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Transient enzyme kinetics: Graph-theoretic approach

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ABSTRACT

A graph-theoretic approach is shown to simplify the analysis of transient enzyme kinetics. The coefficients of the characteristic polynomial for kinetic equations are obtained by graphical construction of directed trees and sub-trees in the kinetic scheme. An example of kinetic schemes, providing a simple time-dependent analytical solution, is demonstrated. This example describes a substrate-inhibited enzymatic reaction and interprets the pH-dependent inhibition of the lactate dehydrogenase. It is shown that rapid equilibrium in some parts of the kinetic scheme can simplify the analysis. The enzyme pre-incubation with a product is shown to be characterized by the non-monotonous transient kinetics. This phenomenon is useful to estimate correctly the kinetic parameters. It is supposed that the lactate dehydrogenase substrate inhibition can be important for switching the glycolytic fluxes.

1. Introduction

Kinetics of enzymatic reactions in solution are studied traditionally in two initial phases: pre-steady-state (transient) phase and pseudo-steady-state phase [1,2].

In both of these phases the substrate concentration is taken in excess not to significantly decrease during the reaction. Such experimental conditions are used to describe transient kinetics by linear differential equations for concentrations of enzyme species as variables. For many linear kinetic equations the analytical solutions, as the sum of exponential time-dependent terms, are well known [1,2].

However, even in the case of exponential kinetics, interpretation of kinetic curves in terms of individual rate constants is often difficult. Therefore, any theoretical methods, simplifying the kinetic analysis, are desirable.

In this paper a graph-theoretic method to simplify the analysis of transient kinetics is discussed. I also apply the Laplace transform which is often used for solution of kinetic equations [3]. I consider only deterministic kinetic equations following the mass action law. No stochastic modifications of kinetic equations are considered, however, stochastic equations can be analyzed with a similar approach [4–7].

Recent finding of slow fluctuations in catalytic activity of single enzyme molecules [8] should be taken into account. However, recent studies [7] show that classical kinetic equations are often valid for fluctuating molecules, individual kinetic parameters being interpreted as effective ones [7]. Moreover, the conformational motions of single enzyme molecules are usually ensemble-averaged in experiments [9,10] not to modify classical kinetic equations.

In our earlier papers [11,12] a graph-theoretic approach had been applied to the analysis of pseudo-steady enzymatic reactions. That approach formulates the known schematic rule by King and Altman [13] with using graph-theoretic notations, thereby allowing various simplifications. A similar our approach has been applied to transient enzyme kinetics [14–16]. Our approach [14–16] was later modified by Kou–Chen Chou [3], who considered an example of one-exponential kinetics. A similar approach was also applied by Hofmeyr [17] to graphical analysis of metabolic pathways for graphical determination of flux and concentration control coefficients.

In this paper I summarize and modify my former results [14–16] to obtain a simple analytical time-dependent solution of kinetic equations. A graph-theoretic approach allows me to define a class of kinetic schemes leading to simple analytical dependences of reaction rates on individual kinetic parameters. One of such schemes is taken to describe the unusual pre-steady-state kinetics for lactate dehydrogenase.

Recent studies [9,10] present the data concerning fast and slow molecular dynamics during the lactate dehydrogenase catalyzed reaction. Atomic motions have been observed on various time scales. However, only the rate-limiting steps are observed in stopped-flow studies [9,10].

It is possible to set up the lactate dehydrogenase system as a reaction system of interconverting enzyme species [10]. Therefore, time-dependent kinetics for stopped-flow experiments can be interpreted by classical linear differential equations. I show that a minimal kinetic scheme can be used to interpret the experimental results [18,19].

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2. Theory

2.1. Graph-theoretic approach

Consider the following kinetic scheme:

$$X_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} X_2 \underset{k_{32}}{\overset{k_{23}}{\rightleftarrows}} X_3 - - - - X_n \tag{1}$$

Here X_i are various enzyme forms, k_{ij} are rate constants for corresponding reaction steps. Some k_{ij} can include the approximately constant substrate (ligand) concentration.

In traditional stopped-flow experiments [20] the reaction starts with the sudden substrate addition in excess to the enzyme solution.

