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THE NONALLOSTERIC MECHANISM OF ENZYME ACTIVITY REGULATION. IS IT THE ONLY TRUE MECHANISM ?

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Abstract: The nonallosteric regulation mechanism of enzyme reaction velocity assumes that the substrate and enzyme interact via a metal cation and form simple and mixed, mono- and multi-nuclear complexes. A solution of equations for individual cases gives a function of initial reaction velocity at any given substrate or modifier concentration. This function can describe kinetic effects that are considered allosteric, as well as phenomena omitted by commonly-accepted models.

Key Words: Enzyme Kinetics, Equilibrium Reaction, Complex, Chelate, Oscillatory Reaction, Control Mechanism, PID Controller

GENERAL MODEL

This paper shows that the mathematical method accepted for fractal geometry leads to a very simple explanation of complicated kinetics [1]. London and Stack's paper [2] had an untimely publication, before fractal theory was evolved, hence this brilliant idea and solution for the proposed equation system remains to this day eclipsed by commonly known models [3, 4]. I have no intention of

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Abbreviations used: S - substrate; P - product; M - modifier (metal cation); SS - substrate dimer or simple complex (MS_2); PP - product dimer or simple complex (MP_2); SP - complex of substrate with product or mixed complex (MSP); E - enzyme; ES - enzyme-substrate complex; ESS, EPP, etc - multinuclear complexes; $v_0(S)$ - function of initial reaction velocity (dependent variable) on substrate concentration (independent variable); S(t) - function of substrate concentration on time; ∂ or d - derivative; kxy - reaction velocity constant for reaction of X with Y; Kxy - equilibrium constant for XY complex; PID - proportional-integral-derivative controller.

Symbols for PID controller are defined in the text. The square brackets denoting concentration are omitted.

discussing the popular models here, but will instead focus on this thus far ignored concept.

If an enzyme changes conformation as the result of binding of a ligand, that is not evidence that changes in the kinetics of the enzyme catalysed reaction result from these conformational changes. The London and Steck model of the regulation of enzyme reaction velocity [2] is free from this shortcoming. In this model, there is a correlation between reaction velocity and the concentration of a substrate or a catalyst (enzyme) or the concentration of any transition complex; this is consistent with kinetic laws.

London and Steck considered a one-substrate reaction catalysed by a one-subunit enzyme. As it was based on the obvious reactions of consecutive complex formation, the model was too complicated for mathematical simulation at the time it was proposed, and therefore was divided into special cases. For a mathematical description of the steady state of each particular case, the authors proposed a system of equations composed of one solution of a second order equation for the reaction of a substrate with a modifier (the lower root of the mass action law equation), and linear equations for the interactions of all reagents with an enzyme. The solution of this system of equations results in a function of initial reaction velocity at any given substrate concentration $v_0(S)$ or a function of v_0 at any given modifier concentration $v_0(M)$. The curves specific to the separate special case functions $v_0(S)$ or $v_0(M)$, in terms of shape, resemble exponential functions with one or several points of inflection. The curves appear: hyperbolic, characteristic of a regular Michaelis-Menten curve; sigmoid, characteristic of multiple interacting enzyme subunits (positive or negative cooperation); monotonic or nonmonotonic with several inflection points, characteristic of inhibition by an excess of substrate or of preservation from inhibition by a substrate; and also monotonic with several inflection points, similar to a titration curve.

This model explains effects interpreted as allosteric. It also explains nonmonotonic curves and an improper fractional Hill coefficient in the context of the reactions' scheme and in the context of the assumed steady state equations. In the considered example, ATP is the substrate and magnesium cation is the modifier. The interactions which occur between organic compounds are similar to those between metal cations and ligands; therefore, this model can be considered a general one for studies of enzyme reactions complicated by equilibrium substrate and enzyme reactions with any component of the reaction medium.

EXAMPLES OF STEADY STATE KINETICS

The reactions controlled by metabolites which are not allosteric effectors are well known. There are reactions catalysed by enzymes which do not have domains capable of binding effectors or effectors that do not influence enzyme conformation. Examples of such enzymes are: muscle pyruvate kinase isozyme,

fructose-1,6-bisphosphatase, thiamine pyrophosphokinase, NADP-isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase. Some enzymes exhibit a nonmonotonic dependency of the initial reaction velocity on the concentration of the substrate, the concentration of bivalent metal cations, or on the concentration of compounds known as effectors. This group of enzymes includes: pyruvate kinase [5-9], phosphofructokinase [10], bifunctional enzyme fructose-2,6-bisphosphate kinase/phosphatase [11, 12], fructose-1,6-bisphosphatase [13, 14]; pyrophosphate moiety transferring enzymes: phosphoribosyl-diphosphate synthase and thiamine pyrophosphokinase [15, 16]; NADP dependent dehydrogenases: of glucose-6-phosphate [17-19], of isocitrate [20] and of malate [21]; protein kinases and phosphatases-controlled enzyme complexes of pyruvate dehydrogenase complex [22] and of ketoglutarate dehydrogenase complex [23, 24]. The leading allosteric enzyme, aspartate transcarbamylase, shows nonmonotonic, two-peaked function of activity against aspartate concentration [25 p. 1393], and this function is explicable using the model of London and Steck (examples are given in Fig. 2); for supplementary justification, see [55, 56].

