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Research Article

Analysis of the Progress of Enzyme-Catalyzed Reaction

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Abstract. The aim of the research is to analyze and model the progress of product accumulation in relation with the kinetic properties of the enzyme and substrate concentration. Conventional methods of analysis are used. The results are as follows: 'linear' decline of high substrate concentration (or rise of the product) is determined by the kinetic properties of the enzyme taking into account initial substrate concentration (Model 1); the exponential decline corresponds to Model 2; the deviations from linearity occurring either because of product inhibition of the reaction, the backward reaction or substrate exhaustion are modelled by algebraic sum of linear and exponential functions. Modification of the conventional scheme of the enzyme-catalyzed reaction, therefore, facilitates deriving the equations highlighting the role of the reaction product and initial substrate concentration in the progress of the reaction. The equations enable estimation of the kinetic parameters of the enzyme from the data of conventional experiments and an additional experiment corresponding to the 'end' of the reaction.

Keywords: Reversible reaction, direct reaction, backward reaction, product inhibition, dissociation constant

1. Introduction

The most essential property of enzymes is their ability to catalyze very specific chemical reactions of biological importance. Some enzymes can increase the rate of a chemical reaction by as much as 10^{12}-fold over the spontaneous rate of the uncatalyzed reaction [1], up to 10^{17}-fold [2, 3], up to 10^{19}-fold [4] or even up to 10^{23}-fold [5]. To quantitatively describe the activity of an enzyme, first of all, the initial rate (or initial velocity) of the enzyme-catalyzed reaction is used. The very notion of the *initial rate* implies that the rate of the reaction in the course of time may be different from the initial one, what may be caused by several factors (see, e.g., [6]). To determine the rate of the reaction (directly being possible only in special cases), accumulation of the reaction product has to be monitored and measured for some time. It is clear that during the time of measurements the rate of product accumulation can change: the greater is the time interval, the greater the change. On the other hand, this interval cannot be chosen very short: the quantity of the product accumulated has to be sufficient to detect it and to measure its quantity. A variety of fine and

sensitive methods are available at present to measure low quantities of chemicals [1]. Because of high sensitivity of these methods, the accuracy of the data obtained cannot be high. It is necessary, therefore, to obtain sufficient amount of experimental data and to apply statistical methods of data analysis.

The methods of data analysis suggested in biochemistry (enzyme) textbooks (see, e.g.,[1, 2, 7, 8]), however, mostly are based on linear models. Essentially non-linear relationships are usually linearized. Quite often that means making use of only a small fragment of available data, those deviating from "theoretical" straight line being discarded. In biochemical laboratories graphical methods (involving the plots of the data on the graph paper and other paper work) of data processing are still routinely used (see, e.g., [1, 6, 8]).

Equally important is the selectivity of the enzyme. That can be characterized by quasi-equilibrium dissociation constant or so-called Michaelis constant. Again, to determine this constant from the experimental data, inadequate methods are used, involving coordinate transformation, the most favourite being the double reciprocal or Lineweaver–Burk (linear) plot (see, e.g., [1, 6, 8]). Linearisation (coordinate transformation) seems to be considered the best way to prepare the data for analysis and to present them, and it is popularized in the textbooks. While that might have been convenient in straight line fitting by hand (in the pre-computer era), it is not justified at present (keeping in mind computer availability and computer-literacy) because of extra difficulties in the interpretation and parameter estimation associated with the transformation-caused non-proportional change of the errors making necessary to introduce (arbitrary) weights to reduce the additional errors.

It is important to relate the conversion of substrate into reaction product to the known properties of the enzyme. A great variety of the enzyme properties have been established up to date (see, e.g., [8]). It is clear that taking into consideration all what is known makes the relationship very cumbersome and virtually impracticable. On the other hand, not all of those properties manifest themselves in the observable progress curves. Presentation of those curves is often misleading, the conditions of the reaction not being specified (see, e.g., [1]).

In the present work, therefore, the simplest possible relationships of the progress of enzyme-catalyzed reaction with the kinetic properties of the enzyme has been analysed. It has been shown that the initial rate of enzymatic reaction (its efficacy) can be determined on the basis of a simple model making use of *all* the experimental data, the estimation being more accurate than that obtained rutinely by conventional methods; the selectivity of the enzyme can be assesed without any transformation, the estimation being better, again. It should be pointed out that the the present analysis concerns mainly the above relationships (not entering into details of enzyme action), the modeling considered to be the most concise and rigorous way to express the relationships.

2. Methods

Standard software was used. The algebraic and differential equations or their systems were solved with *Maple*. Simple *Visual Basic* macro functions for *Microsoft Excel* [9] were used to numerically solve differential equations.

3. Results (General Considerations and Modelling)

3.1. Model 1. Inhibition by the reaction product is taken into account.

3.1.1. States of the enzyme and the model based on the states. The modelling is based on the scheme of interactions presented in Figure 1 (bottom) in which different *states* of the enzyme (being ligand-free or ligand-bound (i.e., substrate- or product-bound) denoted as E, ES and EP) rather than the enzyme *itself* are considered. The states can be characterized quantitatively; probabilities corresponding to certain conditions can be ascribed to the states. The scheme considered here reflects the same relationships as the conventional one (top of Figure 1).

