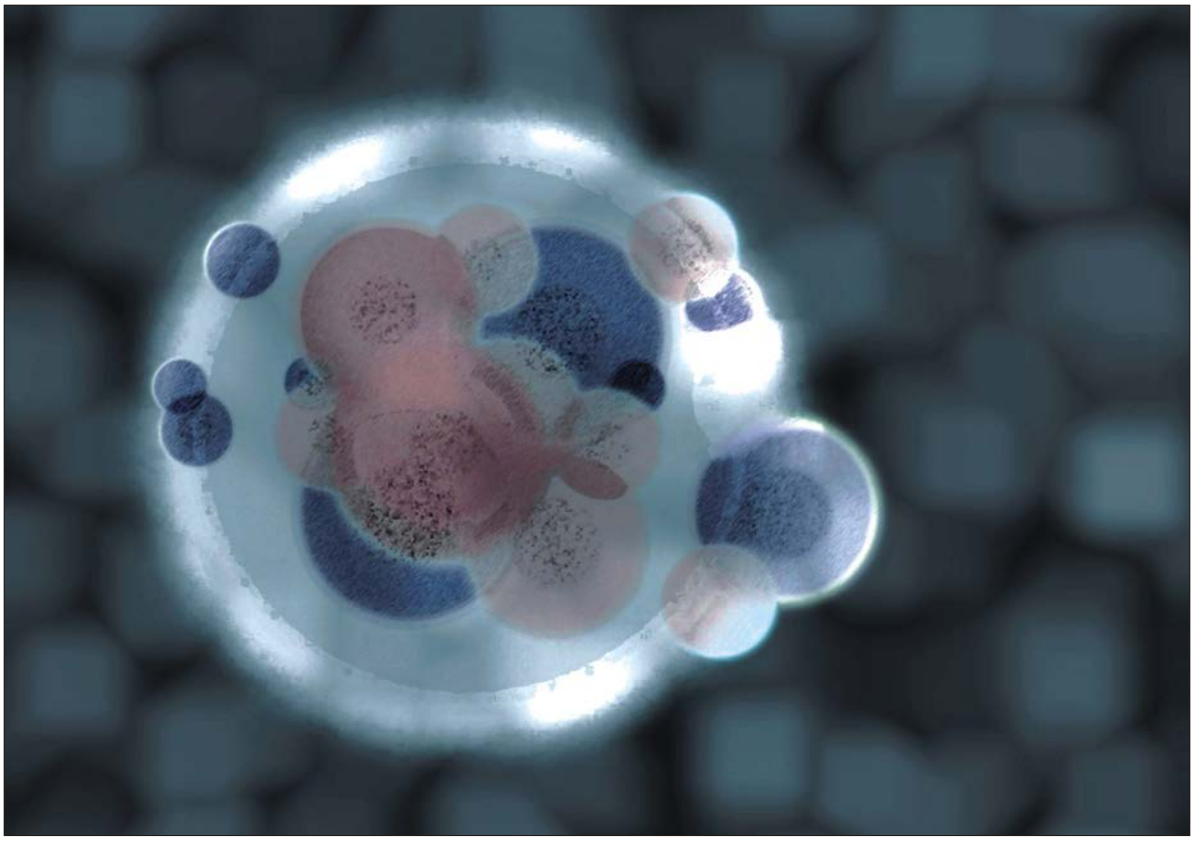

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Additional Possibility of Data Analysis of Enzyme Inhibition and Activation. 8. A Choice of the Equations for Calculation of the Initial Reaction Rates v_i and v_a in Enzyme Inhibition and Activation

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Abstract: The equations for calculation of initial rates of the associative, IV_i type and catalytic, III_i type, of enzyme inhibition are most frequently used in practice of calculation the initial rates of inhibited (v_i) enzymatic reactions (Appendix 1, Eq. A4 and A3). In some cases these equations are used with arbitrary modifications that may lead to incorrect calculation of respective parameters of reactions. Now the equations for calculation of initial rates (v_i) and (v_a) have been deduced for all seven types of inhibited (I_i, II_i, III_i, IV_i, V_i, VI_i, VII_i) and seven types of activated (I_a, II_a, III_a, IV_a, V_a, VI_a, VII_a) enzymatic reactions and it need to discuss the rule of choice (and conditions of applicability) of equation for processing experimental data of each these types of enzymatic reactions. Examples of choice the equations for calculation of the initial reaction rates v_i and v_a (and K_i, K_a) constants are given.

