycle, given by [8]

$$\Phi = \frac{k_{\text{cat}}}{k_d + k_{\text{cat}}} = \frac{k_{\text{cat}}}{k_a K_m}. \tag{11}$$

Enzymes for which $k_d \ll k_{\text{cat}}$ have a Φ close to unity and are called super-efficient [9]. For these enzymes the reaction is nearly diffusion-controlled ($r_t = k_a[S]$) for $[S] \ll K_m$. For enzymes with $\Phi \ll 1$ the kinetic situation corresponds to a pre-equilibrium between reactants and enzyme-substrate complex, and K_m reduces to the equilibrium constant for the dissociation reaction. This is the case originally considered by Michaelis and Menten [1,2]. The inverse of Φ, $\bar{n}_P$, is the average number of cycles required for conversion of one substrate molecule into one product molecule,

$$\bar{n}_P = \frac{1}{\Phi} = 1 + \frac{k_d}{k_{\text{cat}}}. \tag{12}$$

The product yield per cycle is an important parameter for characterizing enzyme activity, but it does not tell at which frequency catalytic cycles proceed. What is then the duration (or period) of a catalytic cycle? This duration is statistical (see Fig. 1) and has an average value τ_c given by [8]

$$\tau_c = \frac{1}{k_a[S]} + \frac{1}{k_d + k_{\text{cat}}} = \frac{1}{k_a}\left(\frac{1}{[S]} + \frac{1}{K_m}\right), \tag{13}$$

see "Appendix A". The cycle duration is the sum of two terms corresponding to the two halves of the catalytic cycle: The first term, $1/(k_a[S])$, is the average duration (or lifetime) of the enzyme in the free state. This duration is inversely proportional to the free substrate concentration. The second term, $1/(k_d + k_{\text{cat}})$, is the average duration (or lifetime) of the enzyme-substrate complex, and is independent of the concentration of substrate.

Using Eqs. 11 and 13 the turnover rate can be rewritten in a simple way [8],

$$r_t = \frac{\Phi}{\tau_c}. \tag{14}$$

The meaning of Eq. 14 is clear: The turnover rate is the number of successful $E \rightarrow ES \rightarrow E$ cycles per unit time and per enzyme molecule. Increase of the substrate concentration does not change the yield of product formation per cycle Φ, but reduces the average cycle duration τ_c, as the free enzyme lasts less time before forming a complex with the substrate. However, above a certain substrate concentration (saturation conditions, $[S] \gg K_m$), the cycle duration attains a constant, minimum value, $1/(k_d + k_{\text{cat}})$, controlled by the complex lifetime, and therefore the turnover rate reaches its maximal value k_{cat}.

Equation (14) also shows that for a given substrate concentration, a super-efficient enzyme may have a turnover rate lower than that of a less efficient one, as the turnover rate is controlled by both the yield of product formation per cycle Φ and the cycle duration τ_c. In kinetic terms it is the turnover rate that defines the kinetic efficiency, and not so much the Φ parameter per se. Under saturation conditions, the turnover rate even reduces to the turnover number k_{cat}. It is thus debatable that enzymes with a Φ close to unity should be called perfect enzymes [9]. Another reason for avoiding this qualifier in general, even for the enzymes with the highest k_{cat}, is that in most cases, relatively slow rates are the adequate ones for the required biological function.

For a reaction in a closed vessel (batch reactor) it may be asked: In how many catalytic cycles is each enzyme (on the average) engaged during the entire course of the reaction? To answer this question, we first note that $[\mathrm{S}]_0/[\mathrm{E}]_0$ substrate molecules are converted into product molecules by each enzyme. In this way, and using Eq. 12, the total average number of cycles per enzyme for the complete course of the reaction is

$$\bar{n}_c = \frac{\bar{n}_P \, [S]_0}{[\mathrm{E}]_0}. \quad (15)$$

For efficient enzymes that are in large excess, one cycle suffices for the reaction to attain completion. In fact, all complexes form almost simultaneously, the cycles are completed at approximately the same time, and the reaction is terminated in one period. If the enzymes are less efficient, several periods are required, but all substrate molecules have a common start and react in a parallel, noncompetitive fashion. The situation $[E]_0 \gg [S]_0$ has therefore little practical interest in the laboratory, as the reaction would be very fast, and even rapid mixing techniques could not be used, except for extremely low enzyme concentrations.