For steady state, supposing the algebraic sum of absolute forward and backward rates of the transitions to be zero and the sum of the probabilities of the enzyme to reside in either state or corresponding relative concentrations being unity, it follows from the scheme that

$$\begin{cases} c_0\alpha s - c_S\beta - c_S\mu + c_P\nu = 0, \\ c_0\gamma s - c_P\delta + c_S\mu - c_P\nu = 0, \\ c_0 + c_S + c_P = 1. \end{cases} \tag{1}$$

Solutions of the above system are impractical to be presented here. For $\mu \ll \beta$ and $\nu \ll \delta$ the cumbersome solutions simplify to

$$c_0 = \frac{\mathfrak{s}}{s + \mathfrak{s} + (\mathfrak{s}/\mathfrak{p})p}, \tag{2}$$

$$c_S = \frac{s}{s + \mathfrak{s} + (\mathfrak{s}/\mathfrak{p})p}, \tag{3}$$

$$c_P = \frac{p}{p + \mathfrak{p} + (\mathfrak{p}/\mathfrak{s})\,s}. \tag{4}$$

Ignoring $c_S\mu$ and $c_P\nu$ directly in System (1) leads to the same equations ((2)–(4)). Here s and p are current concentrations of substrate and reaction product, $\mathfrak{s} = \beta/\alpha, \mathfrak{p} = \delta/\gamma$ and $\mathfrak{q}$ (below), dissociation constants. That will be reasoned in Section 4.

3.1.2. Product accumulation. It is clear that the sum of concentrations of the substrate (s) and that of the product (p) remains constant and equal to the initial concentration (let it

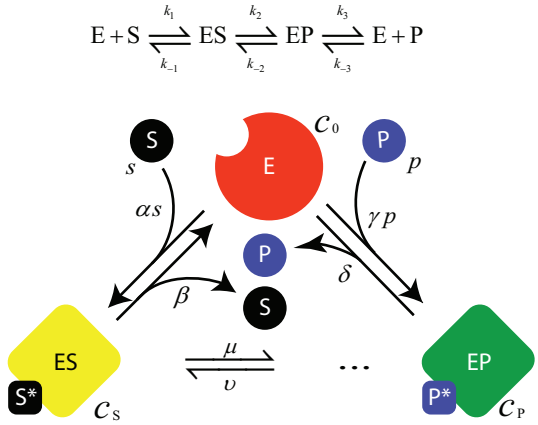

Figure 1: Schematic representation of enzyme-catalyzed reaction. Top: a conventional scheme. Bottom: the scheme equivalent to the former but more convenient for shift to mathematical modelling of the progress of the reaction. The capital non-italic letters (E, ES, EP, S, P, S*, P*) denote the enzyme (free or bound) and the substrate or product (free or bound), the corresponding lowercase italic letters (c_0, c_S, c_P, s, p), the probabilities of the enzyme states and the ligand concentrations, αs, β, γp, δ, μ, υ denoting relative rates of the transitions.

be s_0) during the reaction. That makes one of the equations. At the same time, the rate of the enzymatic conversion of the substrate to product (the forward reaction) is proportional to the concentration of the enzyme (let it be e_0) and the probability of the enzyme to be in state ES (3). The product of the forward reaction is a substrate for the backward one (ES ← EP) whose rate is proportional to the same e_0 and the probability of the enzyme to reside in EP state which is expressed by eq. (4). The net rate makes another equation. Thus

$$\begin{cases} s + p = s_0, \\ \frac{\mathrm{d}p}{\mathrm{d}t} = e_0 \left(\frac{s}{s + \mathfrak{s} + (\mathfrak{s}/\mathfrak{p})p}\mu - \frac{p}{p + \mathfrak{p} + (\mathfrak{p}/\mathfrak{s})\,s}\nu \right). \end{cases} \quad (5)$$

For $\nu = 0$ or $\mathfrak{p} \to \infty$ the solutions are

$$s = \mathfrak{s} W\left(\frac{s_0}{\mathfrak{s}} \exp\left(\frac{s_0}{\mathfrak{s}} - \frac{e_0}{\mathfrak{s}}\mu t \right) \right) \quad (6)$$

and

$$p = s_0 - \mathfrak{s} W\left(\frac{s_0}{\mathfrak{s}} \exp\left(\frac{s_0}{\mathfrak{s}} - \frac{e_0}{\mathfrak{s}}\mu t \right) \right) \quad (7)$$

where W is Lambert W function [10]; see Figure 2.

The 1st term of the right-hand side of the 2nd equation of System (5) models the absolute rate of the forward reaction, while the 2nd term does that of the backward one, the fractions modelling the probabilities of the enzyme to be in states ES and EP (see Figure 1 and Eqs. (3) and (4)). The above terms can be usefully approximated.

3.1.3. Approximations at low product concentration. Product concentration being low ($p \ll s_0$) and $\mathfrak{p}$ being finite, the differential equation of product accumulation is (see Appendix A):

$$\frac{\mathrm{d}p}{\mathrm{d}t} \approx e_0 \left[\frac{s_0}{s_0 + \mathfrak{s}} \left(1 - \frac{\mathfrak{s}p}{(s_0 + \mathfrak{s})\mathfrak{p}} \right) \mu - \frac{\mathfrak{s}p}{(s_0 + \mathfrak{s})\mathfrak{p}}\nu \right] \quad (8)$$

and solution of the above equation under initial conditions (let $s(0) = s_0$, $p(0) = 0$) is:

$$p = \frac{(s_0 + \mathfrak{s})s_0\mathfrak{p}\mu}{\mathfrak{s}\left(s_0\mu + (s_0 + \mathfrak{s})\nu\right)} \times \left[1 - \exp\left(-\frac{e_0\mathfrak{s}}{(s_0 + \mathfrak{s})^2\mathfrak{p}} \left(s_0\mu + (s_0 + \mathfrak{s})\nu \right) t \right) \right]. \quad (9)$$

This equation models product accumulation under various conditions. The model is presented in Figure 2.