The time course of the reaction (1) with approximately constant parameters, k_{ij}, is described by the linear differential equations:

$$\frac{dx_i}{dt} = -x_i \sum_{j \neq i} k_{ij} + \sum_{j \neq i} k_{ji} x_j, \tag{2}$$

where x_i are normalized X_i concentrations, $\sum ix_i = 1$.

By using the Laplace–Carson transform:

$$x_i^*(\lambda) = \lambda \int_0^\infty \exp(-\lambda t) x_i(t) dt \tag{3}$$

where x_i^* is transformed x_i, and λ is transformed time t, we obtain:

$$\left(\frac{dx_i}{dt}\right)^* = \lambda x_i^*(\lambda) - \lambda x_i(0) \tag{4}$$

Here, $x_i(0)$ specify initial $x_i(t)$ values.

Taking in mind the equality, $x_i^* + \sum_{j \neq i} x_j^* = 1$, we obtain from Eq. (4):

$$\frac{1}{\lambda}\left(\frac{dx_i}{dt}\right)^* = x_i^* - x_i(0)\left(x_i^* + \sum_{j \neq i} x_j^*\right), \tag{5}$$

and taking in mind the equality, $\sum_{j \neq i} x_j(0) = 1 - x_i(0)$, we rewrite Eq. (5) as Eq. (6):

$$\frac{1}{\lambda}\left(\frac{dx_i}{dt}\right)^* = x_i^* \sum_{j \neq i} x_j(0) - x_i(0) \sum_{j \neq i} x_j^*. \tag{6}$$

Now, using Eqs. (2), (6), we obtain Eq. (7):

$$x_i^* \sum_{j \neq i} \left(\lambda x_j(0) + k_{ij}\right) = \sum_{j \neq i} x_j^* \left(\lambda x_i(0) + k_{ji}\right). \tag{7}$$

One can see that Eq. (7) look identical to the steady-state equations with only difference in the modified rate constants, including now transformed time, λ, and initial values, $x_j(0)$.

Eq. (7) can be represented by the kinetic graphs, which are similar to the reaction schemes, but involve the additional branches with the values equal to $\lambda x_j(0)$ in parallel to the branches, k_{ij}. The additional branches characterize the reaction dependence on time.

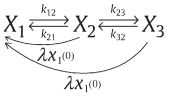
Let us illustrate the graphical procedure by considering a simple kinetic scheme:

$$X_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} X_2 \underset{k_{32}}{\overset{k_{23}}{\rightleftarrows}} X_3 \tag{8}$$

Suppose the only enzyme form, X_1, is present initially:

$$x_1(0) = 1, x_2(0) = x_3(0) = 0.$$

According to Eqs. (7), the following transient graph (9) is constructed:



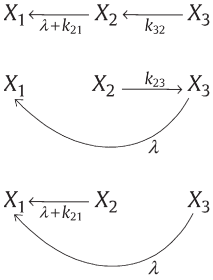
(9)

With applying the graphical rule by King and Altman [13] to the graph (9), the solution is given as:

$$x_i^* = \frac{D_i}{D_1 + D_2 + D_3} \tag{10}$$

where D_i are the sums of the so-called trees [11,12], directed to the graph node $X_i (i = 1, 2, 3)$.

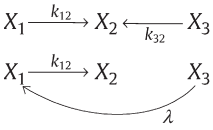
Three trees, directed to X_1, are shown in Eq. (11):



(11)

The sum of these trees is equal to $D_1 = (\lambda + k_{21})k_{32} + \lambda k_{23} + (\lambda + k_{21})\lambda$.

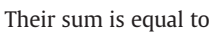
To the node, X_2, two trees are directed:



(12)

Their sum is equal to $D_2 = k_{12}k_{32} + \lambda k_{12}$.

There is a single tree directed to X_3:



(13)

Its value equals to $D_3 = k_{12}k_{23}$.

With using trees (11–13) one can easily write equations for variables x_i^*. For example, x_2^* is equal to:

$$x_2^* = \frac{\lambda k_{12} + k_{12}k_{32}}{\lambda^2 + \lambda(k_{21} + k_{32} + k_{23} + k_{12}) + (k_{32}k_{21} + k_{12}k_{32} + k_{12}k_{23})} \tag{14}$$

It is seen from the denominator of Eq (14) that the coefficient, $(k_{21} + k_{32} + k_{23} + k_{12})$ of the λ-term is constructed as the sum of all branches (1 − trees) of the scheme (8), and the free coefficient, $(k_{32}k_{21} + k_{12}k_{32} + k_{12}k_{23})$, is the sum of paired non-cyclic combinations of the branches (2 − trees) taken from scheme (8).