When it is the substrate that is in excess, however, most substrate molecules must wait for a chance to form a complex with an available enzyme. The reaction takes much longer and needs a different description.

4.2 Substrate in excess

The case where $[S]_0 \gg [E]_0$ is the more common situation, at least in vitro. Typically, enzyme concentrations of 10^{-8} to 10^{-10} M are used, whereas substrate concentrations are usually greater than 10^{-6} M. Under these conditions the enzymes, even when super-efficient, must perform many catalytic cycles. Equations 1, 13, and 14 give

$$\frac{d[P]}{dt} = \frac{k_a \Phi \, [E]_0}{\frac{1}{[S]} + \frac{1}{K_m}} = \frac{k_{\mathrm{cat}} \, [E]_0}{1 + \frac{K_m}{[S]}}. \quad (16)$$

Equation 16 is valid if many catalytic cycles take place for each enzyme before completion of the reaction that is, if substrate concentration changes slowly compared to the cycle duration. Using Eq. 15, this implies that $[S]_0 \gg [E]_0 \, \Phi$, a condition that clearly holds if the total enzyme concentration $[E]_0$ is much less than the substrate concentration $[S]_0$. In order to proceed, it is noted that $[S]_0 \gg [E]_0$ implies that $[S] \cong [S]_0 - [P]$, and using this relation Eq. 16 can be integrated in order to obtain $[P](t)$. The concentration of substrate is next obtained from the same equation $[S] \cong [S]_0 - [P]$. The final result is known in both implicit [10] and explicit [4] forms, see "Appendix B". The explicit form is [4]

$$[S] = K_m W \left[\left(\frac{[S]_0}{K_m} \right) \exp\left(\frac{[S]_0}{K_m} \right) \exp\left(-\frac{k_{\mathrm{cat}} \, [E]_0}{K_m} t \right) \right], \quad (17)$$

where $W(x)$ is the Lambert function. A more general solution encompassing Eq. 17 was recently obtained [3], and is also given in "Appendix B".

A second non-standard derivation also leading to Eq. 17 starts from Eq. 7. Differentiating it with respect to time, and taking into account that during the so-called quasi-steady-state, the concentration of *ES* changes much less rapidly than that of *S*,

$$\frac{d[P]}{dt} \cong -\frac{d[S]}{dt}. \tag{18}$$

Insertion of Eq. 18 into Eq. 16 gives

$$-\frac{d[S]}{dt} = \frac{k_{\text{cat}}\,[E]_0}{1+\frac{K_m}{[S]}}. \tag{19}$$

This equation is identical to that obtained from the QSSA [1,2]. Although the final result is identical, the present derivations are more satisfactory. The mathematically drastic approximation implicit in the QSSA (i.e. equating the derivative of [*ES*] with respect to time to zero) is not necessary for arriving at Eq. 19 or to the integrated solution (see "Appendix B"). According to the first derivation, there is even no need to consider the time derivative of [*ES*], it being enough to take into account that when $[S]_0 \gg [E]_0$ the condition $[ES] \ll [S] + [P]$ is obeyed for all times.

It is interesting at this point to mention two limiting situations:

(i) If $[S]_0 \gg K_m$, the substrate concentration obeys zero-order kinetics (down to $[S] \approx K_m$):

$$[S] = [S]_0 - k_{\text{cat}}\,[E]_0\,t. \tag{20}$$

Historically, the observation of this behavior provided striking kinetic evidence for the existence in solution of a relatively stable enzyme-substrate complex, entity whose existence had already been postulated by Emil Fischer (the famous 1894 lock-key analogy) and others.