Key words: Initial rates of enzymatic reaction, equations for calculation

INTRODUCTION

The equations for calculation of initial rates of inhibited (v_i) and activated (v_a) reactions are rather often used for experimental data processing in enzyme kinetics (Airas, 1976; Bhatnagar *et al*., 1988; Bheemanaik *et al*., 2003; Hara *et al*., 1987; Liu *et al*., 1993; Rose *et al*., 1986; Sung *et al*., 2004; Tian *et al*., 2002). It is remarkable that the equation for calculation of the v_{IVi} initial rates of the associative, IV_i type, of enzyme inhibition is the most preferable:

$$v_{IVi} = \frac{V^0}{1+\frac{K_m^0}{S}\left(1+\frac{i}{K_{IVi}}\right)}, \qquad (1)$$

even when it is used for processing of not only the associative, but also other types of enzyme inhibition. In some cases these equations are arbitrarily modified, which may result in to great errors in the calculation of v_i and K_i constants of enzyme inhibition and mislead the beginning enzymologists as to the mechanism of such enzyme inhibition.

According to the parametric classification (Krupyanko, 1990; Krupyanko, 2005), seven types of inhibited (I_i, II_i, III_i, IV_i, V_i, VI_i, VII_i), seven types of activated enzymatic reactions (I_a, II_a, III_a, IV_a, V_a, VI_a, VII_a) and one, I_0 type, of initial (uninhibited, i = 0 and nonactivated, a = 0) enzymatic reaction were proved to exist in a simple one-substrate Michaelis-Menten scheme (Table 1) and the equations for calculation of the v_i and v_a initial rates of enzyme-catalyzed reactions have been deduced (Krupyanko, 1990). Now it is necessary to consider a rule of a choice of these equations to avoid an incorrectness of their use.

A rule of choice of the equations for calculation of the initial rates of inhibited reactions: The rule presupposes the determination of the type of inhibited reaction. As all equations (Appendix 1) were deduced for the description of a course of change in the v_i parameters in a simple one-substrate system catalyzed by enzymes not exhibiting any features of positive (h>1) or negative (h<1) cooperativity in the mechanism of their action on the substrate, the finding of such features is considered as a preliminary step.

1. Thus, after having plotted a course of change in the v_0 parameters as a function of S in the form of a hyperbolic curve (Fig. 1):

$$v_0 = f(S), \qquad (2)$$

the data obtained should be processed using one of the computing programs, for example, Sigma Plot Version 2000 (USA) as to the absence of cooperativity in the action of the enzyme on the substrate cleaved. It is advisable to use this particular program, because by having applied the equation of Hill (Kurganov, 1978; Palmer, 1985):

Table 1: Equations for calculation of the ν_i and ν_a initial rates of enzyme inhibition and activation