The graphical rule to construct the Laplace-transformed solution for any kinetic schemes has been formulated in my earlier paper [16]:

$$x_i^* = \frac{b_0^{(i)}\lambda^n + b_1^{(i)}\lambda^{n-1} + \ldots + b_n^{(i)}}{\lambda^n + a_1\lambda^{n-1} + \ldots + a_n} = \frac{D_i}{\sum_i D_i} \tag{15}$$

The denominator in Eq. (15) is the characteristic polynomial of kinetic equations [16]. The coefficients, a_i, are constructed by trees and sub-trees of the corresponding kinetic scheme, a_1 being the sum of all branches (1 − trees), a_2 − the sum of 2 − trees, …, a_n − the sum of n-trees (trees of the King–Altman rule [13]). The nominator of Eq. (15) is

constructed by the trees directed to the i-th node in the kinetic scheme. The nominator coefficients depend on the initial conditions, and the denominator coefficients do not. The value of the branch (or simply "branch"), $i \to j$, is equal to the value of the rate constant, k_{ij}. The value of the tree (and sub-trees) is equal to product of its branches. An example of the expressions for the coefficients, a, b, is given in Eq. (14).

To find the original, $x_i(t)$, of the transformed function, $x_i^*(\lambda)$, one needs to find the roots (eigenvalues) of the characteristic polynomial. For example, the time-dependent solution for scheme (8) is:

$$x_2(t) = A_0 + A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} \tag{16}$$

where λ_1, λ_2 are eigenvalues of the characteristic polynomial, that is the denominator of Eq. (14):

$$D = D_1 + D_2 + D_3 = (\lambda + \lambda_1)(\lambda + \lambda_2) \tag{17}$$

The constants, A_0, A_1, A_2, depend on λ_1, λ_2 and initial conditions. The exponents, λ_1 and λ_2, depend on the rate constants, k_{ij}, but simple analytical dependence on k_{ij} is obtained for kinetic schemes of the specific structure only, as exampled in the next section.

For a pseudo-steady state we should take $\lambda = 0$, and obtain:

$$x_i^*(0) = x_i(\infty) = b_n^{(i)} / a_n.$$

2.2. Analytical solution

A graph-theoretic approach of this paper allows us to construct the characteristic polynomial by finding directed trees and sub-trees in the kinetic scheme and allows us also to find specific schemes, having the simple analytical time-dependent solution. Some of solutions are well known [1,2,20]. Others are not mentioned in the literature, but they can be of practical importance. This paper considers an example.

The simple directed sequence of steps:

$$X_1 \to X_2 \to X_3 \ldots X_{n-1} \to X_n \tag{18}$$

has the characteristic polynomial:

$$D = (\lambda + k_{12})(\lambda + k_{23}) \ldots \left(\lambda + k_{(n-1)n}\right) \tag{19}$$

where the exponents of the solution are simply the rate constants. Another kinetic scheme:

$$X_1 \to X_2 \to X_3 \ldots X_{n-1} \rightleftharpoons X_n \tag{20}$$

has the characteristic polynomial:

$$D = (\lambda + k_{12})(\lambda + k_{23}) \ldots \left(\lambda + k_{(n-1)n} + k_{n(n-1)}\right) \tag{21}$$

The time-dependent solution in this case is also simple.

I consider a simple example, applicable to many enzymatic reactions, a partial case of scheme (20), presented by scheme (8) with $k_{21} = 0$.

Consider the following initial condition:

$$x_1(0) \neq 0, x_2(0) \neq 0, x_3(0) = 0 \tag{22}$$

Using the above graphical procedure, we obtain the following solution:

$$x_2(t) = \frac{k_{32}}{k_{32} + k_{23}} + \frac{x_2(0) k_{23} (k_{32} + k_{23} - k_{12} / x_2(0))}{(k_{32} + k_{23})(k_{32} + k_{23} - k_{12})} e^{-(k_{32} + k_{23})t} - \frac{x_1(0)(k_{32} - k_{12})}{k_{32} + k_{23} - k_{12}} e^{-k_{12} t} \tag{23}$$

This Eq. (23) is applied here for interpretation of the transient kinetics for the lactate dehydrogenase.