An approximate equation for the substrate concentration covering both the initial, transient part, and the subsequent steady-state linear time evolution is [11],

$$[S] = [S]_0 - k_{\text{cat}}\,[E]_0\,t - [E]_0\left(1 - e^{-k_a[S]_0 t}\right). \tag{21}$$

(ii) If $[S]_0 \ll K_m$ (but still with $[S]_0 \gg [E]_0$), the substrate concentration obeys first-order kinetics:

$$[S] = [S]_0 \exp\left(-k_a \Phi\,[E]_0\,t\right) = [S]_0 \exp\left(-\frac{k_{\text{cat}}\,[E]_0}{K_m} t\right). \tag{22}$$

The corresponding equations for the product are obtained from $[P] = [S]_0 - [S]$.

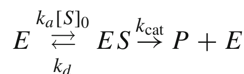
Equations 17, 20 and 22 show that by changing the substrate initial concentration, and by following the time course of the reaction, it is possible to determine the values of k_{cat} and K_m. This is accomplished by a number of data analysis methods [2,12,6,13].

4.3 Pre-equilibrium

Under pre-equilibrium conditions [14] $\Phi \ll 1$, a fast equilibrium between S, E and ES is rapidly established, and the conversion of ES into P takes place at a comparatively much slower rate. When $[S]_0 \gg [E]_0$, the results of the previous section apply. Nevertheless, Eq. 16 is still valid when the substrate is not in excess, provided that $[S]_0 \gg [E]_0\,\Phi$. In such a case, the ES concentration is significant and $[S] \cong [S]_0 - [P]$ is not valid. It is then preferable to write $[S'] = [S]_0 - [P]$, where $[S']$ is the total substrate concentration, $[S'] = [S] + [ES]$, and to change the variable in Eq. 16 to $[S']$. Using the equilibrium constant, $[S]$ can then be related to $[S']$ and the equation finally integrated. The detailed procedure is presented in [3], where a general solution is given.

4.4 The initial transient phase

The full set of three rate constants (k_a, k_d, and k_{cat}) can only be obtained from an additional study of the initial time-dependence of the concentration during the so-called transient phase [12], the initial period that lasts less than the average cycle time τ_c (a few milliseconds at most). During at least the early part of this phase, the substrate concentration is approximately equal to its initial value, and therefore the situation is represented by



$$E \underset{k_d}{\overset{k_a[S]_0}{\rightleftarrows}} ES \overset{k_{\text{cat}}}{\rightarrow} P + E$$

The concentrations of free enzyme and enzyme-substrate complex are

$$[E] = [E]_0 \frac{K_m + [S]_0 \exp\left[-k_a (K_m + [S]_0)\, t\right]}{K_m + [S]_0} = [E]_0 \left(1 - k_a [S]_0\, t\right) + \cdots, \tag{23}$$

$$[ES] = [E]_0 [S]_0 \frac{1 - \exp\left[-k_a (K_m + [S]_0)\, t\right]}{K_m + [S]_0} = k_a [E]_0 [S]_0\, t + \cdots \tag{24}$$

For very short times the free enzyme and the enzyme-substrate complex concentrations evolve linearly with time, and for longer times they nearly stabilize at the steady-state values.