3.2. Model 2. No action of the reaction product is taken into account. In this case the scheme of the reaction is (see [11]):

$$\mathrm{S} \;\&\; \mathrm{E} \leftrightarrow \; \mathrm{ES} \; \rightarrow \; \mathrm{EP}. \quad (10)$$

Here it is sufficient to analyse only the 1st step (S & E ↔ ES) of the process. The model being simple can be extended for any relationship between e_0 and s_0 ($s_0 < e_0 < \infty$). It follows that (see (3b) in [11])

$$c_S = \frac{e_0 + s + \mathfrak{s} - \sqrt{(e_0 + s + \mathfrak{s})^2 - 4e_0 s}}{2e_0} \quad (11)$$

and, consequently,

$$\frac{\mathrm{d}s}{\mathrm{d}t} = -\frac{\mathrm{d}p}{\mathrm{d}t} = -\mu\frac{e_0 + s + \mathfrak{s} - \sqrt{(e_0 + s + \mathfrak{s})^2 - 4e_0 s}}{2}. \quad (12)$$

Solutions of this equation are depicted in Figure 3. For observable (finite) affinity of the product molecule and that of the enzyme Model 2 (scheme (10)) is not applicable.

3.2.1. Approximation at low initial substrate concentration (the single-turnover approach). At low initial substrate concentration ($s_0 \ll \mathfrak{s}$) and in the absence of product action