2.3. Time hierarchy of enzyme systems

In our earlier paper [12] a simplification of the graph-theoretic analysis has been demonstrated for rapid-equilibrium enzymatic multi-cyclic reaction schemes. These reactions can be represented by a single tree of equilibrium steps [12]. One of the equilibrium steps in each cycle of the rapid-equilibrium scheme can be eliminated due to the detailed balance providing interdependence of the kinetic parameters.

This simplification had been modified by Cha [21] in application to the pseudo-steady kinetic mechanisms, involving a number of rapid-equilibrium parts interconnected by more slow steps.

According to the procedure by Cha [21] all rapid-equilibrium parts are represented by single nodes in the new graph, the slow kinetic steps being characterized by the effective rate constants dependent on the relative concentrations of the enzyme forms in the rapid-equilibrium reaction parts.

This paper applies the procedure by Cha [21] to pre-steady-state kinetics. In this way many enzymatic reactions can be reduced to the two-exponential mechanisms, and many can be described by formula (23).

Consider, as an example, a two-substrate reaction, following the ordered mechanism, the reaction catalyzed by the lactate dehydrogenase (LDH), interacting with coenzyme, NADH, and substrate, pyruvate, to produce product, lactate, and coenzyme, NAD [22,23].

The M_4 isozyme of LDH, pre-incubated with NAD, had been shown to be inhibited by its substrate pyruvate, similarly to the known inhibition of the H_4 isozyme of LDH [18,19].

Similar (but not identical) substrate inhibition of M_4 and H_4 isomers of the lactate dehydrogenase depends on pH values. The pH change, pH8 → pH6, induces the enzyme conformational transition to the protonated form, which can be a substrate-inhibited form [18,19].

Substrates, NADH and pyruvate, bind in the protonated enzyme form rapid enough and can be considered as being in rapid equilibrium.

The reaction can be represented by the following simplified scheme:

$$E \overset{\searrow \mathrm{NADH}}{\rightleftharpoons} E \cdot \mathrm{NADH} \overset{\searrow \mathrm{pyr}}{\rightleftharpoons} E_{\mathrm{NADH}}^{\mathrm{pyr}} \nearrow\!\!\to \mathrm{lac}\, E \cdot \mathrm{NAD} \overset{\searrow \mathrm{pyr}}{\rightleftharpoons} E_{\mathrm{NAD}}^{\mathrm{pyr}} \tag{24}$$

Here E is free enzyme, NADH and NAD are coenzymes, pyr denotes pyruvate, lac denotes lactate.

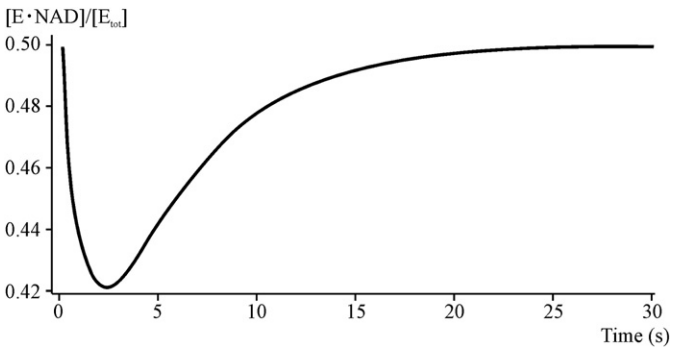


Fig. 1. Normalized concentration, $[E \cdot \mathrm{NAD}] / [E_{\mathrm{tot}}] = x_2(t)$, calculated for scheme (8) with parameters: $k_{21} = 0$, $k_{12} = 0.2\ \mathrm{s}^{-1}$, $k_{23} = 0.4\ \mathrm{s}^{-1}$, $k_{32} = 0.4\ \mathrm{s}^{-1}$. Calculation simulates the LDH catalyzed reaction at pH 8.

In the experiments [19], LDH had been pre-incubated with NAD. The slow reverse catalytic step is neglected. The rapid-equilibrium steps can be represented by the following equilibrium tree:

$$E \longrightarrow K_N[\mathrm{NADH}]\, E\cdot\mathrm{NADH} \longrightarrow K_S[\mathrm{pyr}]\, E^{\mathrm{pyr}}_{\mathrm{NADH}}, \tag{25}$$

where K_N and K_S are the equilibrium association constants.