The concentration of product follows from Eqs. 5 and 24 [12,3],

$$\begin{aligned}[P] &= \frac{k_{\text{cat}} [E]_0 [S]_0}{K_m + [S]_0} \left(t - \frac{1 - \exp\left[-k_a (K_m + [S]_0)\, t\right]}{k_a (K_m + [S]_0)} \right) \\ &= \frac{1}{2} k_a k_{\text{cat}} [E]_0 [S]_0\, t^2 + \cdots \end{aligned} \tag{25}$$

For very short times the product evolves in a quadratic way with time, and for longer times it increases linearly with time. For still longer times it stabilizes and attains a

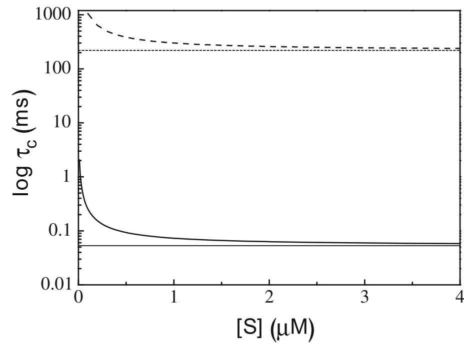


Fig. 2 Average cycle duration (τ_c, given by Eq. 13) for β-galactosidase (*solid line*) and peroxidase (dashed line) as a function of substrate concentration (RGP and H_2O_2, respectively). The *horizontal lines* correspond to the minimum cycle duration for each enzyme-substrate pair, 53 μs and 220 ms, respectively

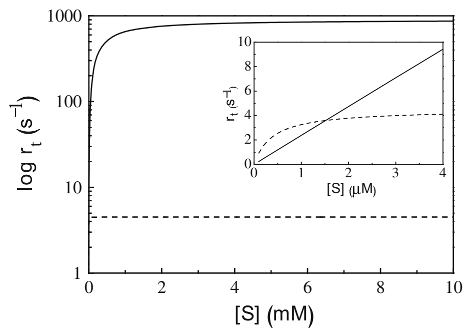


Fig. 3 Turnover rate (r_t, Eqs. 8 and 14) for β-galactosidase (*solid line*) and peroxidase (*dashed line*) as a function of substrate concentration (RGP and H_2O_2, respectively). The inset shows the cross-over of the turnover rates that occurs at low substrate concentration

constant value $[S]_0$ but owing to the assumptions made this final phase is not predicted by Eq. 25. Finally, the concentration of substrate is approximately given by [11]

$$[S] = [S]_0 - \frac{k_{\text{cat}}\,[E]_0\,[S]_0}{K_m + [S]_0} t - \frac{[E]_0\,[S]_0\,(k_d/k_a + [S]_0)}{(K_m + [S]_0)^2}\left(1 - \exp\left[-k_a\,(K_m + [S]_0)\,t\right]\right). \quad (26)$$

5 Application to real systems

Results for two enzyme-substrate pairs, β-galactosidase and RGP (resorufin-β-D-galactopyranoside) at 22°C [15], and peroxidase and H_2O_2 at 25°C and pH = 4 [16, 17], are now discussed and displayed in Figs. 2 and 3. The corresponding kinetic parameters are given in Table 1:

For the first pair, the average lifetime of the enzyme-substrate complex, given by $1/(k_d + k_{\text{cat}})$, is 53 μs, and the product yield per cycle Φ (Eq. 11) is only 5%. The enzyme is thus far from being super-efficient. The minimum cycle duration (53 μs) is attained for substrate concentrations higher than about 40 mM, for which the turnover rate attains its maximum value, $k_{\text{cat}} = 900\ \text{s}^{-1}$.

For the second pair, the enzyme-substrate complex has an average lifetime of about 220 ms, and the product yield per cycle is now 98%, the enzyme being close to super-

Table 1 Kinetic parameters for two enzyme-substrate pairs

Enzyme-substrate pair	$k_a/\mathrm{M}^{-1}\,\mathrm{s}^{-1}$	k_d/s^{-1}	$k_{\mathrm{cat}}/\mathrm{s}^{-1}$	K_m/M	$k_{\mathrm{cat}}/K_m/\mathrm{M}^{-1}\mathrm{s}^{-1}$	Φ
β-galactosidase/RGP [15]	5×10^7	1.8×10^4	900	3.8×10^{-4}	2.4×10^6	0.05
peroxidase/H_2O_2 [16,17]	1.2×10^7	0.1*	4.5	3.8×10^{-7}	1.2×10^7	0.98

*Average value

efficient. The minimum cycle duration (ca. 220 ms) is attained for substrate concentrations higher than about 30 μM, for which the turnover rate attains its maximum value, $k_{\mathrm{cat}} = 4.5\,\mathrm{s}^{-1}$.