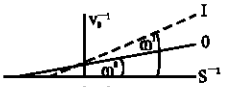
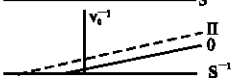
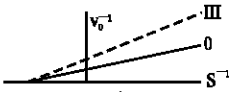
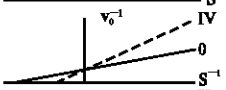
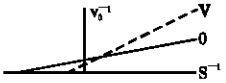
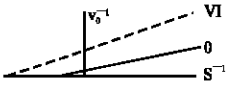
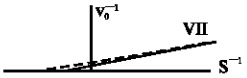
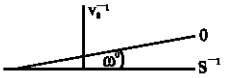
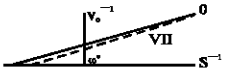
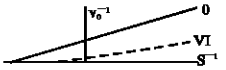
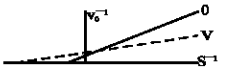
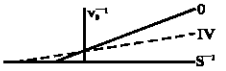
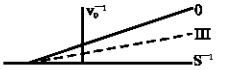
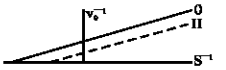
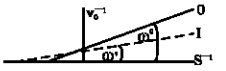
No.	Effect	Type of effect	Correlation between the K'_m and V' parameters	Graphs in the $(\nu_o^{-1}; S^{-1})$ coordinates	Equations for calculation of the ν_i and ν_a initial rates (Appendix 1)
1	Inhibition				
	(i>0)	I_i	$K'_m > K^0_m, V' < V^0$		ν_{Ii} = (Eq. A1)
2		II_i	$K'_m < K^0_m, V' < V^0 (tg\omega' = tg\omega^0)$		ν_{IIi} = (Eq. A2)
3		III_i	$K'_m = K^0_m, V' < V^0$		ν_{IIIi} = (Eq. A3)
4		IV_i	$K'_m > K^0_m, V' = V^0$		ν_{IVi} = (Eq. A4)
5		V_i	$K'_m > K^0_m, V' > V^0$		ν_{Vi}= (Eq. A5)
6		VI_i	$K'_m < K^0_m, V' < V^0 (tg\omega' > tg\omega^0)$		ν_{VIi} = (Eq. A6)
7		VII_i	$K'_m < K^0_m, V' < V^0 (tg\omega' < tg\omega^0)$		ν_{VIIi} = (Eq. A7)
8	None	I_0	$K'_m = K^0_m, V' = V^0$		ν_o = (Eq. A8)
9	Activation (a>0)	VII_a	$K'_m > K^0_m, V' > V^0 (tg\omega' > tg\omega^0)$		ν_{VIIa} = (Eq. A9)
10		VI_a	$K'_m > K^0_m, V' > V^0 (tg\omega' < tg\omega^0)$		ν_{VIa} = (Eq. A10)
11		V_a	$K'_m < K^0_m, V' < V^0$		ν_{Va} = (Eq. A11)
12		IV_a	$K'_m < K^0_m, V' = V^0$		ν_{IVa} = (Eq. A12)
13		III_a	$K'_m = K^0_m, V' > V^0$		ν_{IIIa} = (Eq. A13)
14		II_a	$K'_m > K^0_m, V' > V^0 (tg\omega' = tg\omega^0)$		ν_{IIa} = (Eq. A14)
15		I_a	$K'_m < K^0_m, V' > V^0$		ν_{Ia} = (Eq. A15)

Table 1: Continued

No.	New names of the types of enzymatic reactions	Traditional	Equations for calculation of the K_i and K_a constants
1	Biparametrically coordinated inhibition	Mixed inhibition	$K_{Ii} = \frac{i}{K'_m V^0 / K^0_m V' - 1}$
2	Unassociative inhibition	Uncompetitive inhibition	$K_{IIi} = \frac{i}{K^0_m V^0 / K'_m V' - 1}$
3	Catalytic inhibition	Noncompetitive inhibition	$K_{IIIi} = \frac{i}{V^0 / V' - 1}$
4	Associative inhibition	Competitive inhibition	$K_{IVi} = \frac{i}{K'_m / K^0_m - 1}$
5	Pseudoinhibition		$K_{Vi} = \frac{i}{K'_m V^0 / K^0_m V' - 1}$
6	Discoordinated inhibition		$K_{VIi} = \frac{i}{K'_m V^0 / K^0_m V' - 1}$