The enzyme fraction in the state $E^{\mathrm{pyr}}_{\mathrm{NADH}}$ is calculated according to the tree (25):

$$f = \frac{ns}{1+n+ns}, \tag{26}$$

where n, s are dimensionless concentrations of NADH and pyruvate, correspondingly. If LDH is saturated by coenzyme NADH, Eq. (27) should be used instead of Eq. (26):

$$f = \frac{s}{1+s} \tag{27}$$

A variable x_1 in the simplified scheme (8) is now the following:

$$x_1 = \left([E] + [E\cdot\mathrm{NADH}] + [E^{\mathrm{pyr}}_{\mathrm{NADH}}]\right) / \sum E_i \tag{28}$$

Normalized concentrations, x_2 and x_3, are now the concentrations of $E\cdot$NAD and $E^{\mathrm{pyr}}_{\mathrm{NAD}}$, divided by $\sum E_i$, where $\sum E_i$ is the sum of concentrations for all enzyme forms presented in scheme (24).

In scheme (8), k_{12} represents now the rate constant for a step $E^{\mathrm{pyr}}_{\mathrm{NADH}} \rightarrow E\cdot NAD$ multiplied by the factor f of Eq. (26) or Eq. (27).

The constant, k_{21}, is neglected, as representing the slow reverse catalysis, and k_{23}, k_{32} are constants for the rates of the reversible substrate inhibition.

One can see that Eq. (23) can simulate the pre-steady-state $E\cdot NAD$ formation in the LDH-catalyzed reaction.

2.4. Enzyme pre-incubation with the product

Two-exponential transient rate equations, known in the literature [1,2], describe monotonous kinetic curves. These equations relate to enzymatic reactions started with a single non-zero enzyme form (free enzyme).

Non-monotonous behaviour has been observed for the lactate dehydrogenase pre-incubated with two coenzymes, NADH and NAD [19]. For the reaction, presented by scheme (8) this pre-incubation leads to the two non-zero initial concentrations, $x_1(0) \neq 0$, $x_2(0) \neq 0$. Pre-incubation stimulates the abortive inhibition started immediately after the sudden pyruvate addition.

Figs. 1 and 2 show the simulation of the experimental kinetics for the pre-incubated LDH with using Eq. (23). The calculated variable, $x_2 = [E\cdot\mathrm{NAD}] / [E_{\mathrm{tot}}]$, describes the LDH transient activity. Fig. 1 shows a typical non-monotonous dependence similar to the observed at pH 8 for the lactate dehydrogenase, when the substrate inhibition has been less intensive.

The lower constant, $k_{32} = 0.1\ \mathrm{s}^{-1}$, in Fig. 2 (instead of $k_{32} = 0.4\ \mathrm{s}^{-1}$, in Fig. 1), induces typical monotonous inhibition as observed experimentally for LDH at pH 6.

Other parameters are taken the same in the both Figs. 1 and 2, and are taken close to the known values for the lactate dehydrogenase: $k_{12} = 0.2\ \mathrm{s}^{-1}$, $k_{23} = 0.4\ \mathrm{s}^{-1}$ [19].

The initial conditions for the pre-incubation are taken the following:

$$x_1(0) = x_2(0) = 0.5, x_3(0) = 0 \tag{29}$$

Comparison of the two drastically different figures, Figs. 1 and 2, allows us to correctly estimate the pH-influence on the LDH substrate inhibition. The simulation explains how the substrate inhibition can be fully eliminated after a short time period, when the pH value changes to pH 8. Both figures are obtained for equally pre-incubated LDH.

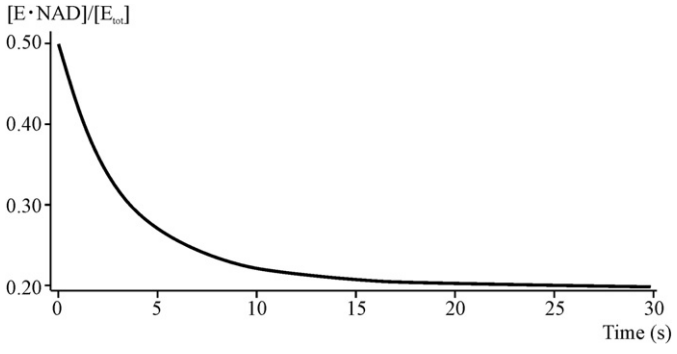


Fig. 2. Similar dependence as in Fig. 1 for changed parameter, $k_{32} = 0.1\ \mathrm{s}^{-1}$. The changed parameter simulates the LDH substrate inhibition at pH 6.