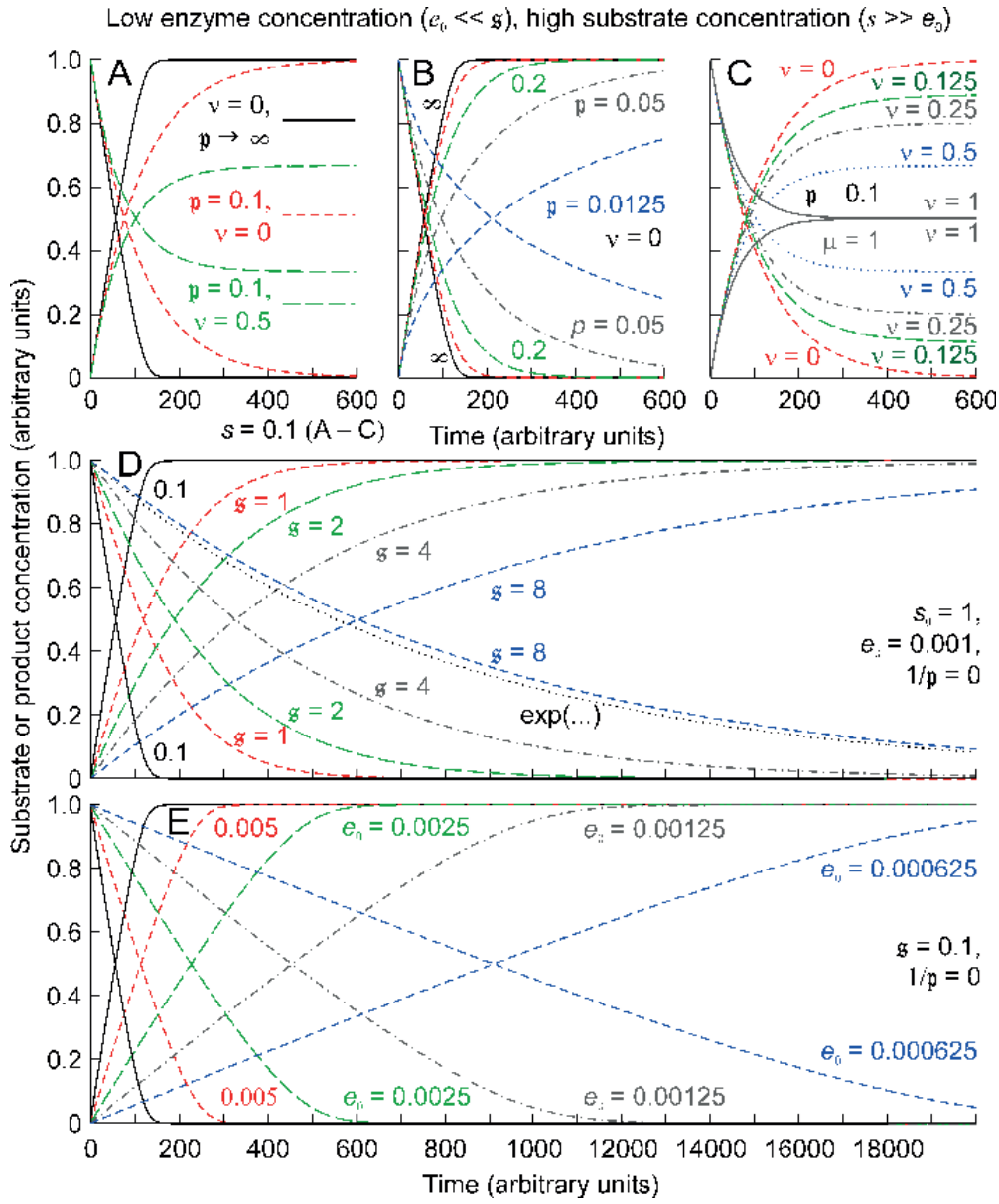

Figure 2: Reaction product accumulation (rising) and substrate exhaustion (declining; high substrate concentration). A. No effect of product on the reaction progress being taken into account; inhibition of the reaction by the product and the backward reaction being taken into account. B. Various product dissociation constants, $\mathfrak{p}$. C. Various rates of the backward reaction, ν, the dissociation constants of substrate and product with the enzyme being equal ($\mathfrak{p}/\mathfrak{s} = 1$). D. Various dissociation constants of the substrate with the enzyme, s, initial substrate and enzyme concentrations being constant ($s_0 = 1, e_0 = 0.001$), no inhibition by the reaction product ($\mathfrak{p} \to \infty$); the exponential decline is also depicted (exp(…)). E. Various concentrations of the enzyme, no inhibition by the reaction product ($\mathfrak{p} \to \infty$).

on the enzyme ($\mathfrak{p} \to \infty$) the rate of substrate exhaustion and product accumulation can be approximated (see (12) and Appendix A) as follows:

$$\frac{\mathrm{d}s}{\mathrm{d}t} = -\frac{\mathrm{d}p}{\mathrm{d}t} \approx -\frac{e_0}{e_0 + \mathfrak{s}}\mu s \tag{13}$$

the solution of the above equation being exponential (see Figure 3).

3.3. The case of 2 reaction products. The scheme depicted in Figure 1 represents a reaction in which consideration of a single product is sufficient. A more sophisticated enzyme-catalyzed reaction still feasible for analysis would be a reaction resulting in 2 products (Figures 4 and 5). 5 states of the enzyme have to be considered (Figure 4) now: ligand-free (E), substrate-bound (ES), product$_1$-bound (EP), product$_2$-bound (EQ) and both products-bound (EPQ). Correspondingly, a system (analogous to System (1)) of 5 algebraic equations with regard to those states has to be

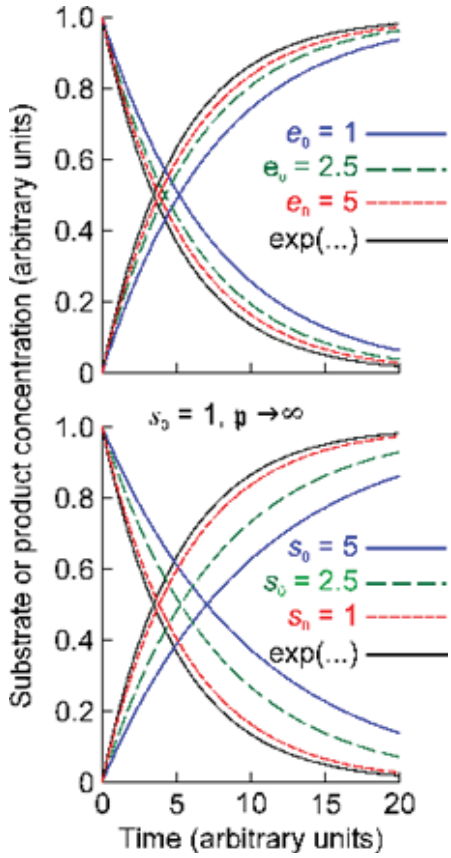

Figure 3: Reaction product accumulation (rising) and substrate exhaustion (declining; low substrate concentration) at constant enzyme concentration, fixed substrate–enzyme dissociation constant ($e_0 = 100$, $\mathfrak{s} = 100$) and varying initial substrate concentrations, no inhibition by the reaction product ($\mathfrak{p} \to \infty$); the exponential decline and corresponding rise is also depicted.

composed. Solutions of this system with respect to states ES and EP (assuming $q \equiv p$) are:

$$c_S = \frac{s}{s + \mathfrak{s} + (\mathfrak{s}/\mathfrak{p})p + (\mathfrak{s}/\mathfrak{q})p + (\mathfrak{s}/\mathfrak{pq})p^2}; \qquad (14)$$

$$c_P = \frac{p^2}{p^2 + \mathfrak{pq} + (\mathfrak{p} + \mathfrak{q})p + (\mathfrak{pq}/\mathfrak{s})s}. \qquad (15)$$

On the basis of (14) and (15) and taking into account that $s + p = \text{const}$ (it is assumed to be s_0) a system of differential equations analogous to System (5) has been composed. Model curves corresponding to the solutions of the system are presented in Figure 5.

4. Discussion (Model Analysis and Further Modelling)

Not using the subscripts in the notations of rate constants, arithmetic signs (+) in the scheme and avoiding to use the brackets (denoting the probabilities of the states and the relative concentrations by lowercase letters (c_0, c_S, c_P, s, p) corresponding to the states (E, ES, EP, S, P)) facilitates the "translation" of the scheme into mathematical equations expressing the relationships of the states, rates and concentrations; besides, the modified scheme is visually suggestive. Switching to the equations from the conventional schemes (with multi-character symbols) would be neither simple nor clear. (9), e.g., would become unintelligible. Moreover, in the textbooks the chemical transformation of the complexed enzyme is often ignored in the scheme, the complex EP (Figure 1, top) being omitted (see, e.g., [1, 2, 8];). The rates of transitions sometimes are omitted from the schemes although used in the text [12] or denoted rather inaccurately (the direct reaction rates being denoted as k_{-1}, k_{-2},…, i.e., with the "minus" signs) [13].