Table 1: Continued

No	New names of the types of enzymatic reactions	Traditional	Equations for calculation of the K_i and K_a constants
7	Transient inhibition		$K_{VIIi} = \frac{i}{K_m^0 V' / K_m' V^0 - 1}$
8	Initial (uninhibited i = 0 and nonactivated a = 0) enzymatic reaction		
9	Transient activation		$K_{VIIa} = \frac{a}{K_m' V^0 / K_m^0 V' - 1}$
10	Discoordinated activation		$K_{VIa} = \frac{a}{K_m^0 V' / K_m' V^0 - 1}$
11	Pseudoactivation		$K_{Va} = \frac{a}{K_m^0 V' / K_m' V^0 - 1}$
12	Associative activation	Competitive activation	$K_{IVa} = \frac{a}{K_m^0 / K_m' - 1}$
13	Catalytic activation	Noncompetitive activation	$K_{IIIa} = \frac{a}{V' / V^0 - 1}$
14	Unassocaitive activation	Uncompetitive activation	$K_{IIa} = \frac{a}{K_m' V' / K_m^0 V^0 - 1}$
15	Biparametrically coordinated activation	Mixed activation	$K_{Ia} = \frac{a}{K_m^0 V' / K_m' V^0 - 1}$

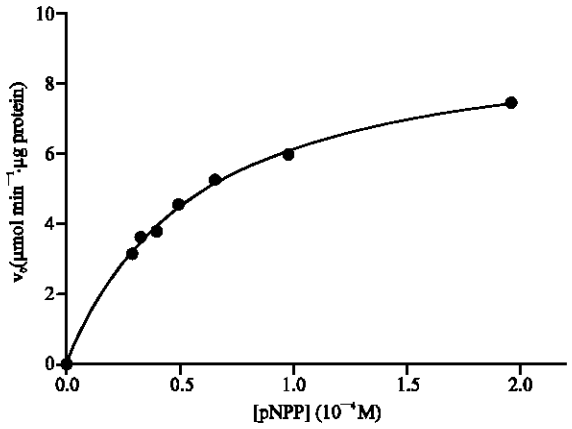


Fig. 1: Dependence of a course of change of the initial rates (v_0) upon pNPP concentrations in the reaction catalyzed by porcine alkaline phosphatase

$$v_0 = V_m \cdot \frac{|S|_0^h}{|S|_0^h + |S|_{0.5}^h} \quad (3)$$

one may calculate the value of Hill coefficient (h) and also the other two parameters $S_{0.5}$ and V_m of catalyzed reaction. The obtained value of a pure h coefficient (if it is equal to 1 or near 1) will indicate the absence of cooperativity in the action of the tested enzyme on the substrate. Evidently, if coefficient h = 1, Eq. (3) will be simplified to the known equation of Michaelis-Menten:

$$v_0 = V^0 \cdot \frac{S}{S + K_m^0} \quad (4)$$

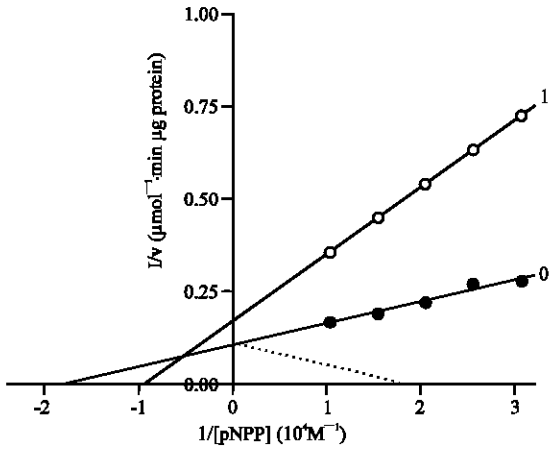


Fig. 2: Graphs of inhibitory effect of Na_2WO_4 on the initial rates (v_0) of pNPP cleavage catalyzed by porcine alkaline phosphatase in the coordinates of Lineweaver-Burk. Line 1-The concentration $Na_2WO_4 \cdot 10^{-5}$ M, line (0)-The inhibitor is absent. The position of the point $1/K_m^0 \approx 1/S_{mid}$ is marked with a dotted line for determination of an appropriate interval of concentrations of cleaved substrate at minimal error in the calculation of K_m^0 and V^0 parameters of catalyzed reaction