3. Discussion

A graph-theoretic approach in this paper simplifies the analysis of transient enzyme kinetics by graphical construction of coefficients in the characteristic polynomial of kinetic schemes. This graph-theoretic approach leads here to a simple analytical solution of kinetic equations for two-substrate enzymatic reactions, inhibited by the abortive enzyme-substrate complex formation. This solution describes the kinetic effect of enzyme pre-incubation with the product and interprets the observed non-trivial LDH transient kinetics. A kinetic curve with the intermediate minimum, observed for the lactate dehydrogenase, is interpreted on the basis of a simple kinetic scheme with using realistic values of the rate constants.

The pH-dependent substrate inhibition of the lactate dehydrogenase should be important for switching the pH-dependent branched aerobic/anaerobic glycolytic fluxes. Indeed, our modified scheme [24], including the pyruvate influx in addition to the scheme discussed in this paper, describes a switch-like kinetic behaviour of the lactate dehydrogenase.

The pyruvate influx can be realized *in vitro* as well as *in vivo* for some experimental situations. The known spontaneous pH-dependent transitions between pyruvate isomers can produce the pyruvate influx from inactive isomers to active keto-pyruvate [24]. Possibly, this situation had been observed for the lactate dehydrogenase isolated from the non-mammalian sources, where the switch-like substrate inhibition had been demonstrated [25,26].

It should be mentioned that the pyruvate concentration in the metabolic system can be a variable similarly to variable enzyme concentrations. Therefore, the enzymatic reaction *in vivo* can be non-linear in contrast with the linear pre-steady-state reaction, discussed in this paper.

A graph-theoretic approach can be applied to the analysis of the linearized non-linear kinetic equations [27–29]. Coefficients of the characteristic polynomial in the last case are constructed not only by the non-cyclic structures (trees), but by cycles, arising from substrate participation in the inhibition step. The competition of the same substrate for catalysis and inhibition produces the graphical cycle in the kinetic scheme, generating the negative coefficient in the characteristic polynomial [29]. The substrate inhibition can be of critical type in this case. The non-linear substrate-inhibited reaction, catalyzed by the lactate dehydrogenase, can produce the critical inhibition (very rapid and hysteretic), as could be needed to switch glycolytic fluxes.

The pre-steady-state LDH kinetics, simulated in this paper, provides necessary information concerning the pH-dependent

substrate inhibition, which has been observed in the closed system [18,19], but can be important for the open system.