The dissociation constants used in the equations are related to corresponding ligands and have the same dimension as the ligands. The notations used ($\mathfrak{s}, \mathfrak{p}, \mathfrak{q}$), therefore, seem to be quite justified.

The assumption that $\mu \ll \beta$ and $\nu \ll \delta$ means that the rate constants of the reversible chemical transitions of the enzyme–ligand complexes (ES ↔ EP) are considerably lower than those of ligand un-binding (E ← ES or E ← EP). That corresponds to reducing the closed scheme (Figure 1) to the open one in which the transitions μ and ν are omitted or considering the *rapid equilibrium* model rather than the *steady state one*. The enzyme-catalyzed reaction is considered, therefore, to proceed as two separate and independent processes: 1) interactions between ligand and enzyme molecules and 2) chemical transitions of the enzyme–ligand complexes (ES ↔ EP). In the fast processes of interactions the low rate constants (μ and ν) can be ignored ((2)–(4)) while these constants determine the progress of (slow) chemical reaction (System (5)), the states (E, ES and EP) and corresponding probabilities (e_0, e_S and e_P) being determined by (2)–(4). The processes of interactions correspond to the *specificity* of the enzyme, the following, to its *efficacy*. Such division may be useful when the ligand molecule does not undergo any transformation beyond the binding or chemical transformation of it is not considered (see, e.g., [14–19]).

It is interesting to note that neither the above schemes nor System (5) incorporate the rate of chemical reaction proceeding spontaneously (without the enzyme), neither the specificity nor efficacy depending on this rate. Conversely, therefore, the enzyme-caused increase in the above rate occurs, presumably, due to high specificity (low dissociation constant, $\mathfrak{s}$), high efficacy (high rate of the conversion, μ), each independently, and both independently of the spontaneous rate.

System (5) is equivalent to the scheme and adequately models the conversion of substrate to product (S* → P*) containing 4 independent parameters ($\mathfrak{s}, \mathfrak{p}, \mu$ and ν) which can be estimated by comparing the model with experimental data. (6) and (7) involve Lambert W function which is

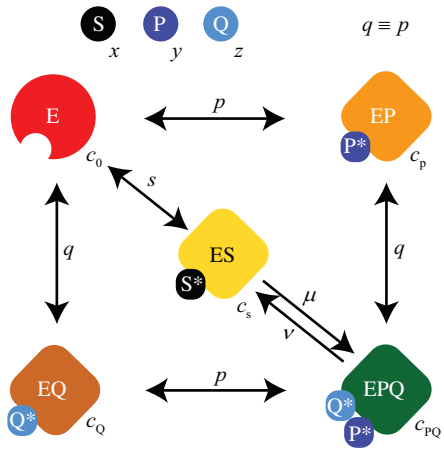

Figure 4: Simplified representation of enzyme-catalyzed reaction producing 2 products. Here $\mathfrak{s}$, $\mathfrak{p}$, and $\mathfrak{q}$ are dissociation constants of substrate and products with the enzyme.

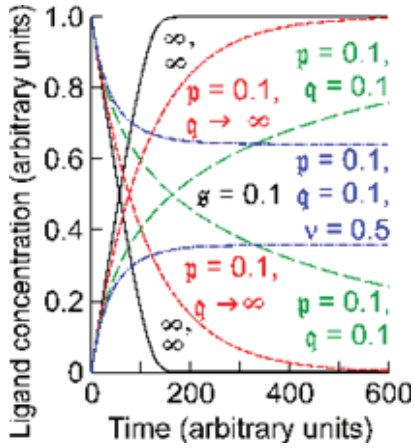

Figure 5: Product accumulation and substrate exhaustion in the reaction resulting in 2 products. Enzyme concentration, substrate and enzyme dissociation constant and initial substrate concentration ($e_0 = 0.01$, $s = 0.1$ and $s_0 = 1$ arbitrary unit) are the same throughout. No effect of product on the reaction progress being taken into account ($\mathfrak{p} \to \infty$, $\mathfrak{q} \to \infty$), inhibition of the reaction by its product ($\mathfrak{p} = 0.1$ and $q \to \infty$, $\mathfrak{p} = 0.1$ and $q = 0.1$), the backward reaction ($\mathfrak{p} = 0.1$ and $\mathfrak{q} = 0.1$, $\nu/\mu = 0.5$) being taken into account.

solution of the transcendental equation $\mathrm{W}(z) \times \exp(\mathrm{W}(z)) = z$ and cannot be expressed via elementary functions, besides, these equations are simplifications. (The curves modelled by these equations can be plotted using *Maple*, *Mathematica* or others. System (5) can be solved numerically using *Microsoft Excel* or other software.)

As seen from (9), reaction product accumulation (*product accumulation* rate rather than *reaction velocity* seems to be more appropriate notion in the present analysis) deviates from linearity for any observable affinity of the product molecule and that of the enzyme ($1/\mathfrak{p} \neq 0$), independently of taking place or not of the backward reaction ($\nu \neq 0$ or $\nu = 0$), the deviation depending both on the enzyme and initial substrate concentrations (e_0 and s_0). It is clear that the deviation can be ignored for a short period of time. Although (9) at first glance may look cumbersome, its parameters of similar meaning are denoted by similar symbols and grouped together making the equation clear and simple. Expression of the same relationship using the conventional notations would make the equation incomprehensible.