2. Then, one should determine the type of inhibited reaction by the position of plot 1 of inhibited (i>0) reaction:

$$\frac{1}{v_i} = \frac{K_m'}{V'} \cdot \frac{1}{S} + \frac{1}{V'} \quad (5)$$

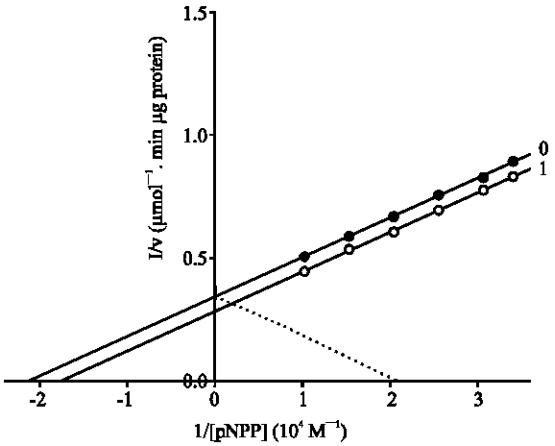


Fig. 3: Activating effect of guanosine on the initial rates (v_a) of pNPP cleavage catalyzed by canine alkaline phosphatase in the coordinates of Lineweaver-Burk. Line 1-The concentration of guanosine-2 10^{-3} M, line (0)-The activator is absent

relative to the position of plot (0):

$$\frac{1}{v_0} = \frac{K_m^0}{V^0} \cdot \frac{1}{S} + \frac{1}{V^0} \qquad (6)$$

of the initial uninhibited (i = 0) enzymatic reaction in the (v^{-1}; S^{-1}) coordinates of Lineweaver-Burk (Fig. 2).

One should bear in mind that during analysis of substrate curves calculated by the Hill equation (Eq. 3) the origin point $v_0 = 0$ at ($S_0 = 0$) and a considerably greater number of experimental values of the v_0 dependence on S in a wider range of concentrations of the substrate cleaved may be included in data processing. As for plotting graph using Eq. (6) in the (v^{-1}; S^{-1}) coordinates of Lineweaver-Burk, it is advisable to make as few errors as possible in the determination of the K_m^0 and V^0 parameters of catalyzed reaction, i.e., to select a range of concentrations of the cleaved substrate in such a way that an extrapolated fragment of the line (Eq. 6) turned to the right by 180° would be located in the middle of a range of reverse concentrations of the cleaved substrate (Krupyanko and Krupyanko, 1999):

$$\frac{1}{S_{fin}} = \left(\frac{1}{S_{mid}} = \frac{1}{K_m^0}\right) = \frac{1}{S_{init}} \qquad (7)$$

or placed near the point $1/S_{midl} \approx 1/K_m^0$ (Fig. 2 and 3).

Based on the results obtained by both approaches, it seems easy to establish the type of enzyme inhibition using Table 1 as well as an equation for calculation of a course of change of v_i (Appendix 1) and also the respective K_i constants of enzyme inhibition and K_a constants of activation (Table 1), which are of much interest for experimental enzymology.

All the above is analogous and for enzyme activation. The determination of Hill coefficient (h) and a correct choice of the equation for calculation of the v_a initial rates of activated reactions will help to avoid unjustified errors in the calculation of v_a and K_a parameters of enzyme activation.

Let us consider applicability stated above rules 1 and 2 to processing experimental data of enzyme inhibition and activation.

MATERIALS AND METHODS

Chemicals: Porcine intestinal alkaline phosphatase (EC 3.1.3.1) and canine (dog) intestinal alkaline phosphatase (EC 3.1.3.1)-are products of Sigma (USA).

Substrate: p-Nitrophenylphosphate 2CHA salt (pNPP)-a product of Serva (Germany).

Inhibitors: Disodium of tungstic acid ($Ma_2WO_4 \cdot 2H_2O$)-a domestic preparation of high purity grade, guanosine (Guo)-a product of Sigma (USA).