In order to explain the nonmonotonic, exponential function of the activity of thiamine pyrophosphokinase against ATP or magnesium salt concentration, Vinogradov and Strumilo [16] assumed a simple scheme of the interaction of the enzyme-substrate complex with the magnesium ion and via the magnesium ion bridge with the excess of the substrate. A special case function of the London-Steck model is a good fit for dozens of the measured values of initial velocity at different ATP and magnesium sulphate concentrations. The same function was used to describe the activity of pyruvate kinase from the adrenal cortex, and its activation by a low level and inhibition by a high level of ADP or Mg^{++} [26]. This function fits the dependency of glutamine synthetase activity on ATP and on Mg^{++} concentration [27] and the activity of hypoxanthine/guanine phosphoribosyltransferase [28].

Since the authors only considered a one-substrate reaction, i.e. at pseudo first order conditions, a certain number of models proposed for the explanation of enzyme bisubstrate or for enzyme coupled reactions can be considered as expanded particular special cases of the general London-Steck model. The inhibition of glucose-6-phosphate dehydrogenase by an excess of the substrate (NADP) [18] and the mutual inhibition of pyruvate kinase and lactate dehydrogenase coupled reaction by the substrates [8, 9] may be examples of this case. The scheme postulated for the explanation of the inhibition of gluconate kinase by ATP assumed the formation of binary and tertiary, simple and mixed magnesium complexes with the enzyme, gluconate and ATP [29]. The mechanism of cytochrome P450 interaction, in a disubstrate reaction, in which the first and second substrate might react with the enzyme and with the ES complex [30], is also derivable from the general London-Steck model.

CONVERGENCE

The schemes mentioned above explain only a part of the observed phenomena. Studies of enzyme reaction kinetics, based on analysis of the entire reaction progress curve were occasionally reported on. The majority of enzyme kinetic studies only considered the initial reaction velocity and hence oscillatory reaction progress was frequently omitted, and for enzyme bisubstrate reactions was only occasionally reported on [8, 9, 13, 31-35 and cited therein].

The maximum coordination number of bivalent metal cations is 4 or 6, hence bivalent cations can form chelate polymers with multidentate ligands such as the pyrophosphate moiety of ATP and ADP, or phosphate esters containing more than one phosphate moiety. Thus, in a solution containing magnesium or calcium salts and pyrophosphate esters, the slow velocity of the dissociation reaction of the chelate polymers of the substrates, products and enzyme-substrate complexes with bivalent cations corresponds to a slow dissociation velocity of tightly binding inhibitors such as transition state analogs [36 and cited therein]. The simple mechanism of slow, reversible enzyme inhibition was proposed for the reaction with damped velocity oscillations [37]. If one assumes that an enzyme is inhibited by its substrate or product, i.e. that an active ES complex can form inactive ESS or ESP complexes, the mechanism can be thought to be a part of the general model.

Considering the influence of phosphate and pyrophosphate on the general progress of a pyruvate kinase reaction, the formation of the same magnesium complexes in solution was postulated, such as the one postulated in the enzyme active centre, i.e. the one assumed to be formed by a sequential bisubstrate reaction mechanism. In this case, both mixed and simple complexes should be formed. Taking into account that it is impossible to distinguish the enzyme inhibition process from equilibrium reactions of the association-dissociation of substrate complexes [38], and that the stability constants of mixed complexes are unknown, the London and Steck solution is judged as a minimal equation system for the dependence of pyruvate kinase activity on chelating agents, such as pyrophosphates [9].

In the case of an enzyme reaction preceded by equilibrium reactions, if the velocity of the general reaction is relatively high, and the consecutive equilibrium reactions of the substrates and products are relatively slow, the initial conditions of the reaction change along with the progress of the reaction. Thus, the general kinetics of such reaction can be described by a kinetic equation with a time dependent rate coefficient [39]. The commonly accepted modifications of the Michaelis-Menten equation limit all the equilibria existing in the reacting solution to the enzyme's interaction with a substrate, with a modifier (or inhibitor), resulting in an unclear improper fractional Hill coefficient. The Hill coefficient is frequently dependent on the reaction conditions [40], i.e. on the initial substrate concentration. Considering the Hill coefficient as a reaction order, such a dependency discloses relatively slow,

reversible reactions of the substrate, independent of the enzyme, [41]. A good example is phosphatase kinetics [42] for which, as is usual for a detergent, the aggregation of aromatic phosphate is acceptable. According to fractal kinetics theory, the order of such a general reaction is determinable [39, 43].