It should be noted that the reaction progress curves corresponding to high initial substrate concentrations (all the other parameters including the dissociation constant(s) being the same) are not convenient to present on the same graph because of the scale. For this reason, the initial substrate concentration is assumed to be the same (1 arbitrary unit) for all the curves, the dissociation constant(s) for different curves being different. Numerical solutions of system (5) for fixed initial conditions ($s(0) = s_0$ and $p(0) = 0$) and various values of $\mathfrak{s}$, $\mathfrak{p}$, μ and ν are presented in Figure 2.

In spite of the non-linearity (both theoretical and experimental), usually only the so-called initial velocity of the reaction is estimated from experimental data by drawing the straight line tangent to the curve approximating (rather arbitrarily) the data, valuable information (often most of it) contained in the data not being used. Use of (9) for comparison with experimental data of product accumulation to extract information concerning the unknown parameters is impractical, however, because of too many parameters ($\mathfrak{s}$, $\mathfrak{p}$, μ and ν) contained in it.

It should be noted that presenting the enzyme-catalyzed reaction as in the above schemes (either the conventional one or its modification, Figure 1, Model 1) assumes tacitly that the substrate concentration is high enough enabling to ignore the difference in the concentrations of free and enzyme-bound substrate. Usually, it is assumed that the enzyme concentration is low compared to that of the substrate ($e_0 \ll s_0$). The assumption that the reaction is not inhibited

by the product (its dissociation constant, $\mathfrak{p}$, being high) enables to simplify the reaction scheme as shown in the scheme (10) and that in turn allows to extend the range of substrate concentration ($0 < s < \infty$) independently of e_0 considering the binding rate to be proportional to $s - e_0$ (Model 2). (Similar extension of Model 1 would make it too cumbersome).

4.1. Comparing of approximate models with experimental data. Modelling the non-linear product accumulation (the non-linearity being caused solely by product accumulation) is rather straightforward. Indeed, let the substrate concentration be high ($s_0 >> e_0$) and the rate of substrate conversion into the product (without taking into account the effect of the product on the reaction) be proportional to $\mu s_0/(s_0 + \mathfrak{s})$, (the fraction considered as the degree of saturation of the enzyme by the substrate). It seems reasonable to assume the result of the product action on the deviation of product accumulation from the straight line to be proportional to its concentration, p, independently of the causes of the non-linearity (the causes may be different from those considered here (e.g., thermal degradation of the enzyme, product metabolism by other enzyme(s) etc., see, e.g., [15, 16])); let the coefficient of proportionality be ω. Then

$$\frac{\mathrm{d}p}{\mathrm{d}t} = e_0 \frac{s_0}{s_0 + \mathfrak{s}} \mu - \omega p. \tag{16}$$

The right-hand side of the above equation is of the same form as that of (8). Its solution under the same conditions is

$$p = e_0 \frac{s_0}{s_0 + \mathfrak{s}} \frac{\mu}{\omega} \left(1 - \exp(-\omega t)\right), \tag{17}$$

i.e., the solution is of the same form as that of (9) and, as a matter of fact, equivalent to (9). It can be reminded that the latter follows from System (5) in which (3) and (4), i.e, approximations concerning the rate constants are used. Parameter ω here is auxiliary. (17) (as well as (9)) models both the dependence of the reaction progress on the initial substrate concentration (the fraction) and non-linearity (the bracketed term). It can be compared with experimental data. In such a comparison *all* the experimental data can be used. To obtain the best accuracy, the least squares method (minimization of sum of the squares of differences between the model and the data) can be applied; that can be done automatically by *Microsoft Excel* add-in "*Solver*" (see, e.g., [20–22]). Fitting of numerically integrated rate equations to the data is widely used (see, e.g., [23]).

Initial substrate concentration being much lower than that of the enzyme ($s_0 \ll e_0$) and, as a consequence, the backward reaction being absent, corresponds to so-called *single-turnover* experiment (see [8]). Under these conditions the substrate practically can be only in enzyme-bound (but not in enzyme-free) state, the transition being possible only from the enzyme-bound substrate to the enzyme-bound product (S* → P*). The reaction is modelled by the solution of (13). The substrate depletion under these conditions is exponential. In the *single-turnover* approach, however, the concentrations of the substrate and the product are of the same order but no product action (assumed to be absent in this approach) on the reaction can be taken into account. Along with the initial enzyme concentration, e_0, the substrate–enzyme dissociation constant, $\mathfrak{s}$, is of importance in the *single-turnover* approach (see (13) and Figure 3).

4.2. Selectivity of the enzyme. Equation (3) adequately models the selectivity. Product action on the reaction being negligible ($1/\mathfrak{p} \to 0$), (2) and (3) are reduced to expressions which are usually referred to as Henri–Michaelis–Menten [1, 8] or even just Michaelis–Menten equations [2]. Frère [24] and Kühl [25] remind that the equation was derived by Henri (in 1903) and published 10 years earlier than by Michaelis and Menten (in 1913). Equations (2)–(4) can be considered as extension of Henri–Michaelis–Menten relationships. It should be pointed out that $\mathfrak{s} = \beta/\alpha$ is *dissociation* rather than so-called *Michaelis* or *steady state kinetic* [1] *constant* (K_m) which in the present notations would be $K_m = (\beta + \mu)/\alpha$.It is believed that "$K_m$ is less than, greater than, or equal to" $\mathfrak{s}$ [1]. It is easy, however, to see that $\mathfrak{s} = \beta/\alpha \le (\beta + \mu)/\alpha = K_m$ for any $0 \le \mu \le \infty$ and s $\approx K_m$ for $\mu \ll \beta$. This conclusion seems reasonable, since the transition of the enzyme from substrate- to product-bound state (ES → EP) is supposed to involve conformational change which is thought to be slower than substrate association or dissociation (E ↔ ES). The possibility of the opposite inequality is also formally considered [12].