A process of pNPP cleavage was recorded by a CF-4 DR double-beam spectrophotometer (Optica Milano CF-4D, Italy). Reactions were carried out in 0.05 M Tris-HCl buffer (pH 9.0) with ionic strength of 0.1 by NaCl (high purity) at constant stirring and thermostatting (37°C) (Krupyanko, 1990). The kinetic curves were registered by increase ($+\Delta A_{400}$) in the absorption of solution containing the substrate, enzyme and inhibitor against a solution of the same composition, but without the enzyme.

The concentration of pNPP was changed from $0.327 \cdot 10^{-4}$ to $0.98 \cdot 10^{-4}$ M, the concentration of porcine alkaline phosphatase was 2.1 μg mL^{-1}, the concentration of canine alkaline phosphatase was 1.64 μg mL^{-1}, the concentration of pNPP was changed from $0.294 \cdot 10^{-4}$ to $0.98 \cdot 10^{-4}$ M, the concentration of inhibitor and activator are given in the legends to Fig. (2) and (3). The selection of substrate concentration was stipulated by an interval of minimal error in the determination of and parameters (Krupyanko and Krupyanko, 1999).

Determination of enzyme activity: The initial reaction rates (v) were calculated by a slope angle of tangents to initial segments of curves representing substrate cleavage in at least five parallel experiments. The K_m and V parameters were determined by respective plots in the (v^{-1}; S^{-1}) coordinates using the computer program Sigma Plot Version 2000 (USA). Root-mean-square deviation at five-fold determination was as follows: v = 2.5%, K_m and V = ±7.5%, respectively.

RESULTS AND DISCUSSION

Determination of the type of inhibited reaction: The study revealed that initial rates (v_i) of pNPP cleavage catalyzed by porcine alkaline phosphatase in the presence of $1\cdot10^{-5}$ M WO_4^{2-} decreased $v_i<v_0$ within the whole interval of concentrations of substrate cleaved (Fig. 2), i.e., this is inhibited reaction.

Testing for the presence of any feature of cooperativity in the mechanism of action of the above enzyme on pNPP made by using the program Sigma Plot 2000 (Appendix 2 and Table 2) showed that in that case h = 1.042, (V^0 = 9.3467 μmol/min·μg protein, $S^h_{0.5} = K^0_m$ = 5.415-10^{-5} M). Thus, it was possible to construct plots in the (v^{-1}; S^{-1}) double reciprocal coordinates, which permitted to establish that experimental lines (Fig. 2) intersect the coordinate axes in the point:

$K'_m > K^0_m$, $V'<V^0$ which correspond to all the features of the, I_i type, of enzyme inhibition by WO_4^{2-} anions (Table 1, line 1). One should use Eq. (1) to calculate the K_{Ii} constant of inhibition.

Substitution of all the appropriate parameters: $K^0_m = 5.45\cdot10^{-5}$ M, V^0 = 9.363 μmol/min·μg protein and $K'_m = 10.62\cdot10^{-5}$ M, V' = 5.86 μmol/min·μg protein, i = $1\cdot10^{-5}$ M in Eq. (1) (we must use these parameters, such as they were calculated from one and the same substrate cleavage intervals (Fig. 2) allows to deduce the following equation:

$$K_{Ii} = \frac{1\cdot10^{-5}\,M}{\frac{10.62\cdot9.363}{5.45\cdot5.86}-1} = 4.73\cdot10^{-6}\ M \qquad (8)$$

which indicates a more strong binding of the enzyme to WO_4^{2-} (5.45/0.473 = 11.5) than to the cleaved substrate.

An attempt to calculate the constant of this type of enzyme inhibition using Eq. (3) would yield (K'_{IIIi}) = $1.67\cdot10^{-5}$ M, which would differ by more than 3 times from the K_{Ii} calculated by Eq. (1). This happens, because a ratio of the K'_m and K^0_m parameters of this reaction is not taken into consideration.

In literature there are examples of calculation of analogous (K'_{IIIi}) constants of enzyme inhibition and this type often is refereed to the noncompetitive type of enzyme inhibition (Beaudet and Mackenzie, 1975; Maxwell *et al*., 1992; Lokshmann *et al*., 1992).