References

[1] C.F. Bernascony, Relaxation Kinetics, Academic Press, New York, 1976.
[2] A. Fersht, Structure and Mechanism in Protein Science: A guide to Enzyme Catalysis and Protein Folding, Freeman and Co., New York, 1999.
[3] K.C. Chou, Graphic rules in steady and non-steady state enzyme kinetics, J. Biol. Chem. 264 (1989) 12074–12079.
[4] O. Flomenbom, J. Klafter, Closed-form solution for continuous time random walks on finite chains, Phys. Rev. Lett. 95 (2005) 098105.
[5] O. Flomenbom, K. Velonia, D. Loos, S. Masuo, M. Cotlet, Y. Engelborghs, J. Hofkens, A.E. Rowan, R.J.M. Nolte, M. Van der Auweraer, F.C. de Schryver, J. Klafter, Stretched exponential decay and correlations in the catalytic activity of fluctuating single lipase molecules, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 2368–2372.
[6] S.C. Kou, B.J. Cherayil, W. Min, B.P. English, X.S. Xie, Single-molecule Michaelis–Menten equations, J. Phys. Chem. B 109 (2005) 19068–19081.
[7] W. Min, I.V. Gopich, B.P. English, S.C. Kon, X.S. Xie, A. Szabo, When does the Michaelis–Menten equation hold for fluctuating enzymes? J. Phys. Chem. B 110 (2006) 20093–20097.
[8] H. Yang, G. Luo, P. Karnchanaphanurach, T.M. Louie, I. Rech, S. Cova, L. Xun, X.S. Xie, Protein conformational dynamics probed by single-molecule electron transfer, Science 302 (2003) 262–266.
[9] H. Deng, S. Brewer, D.M. Vu, K. Clinch, R. Callender, R.B. Dyer, On the pathway of forming enzymologically productive ligand–protein complexes in lactate dehydrogenase, Biophys. J. 95 (2008) 804–813.
[10] N. Zhadin, M. Gulotta, R. Callender, Probing the role of dynamics in hydride transfer catalyzed by lactate dehydrogenase, Biophys. J. 95 (2008) 1974–1984.
[11] M.V. Volkenstein, B.N. Goldstein, A new method for solving the problems of the stationary kinetics of enzymological reactions, Biochim. Biophys. Acta 115 (1966) 471–477.
[12] M.V. Volkenstein, B.N. Goldstein, Allosteric enzyme models and their analysis by the theory of graphs, Biochim. Biophys. Acta 115 (1966) 478–485.
[13] E.L. King, C. Altman, A schematic method of deriving the rate laws for enzyme-catalyzed reactions, J. Phys. Chem. 60 (1956) 1375–1378.
[14] M.V. Volkenstein, B.N. Goldstein, V.E. Stefanov, Investigation of non-stationary enzymatic reactions by graph-theoretic method, Molek. Biol. (Moscow) 1 (1967) 52–58.
[15] B.N. Goldstein, M.V. Volkenstein, Investigation of non-stationary complex monomolecular reactions by graph-theoretic method, Russ. Dokl. 178 (1968) 386–388.
[16] B.N. Goldstein, Analysis of cyclic enzyme reaction schemes by the graph-theoretic method, J. Theor. Biol. 103 (1983) 247–264.
[17] J.H.S. Hofmeyr, Control-pattern analysis of metabolic pathways, Eur. J. Biochem. 186 (1989) 343–354.
[18] L.O. Yagodina, E.A. Saburova, Biphase reaction of the enzyme-catalyzed pyruvate reduction during inhibition, Molek. Biol. (Moscow) 18 (1984) 653–658.
[19] L.O. Yagodina, E.L. Shevelev, T.T. Smolyninova, B.N. Goldstein, D.S. Markovich, Investigation of kinetic manifestation of slow conformational transitions in the lactate dehydrogenase, Molek. Biol. (Moscow) 20 (1986) 61–71.
[20] H. Gutfreund, Transient and relaxation kinetics of enzyme reactions, Annu. Rev. Biochem. 40 (1971) 315–344.
[21] S. Cha, A simple method for derivation of rate equations for enzyme-catalyzed reactions under rapid equilibrium assumption or combined assumptions of equilibrium and steady-state, J. Biol. Chem. 243 (1968) 820–825.
[22] J.J. Holbrook, A. Lijas, S.J. Steindel, M.G. Rossman, Lactate dehydrogenase, Enzymes 11 (1975) 191–292.
[23] J. Everse, N.O. Kaplan, Lactate dehydrogenase: structure and function, Adv. Enzymol. 37 (1973) 61–133.
[24] S.R. Saifullin, B.N. Goldstein, Slow substrate transitions in the enzyme reaction. 1. Effect of the pyruvate forms in the steady-state reaction catalyzed by the lactate dehydrogenase, Molek. Biol. (Moscow) 19 (1985) 1092–1099.
[25] G.N. Somero, Thermal modulation of pyruvate metabolism in the fish *Gillichthys mirabilis*: the role of lactate dehydrogenase, Comp. Biochem. Physiol. B 44 (1973) 205–209.
[26] J. Soler, D. de Arriaga, Q. Cadenas, E. Cadenas, Substrate inhibition of lactate dehydrogenase from *Phycomyces blakesleeanus*: NADH dependence, Exp. Mycol. 5 (1981) 357–362.
[27] B.N. Goldstein, A.N. Ivanova, Hormonal regulation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: kinetic model, FEBS Lett. 217 (1987) 212–215.
[28] B.N. Goldstein, A.A. Maevsky, Critical switch of the metabolic fluxes by 6-phosphofructo-2-kinase:fructose-2-6-bisphosphatase. A kinetic model, FEBS Lett. 532 (2002) 295–299.
[29] B. Goldstein, Switching mechanism for branched biochemical fluxes: Graph-theoretical analysis, Biophys. Chem. 125 (2007) 314–319.