CONTROL SYSTEM

The proportional-integral-derivative control (PID control) is a widely used control strategy for various automated systems in industry [44-47]. Let us consider a steady state process (Fig. 1.) for this, when the input is equal to the output.

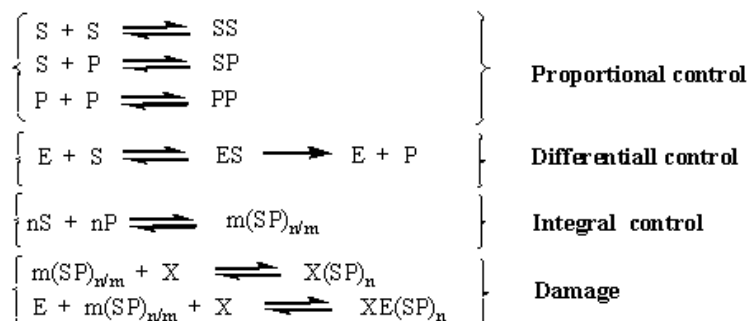
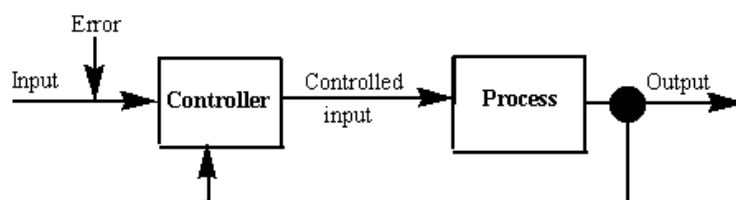


Fig. 1. A simple feedback loop. Reversible reactions function as a PID controller. E - enzyme, S - substrate, P - product, X - nucleation agent. The equations of proportional control and differential control represent the relations: $E + SS = ESS$, $E + PP = EPP$ and $E + SP = ESP$ (for explanation see [38 p. 162]). For a simulation see Fig. 2 D.

A PID controller consists of three elements:

P - proportional control - introduces a correction to the input. The correction (C_p) is in proportion to the error:

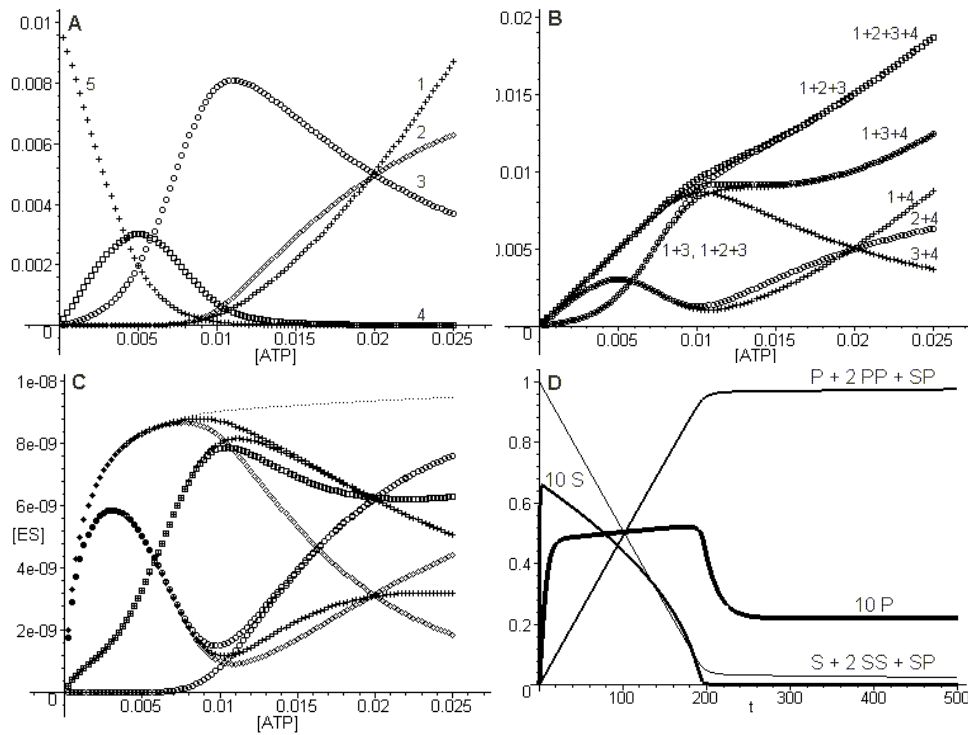
$$E = O_s - O_a$$

$$C_p = K_p E$$

when: E - error, O_s - set point output, O_a - actual output, K_p - proportionality factor.