A crossing of the turnover rates is apparent in the inset of Fig. 3: For low substrate concentrations, peroxidase displays the highest values, as it has the highest cycle efficiency Φ; for higher substrate concentrations the reverse happens however, owing to the more elevated turnover number k_{cat} of the β-galactosidase system. The overall efficiency for a given enzyme-substrate pair is therefore better measured by the turnover rate, which is a function not only of the kinetic parameters but also of the substrate concentration.

6 Conclusions

A different mathematical approach to Henri-Michaelis-Menten enzyme kinetics was presented and applied to real systems. This pedagogically useful description stresses the cyclic nature of the catalytic process. The approach provides parameters that define enzyme efficiency (product yield per cycle, Eq. 11) and speed (average cycle duration, Eq. 13). The turnover rate can be described in terms of these two parameters by an equation (Eq. 14) that has a clear dynamical meaning. The total number of cycles per enzyme was also obtained (Eq. 15). The time-dependence of the substrate concentration after the initial transient phase (Eq. 17) is derived in a simple way that does not require the quasi-steady-state approximation. An approximate equation for the substrate concentration covering both the transient phase and the subsequent steady-state part is also given (Eq. 26). Application of the results to two real systems allowed obtaining numerical values of the parameters defined, and concluding that enzyme kinetic efficiency is best measured by the turnover rate, which is a function of substrate concentration. It is important to note that both the QSSA and the present derivation fail to correctly describe HMM kinetics when substrate and enzyme concentrations are comparable. In this case a more complex approach is required, see [3] and references therein, an approximate result being for instance Eq. B5.

Appendix A

The average duration of a catalytic cycle, Eq. 13, can be derived as follows: Consider the cycle sequence $E \to ES \to E$ for an individual enzyme, as shown in Fig. 1.

Its duration t_c, for a particular realization, is

$$t_c = t_E + t_{ES}, \tag{A1}$$

where t_E and t_{ES} are the durations of the two steps, i.e., of the enzyme and enzyme-substrate complex species. As these durations are independent, the average duration is

$$\tau_c = \bar{t} = \bar{t}_E + \bar{t}_{ES}, \tag{A2}$$

where $\bar{t}_E$ and $\bar{t}_{ES}$ are the average durations of the two species. The probabilities that enzyme and enzyme substrate react between t and $t + dt$ after their formation are given by exponential distributions [8], as both decay by genuine first-order or pseudo first-order processes,

$$f_E(t) = k_a[S] \exp\left(-k_a[S]t\right), \tag{A3}$$

$$f_{ES}(t) = (k_d + k_{\text{cat}}) \exp\left[-\left(k_d + k_{\text{cat}}\right)t\right], \tag{A4}$$

hence the average durations are

$$\bar{t}_E = \int_0^\infty t f_E(t)dt = \frac{1}{k_a[S]}, \tag{A5}$$

$$\bar{t}_{ES} = \int_0^\infty t f_{ES}(t)dt = \frac{1}{k_d + k_{\text{cat}}}, \tag{A6}$$

and Eq. 13 is obtained. Note that $[S]$ varies in the course of the reaction but is assumed not to change significantly for the average duration of a cycle, in agreement with the hypothesis that $[S]_0 \gg [E]_0$.