The relationship between the initial rate of product accumulation and substrate concentration resulting from the reaction corresponds to the dependence of probability of the enzyme to be in state ES on substrate concentration ignoring any possible action of the product on the reaction (ignoring any affinity of the product to the enzyme, $\mathfrak{p} \to \infty$). Simplification of (3) results in

$$c_S \approx \frac{s}{s + \mathfrak{s}}. \tag{18}$$

Direct application of this model for analysis of experimental data (without any transformation), being very simple and clear (being no problem if any computer spreadsheet is used), enables the best accuracy of the parameter $\mathfrak{s}$ estimation. For the best fit of the model to the data the least squares method can be applied [20–22]. If experimental data suggest a decline after the rise (probably caused by excess of substrate concentration (see [14, 17])), these data points should not be used for parameter estimation. If the data suggest a different slope of the curve (perhaps because of allostericity), the model (18) is not applicable (see, e.g., [19]).

4.3. Parameter estimation. Marangoni [6] suggests that model parameters (only the transition rate constant and the affinity of the substrate and enzyme molecules (μ and $\mathfrak{s}$,

in the present notations) are considered, the reaction being non-reversible) can be estimated from a single progress curve. Indeed, (6) and (7) contain both μ and $\mathfrak{s}$, these parameters determining the shapes of the curves; conversely, the parameters being possible (in principle) to be estimated from the curves. There is no problem to estimame the rate of the reaction. It should be reminded, however, that the shape of the curves also depends on the enzyme and initial substrate concentrations (e_0 and s_0): at high substrate concentration ($s_0 > \mathfrak{s}$) its decline is with a clear break (and, therefore, most informative), deviating considerably from the exponential, but without a break at low substrate concentration (see Figs. 2D and 3). That makes the use of the information contained in the curve possible only at high substrate concentration. Besides, experimental data of the reaction progress "until its end" are necessary. Marangoni [6] suggests graphical estimation of the parameters. Goudar et al. [10] suggest direct fitting to the (algorithmic) solution the transcendental equation leading to the Lambert *W* function. Approximations of System (5) or (17) are clearer and more useful.

Parameter $\mathfrak{s}$ can be estimated from comparison of (18) with the corresponding data, direct fitting enabling better estimation as stated above. From comparison of the solution of (14) with experimental data, parameter μ can be estimated; additionally, the estimate of the auxiliary parameter (ω) characterising the shape of the reaction progress curve determined by the action of the product on the enzyme (independently of the backward reaction) follows immediately from the comparison of (17) with the data. (This additional parameter can be used in practice without separation of the dissociation constant of the product and the enzyme from the rate constant of the backward reaction.) The parameter ω used in (16) and (17) and characterising the non-linearity of the product accumulation is related to the intrinsic parameters ($\mathfrak{p}$ and ν) of the enzyme (see Figure 1, System (5) and (9)); that relation makes an equation necessary to find the latter parameters. Another equation is based on the relationship between the substrate and product concentrations resulting from the indefinite progress of the reaction. It is clear that under these conditions $e_S/e_P = \nu/\mu$ (see Figure 1 and (3) and (4)) in the transition ES $\leftrightarrow$ EP. Thus (see Appendix A):

$$\mathfrak{p} = \mathfrak{s}\frac{e_0 s_0 p_\infty \mu}{(s_0 + \mathfrak{s})\left(p_\infty(s_0 + \mathfrak{s})\omega_0 - e_0\mu s_\infty\right)} \tag{19}$$

$$\nu = \frac{e_0 s_0 p_\infty \mu^2}{(s_0 + \mathfrak{s})\left(p_\infty(s_0 + \mathfrak{s})\omega_0 - e_0\mu s_\infty\right)} \tag{20}$$

where ω_0 is experimentally established parameter corresponding to e_0 and s_0; s_∞ and p_∞ are substrate and product concentrations resulting from the indefinite progress of the reaction. (19) and (20) enable, therefore, estimation of the constants of the backward reaction ($\mathfrak{p}$ and ν).

Numerical solving of differential equations is not necessary for parameter estimation (used here only to present the curves on the graphs). All the parameters of the system (i.e., the kinetic parameters of the enzyme, see Figure 1 and System (5)) can be estimated, therefore, from comparing the reaction product accumulation with simple models (ordinary algebraic functions). No experiments carried out under different conditions are required to estimate the parameters characterizing the inhibition of the reaction, only the 'final' substrate and product concentrations being necessary.