After correction, the output signal oscillates around a set point.

D - differential control - introduces the C_D correction, which depends on the intensity of the error change in time.



$$eq1 := \frac{\partial}{\partial t} S(t) = -2 k_{ss} (S(t)^2 - K_{ss} SS(t)) - k_{sp} (S(t) P(t) - K_{sp} SP(t)) - \frac{V_m S(t)}{K_m + S(t)}$$

$$eq2 := \frac{\partial}{\partial t} P(t) = \frac{V_m S(t)}{K_m + S(t)} - k_{sp} (S(t) P(t) - K_{sp} SP(t)) - 2 k_{pp} (P(t)^2 - K_{pp} PP(t))$$

$$eq3 := \frac{\partial}{\partial t} PP(t) = k_{pp} (P(t)^2 - K_{pp} PP(t))$$

$$eq4 := \frac{\partial}{\partial t} SP(t) = k_{sp} (S(t) P(t) - K_{sp} SP(t))$$

$$eq5 := \frac{\partial}{\partial t} SS(t) = k_{ss} (S(t)^2 - K_{ss} SS(t))$$

$$init := S(0) = 0, P(0) = 0, SS(0) = .5, PP(0) = 0, SP(0) = 0$$

$$[V_m = .005, K_m = .001, k_{ss} = 10., k_{pp} = 1., k_{sp} = .1, K_{ss} = .01, K_{pp} = .001, K_{sp} = .01]$$

Fig. 2. Simulated curves obtained for the London and Steck model. A. The calculated concentration of calcium cation complexes with ATP: 1) ATP (aquo-complex), 2) $\text{Ca}(\text{ATP})_2$, 3) CaATP , 4) Ca_2ATP , 5) Ca^{2+} (aquo-complex). B. The sum of the concentrations of the complexes. Assumed $-\lg(K)$ for equilibria: $\text{Ca}^{2+} + \text{ATP} = \text{CaATP}$ 5.28, $\text{CaATP} + \text{Ca}^{2+} = \text{Ca}_2\text{ATP}$ 2.29, $\text{CaATP} + \text{ATP} = \text{Ca}(\text{ATP})_2$ 2.89 (the stability constants were taken from [54]); total concentration of $\text{Ca}^{2+} = 0.01$. C. A simulation of ES concentration on the assumption that the enzyme can react with one or more than one complex of ATP, $K_s = 1$ mM. D. The simulation of the progress of the London and Steck model reactions; for these S and P are the modifier. The parameters and equations are shown below the graphs. (Brackets denoting concentrations are omitted).

$$C_D = K_d \frac{dE}{dt}$$

K_d - damping constant. The correction damps the output signal oscillation.

I - Integral control - introduces a correction proportional to the error integral.

$$C_I = K_i \int E(t)dt$$

This correction corrects the steady state, reset level.

General correction: $C = C_p + C_D + C_I$

The fading oscillations of the reaction velocity presented for the pyruvate kinase reaction [9] are similar to the oscillations of the PID controller [44-47]. The assumed reversible reactions of the substrates, resulting in chelate oligomers, function as a proportional control. The reactions of the substrates and products with the enzyme function as a differential control, and the formation reactions of the inert chelate oligomers function as an integral control. Thus, the scheme presented for pyruvate kinase resembles a PID controlled process. The general London-Steck model consists of two elements, a proportional control and a differential control; when supplemented with the formation reactions of inert complexes of substrates and products, i.e. chelate oligomers, the model seems to be a general one for automatic control system of cell metabolism (for a simulation, see Fig. 2; for a simulated PID controller, see ref 45).

CONCLUSION

The functions of this model ($v_0(S)$ and $v_0(M)$) not only fit the dependency of enzyme reaction velocity, but also fit the dependency of ribozyme reaction velocity on the concentration of salts of bivalent metals [9, 48, 49]. These functions can be useful to describe general consecutive, reversible and catalytic reactions. Kinetic laws derive from probability calculus [39], so the functions of this model cannot describe kinetics in extremely diluted solutions or in trace volume.

The model enables the interpretation of many observed phenomena that seem to show a real mechanism of regulation. Disruption of this controlling mechanism by compounds which can form inert simple or inert mixed complexes with substrates such as phosphates, pyrophosphates and with metal cations seems to be recognised. Fluorides, aluminium salts and silicates may be examples of such compounds and can promote a heterogeneous nucleation of inert complexes of bivalent metals with phosphates [50-53].

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