Appendix B

Equation 19 can be integrated by separation of variables to give

$$\ln \frac{[S]_0}{[S]} + \frac{[S]_0 - [S]}{K_m} = \frac{k_{\text{cat}}\,[E]_0}{K_m} t. \tag{B1}$$

This integrated form has been known for a long time: A form analogous to Eq. B1 was already obtained by Henri in 1903 [18] under the assumption of a pre-equilibrium. For the result free from this assumption see [10] and references therein. More recently, and with the help of technical software, Schnell and Mendoza [4] recognized that the substrate concentration can be explicitly given as a function of time in terms of the

Lambert W function. This function is defined as the inverse function of xe^x, that is, if $y = W(x)$ then $ye^y = x$ Indeed, Eq. B1 can be rewritten in the form

$$\left(\frac{[S]}{K_m}\right)\exp\left(\frac{[S]}{K_m}\right) = \left(\frac{[S]_0}{K_m}\right)\exp\left(\frac{[S]_0}{K_m}\right)\exp\left(-\frac{k_{cat}\,[E]_0}{K_m}t\right), \tag{B2}$$

hence [4]

$$[S] = K_m W\,[F(t)]\,, \tag{B3}$$

with

$$F(t) = \left(\frac{[S]_0}{K_m}\right)\exp\left(\frac{[S]_0}{K_m}\right)\exp\left(-\frac{k_{cat}\,[E]_0}{K_m}t\right). \tag{B4}$$

The physical content of Eq. B3 is of course identical to that of Eq. B1, but the explicit form is more elegant and also more convenient for computational purposes, as the Lambert function is implemented in technical computing software (e.g. as *ProductLog*[x] in *Mathematica*). Equations 22 and 20 follow from Eq. B3, as for small x the Lambert function becomes $W(x) = x$, and for large x the function is $\mathrm{W}(x) \approx \ln x$.

A more general solution, accurate after the transient phase, and encompassing Eq. B3 is [3],

$$[S] = K_m W\,[F(t)] - [E]_0 \frac{W\,[F(t)]}{1 + W\,[F(t)]}. \tag{B5}$$

References

1. J.W. Moore, R.G. Pearson, *Kinetics and Mechanism*, 3rd edn. (Wiley, New York, 1980)
2. A.R. Schulz, *Enzyme Kinetics* (Cambridge University Press, Cambridge, 1994)
3. M.N. Berberan-Santos, MATCH Commun. Math. Comput. Chem. **63**, 283 (2010)
4. S. Schnell, C. Mendoza, J. Theor. Biol. **187**, 207 (1997)
5. A.R. Tzafriri, Bull. Math. Biol. **65**, 1111 (2003)
6. S. Schnell, P.K. Maini, Comm. Theor. Biol. **8**, 169 (2003)
7. B. Li, Y. Shen, B. Li, J. Phys. Chem. A **112**, 2311 (2008)
8. C. Baleizão, M.N. Berberan-Santos, ChemPhysChem **10**, 199 (2009)
9. M.E. Stroppolo, M. Falconi, A.M. Caccuri, A. Desideri, Cell. Mol. Life Sci. **58**, 1451 (2001)
10. H.N. Fernley, Eur. J. Biochem. **43**, 377 (1974)
11. M.N. Berberan-Santos, MATCH Commun. Math. Comput. Chem. **63**, 603 (2010)
12. H. Gutfreund, *Kinetics for the Life Sciences* (Cambridge University Press, Cambridge, 1998)
13. R.J. Jennings, C. Niemann, J. Am. Chem. Soc. **77**, 5432 (1953)
14. M. Rae, M.N. Berberan-Santos, Chem. Phys. **280**, 283 (2002)
15. B.P. English, W. Min, A.M. van Oijen, K.T. Lee, G. Luo, H. Sun, B.J. Cherayil, S.C. Kou, X.S. Xie, Nat. Chem. Biol. **2**, 87 (2006)
16. A.R. Tzafriri, E.R. Edelman, J. Theor. Biol. **245**, 737 (2007)
17. B. Chance, J. Biol. Chem. **151**, 553 (1943)
18. V. Henri, *Lois Générales de l'Action des Diastases* (Hermann, Paris, 1903)