In the case of the reaction resulting in 2 products (see Figure 4 and (14) and (15)), molar concentrations of both products are the same ($q \equiv p$), their affinities to the enzyme, however, in general are not identical ($\mathfrak{q} \neq \mathfrak{p}$). As seen from (14), if the action of the reaction products on its progress is negligible (both $\mathfrak{p} \to \infty$ and $\mathfrak{q} \to \infty$), it is reduced to the Henri hyperbola (18); substrate exhaustion and product accumulation are modelled by (6) and (7) like in the one-product reaction (cf. Figure 2A, B, D, E and Figure 5). If the action on the enzyme of any one reaction product can be ignored (either $1/\mathfrak{p} = 0$ or $1/\mathfrak{q} = 0$), (14) is reduced to (3); approximations (subsubsection 3.1.3) hold true in this case (cf. Figures 2A and 5). It is clear as well (see (15)) that the backward reaction is possible only when the affinities of both products to the enzyme are finite (both $1/\mathfrak{p} \neq 0$ and $1/\mathfrak{q} \neq 0$). If one of the reaction products is equivalent to that present in the solution (e.g., H_2O) whose concentration is practically independent of the reaction, it can be considered as one product reaction analysed above.

5. Conclusions

Modification of the conventional scheme of the enzyme-catalyzed reaction facilitates the use of mathematics. The 'linear' decline of the substrate concentration (or rise of product concentration) is modelled by the equation depending on the enzyme and initial substrate concentrations (Model 1); the exponential decline corresponds to Model 2. Numerical solving of differential equations is not necessary for parameter estimation.

1. Approximations of the general equation enable estimation of the kinetic parameters of the enzyme (including those characterizing inhibition of the reaction by the product and the backward reaction) from the data of conventional experiments and an additional experiment carried out under the same conditions corresponding to the 'end' of the reaction.
2. The equation obtained enables modelling both linear and non-linear product accumulation.
3. Initial rate of *product accumulation* rather than *initial velocity* of enzyme-catalyzed *reaction* is more appropriate notion concerning the process.
4. Direct application of non-linear equation to experimental data of product accumulation enables better estimation of the enzyme turnover.

5. Direct fitting (without any transformations) of Henri hyperbola to experimental data concerning the dependence of product accumulation rate on substrate concentration enables better estimation of the enzyme selectivity.

Appendix A

For $p \ll s_0$ (thus for $s = s_0 - p \approx s_0$) and $1/\mathfrak{p} > 0$, the fractions of the 2nd equation of System (5) are (see subsection 3.1.2):

$$\begin{aligned} \frac{s}{s+\mathfrak{s}+(\mathfrak{s}/\mathfrak{p})p} &= \frac{s_0}{(s_0+\mathfrak{s})\left(1+(\mathfrak{s}/\mathfrak{p})p/(s_0+\mathfrak{s})\right)} \\ &\approx \frac{s_0}{s+\mathfrak{s}}\left(1-\frac{\mathfrak{s}}{\mathfrak{p}(s_0+\mathfrak{s})}p\right) \end{aligned} \tag{A.1}$$

for the forward reaction, and for the backward one:

$$\frac{p}{p+\mathfrak{p}+(\mathfrak{p}/\mathfrak{s})\,s} \approx \frac{p}{\mathfrak{s}\mathfrak{p}/\mathfrak{s}+s_0\mathfrak{p}/\mathfrak{s}} = \frac{\mathfrak{s}}{\mathfrak{p}(s_0+\mathfrak{s})}p. \tag{A.2}$$

It follows from System (5), taking into account expressions (A.1) and (A.2):

$$\frac{\mathrm{d}p}{\mathrm{d}t} \approx e_0\left[\frac{s_0}{s_0+\mathfrak{s}}\left(1-\frac{\mathfrak{s}}{(s_0+\mathfrak{s})\mathfrak{p}}p\right)\mu-\frac{\mathfrak{s}}{(s_0+\mathfrak{s})\mathfrak{p}}p\nu\right]. \tag{A.3}$$

It follows from equation (11) that

$$\begin{aligned} &\frac{\mathrm{d}}{\mathrm{d}s}\frac{e_0+s+\mathfrak{s}-\sqrt{(e_0+s+\mathfrak{s})^2-4e_0s}}{2e_0} \\ &\qquad = \frac{1-\dfrac{s-e_0+\mathfrak{s}}{\sqrt{(e_0+s+\mathfrak{s})^2-4e_0s}}}{2e_0} \end{aligned} \tag{A.4}$$

and for low s

$$\lim_{s\to 0}\frac{1-\dfrac{s-e_0+\mathfrak{s}}{\sqrt{(e_0+s+\mathfrak{s})^2-4e_0s}}}{2e_0} = \frac{1}{e_0+\mathfrak{s}} \tag{A.5}$$

From which follows equation (13) in Subsection 3.2.1 of the main text. Comparing the exponential terms of equations (9) and (17) and taking into account $c_S/c_P = \nu/\mu$ (see equations (3) and (4) in Subsection 3.1) results in the system

$$\begin{cases} \dfrac{e_0\mathfrak{s}\left(\dfrac{s_0}{s_0+\mathfrak{s}}\mu+\nu\right)}{(s_0+\mathfrak{s})\mathfrak{p}} = \omega_0, \\ \dfrac{s_\infty\left(p_\infty+\mathfrak{p}+(\mathfrak{p}/\mathfrak{s})s_\infty\right)}{p_\infty\left(s_\infty+\mathfrak{s}+(\mathfrak{s}/\mathfrak{p})p_\infty\right)} = \dfrac{\nu}{\mu}. \end{cases} \tag{A.6}$$

Solution of this system results in equations (19) and (20) in Subsection 4.3 of the main text.

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