Determination of the type of activated reaction: It was shown that initial rates v_0 of pNPP cleavage catalyzed by canine alkaline phosphatase in the presence of $2\cdot10^{-3}$ M Guo increased $v_a>v_0$ within the whole interval of concentrations of the substrate cleaved (Fig. 3), which is a feature of enzyme activation.

A mechanism of action of the enzyme on pNPP as to the presence of any features of cooperativity was studied using the same program Sigma Plot 2000 in enzyme activation and also with the aim to find out the possibility of plotting graphs in the (v^{-1}; S^{-1}) double reciprocal coordinates. The study allowed to establish that in this case the Hill coefficient is equal to a unit: h = 0.993.

Plotting of dependencies in the above coordinates (Fig. 3) revealed that the experimental line 1 of activated reaction is located below and parallel to the line (0) of initial (nonactivated, a = 0) reaction at the ratio of parameters: $K'_m > K^0_m$, $V'>V^0$, (with correlation $K'_m/V' = K^0_m/V^0$), i.e., these lines would never intersect. As is easily seen from Table 1, (line 14), this corresponds to all the features of the, II_a type, of unassociative activation and to calculate a course of change in v_a as a function of (Eq. A14) must be used (Appendix 1). As for calculation of the K_{IIa} constant of activation, Eq. (14) must be used (Table 1). Substitution of the obtained from (Fig. 3) parameters: $K^0_m = 4.69\ 10^{-5}$ M, V^0 = 2.92 μmol min^{-1}·μg protein, $K'_m = 5.67\cdot10^{-5}$ M, V' = 3.53 μmol/min·μg protein and Guo = $2\cdot10^{-3}$ M of enzyme activation in Eq. (14) yields an equation:

$$K_{IIa} = \frac{2\cdot10^{-3}\,M}{\frac{5.67\cdot3.53}{4.69\cdot2.92}-1} = 4.33\cdot10^{-3}\ M \qquad (9)$$

that shows that the binding of this enzyme to guanosine (K_{IIa}/K^0_m = 433/4.69 = 92.3) is by two orders lower than to the substrate. One might have expected this, since there is no any similarity between the uncharged molecule of Guo and negatively charged substrate (pNPP).

APPENDIX 1

The equations for calculation of the initial rates of enzymatic reactions.

Inhibited reactions:

No. 1. (I_i type, biparametrically coordinated inhibition)

$$v_{Ii} = \frac{V^0\cdot\frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K^0_m}{S}\cdot\left(1+\frac{i}{K_{IVi}}\right)} \qquad (A1)$$

No. 2. (II_i type, unassociative inhibition)

$$v_{IIi} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{i}{K_{IVa}}\right)}} \quad (A2)$$

No. 3. (III_i type, catalytic inhibition)

$$v_{IIIi} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}} \quad (A3)$$

No. 4. (IV_i type, associative inhibition)

$$v_{IVi} = \frac{V^0}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{i}{K_{IVi}}\right)} \quad (A4)$$

No. 5. (V_i type, pseudoinhibition)

$$v_{Vi} = \frac{V^0 \cdot \left(1+\frac{i}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{i}{K_{IVi}}\right)} \quad (A5)$$

No. 6. (VI_i type, discoordinated inhibition)

$$v_{VIi} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{i}{K_{IVa}}\right)}} \quad (A6)$$

No. 7. (VII_i type, transient inhibition)

$$v_{VIIi} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{i}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{i}{K_{IVa}}\right)}} \quad (A7)$$

Initial (uninhibited and nonactivated) reaction

No. 8. (initial i = 0 and a = 0 reaction)

$$v_0 = \frac{V^0}{1+\frac{K_m^0}{S}} \quad (A8)$$

Activated reactions:

No. 9. (VII_a type, transient activation)

$$v_{VIIa} = \frac{V^0 \cdot \left(1+\frac{a}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{a}{K_{IVi}}\right)} \quad (A9)$$

No. 10. (VI_a type of discoordinated activation)

$$v_{VI_a} = \frac{V^0 \cdot \left(1+\frac{a}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}\cdot\left(1+\frac{a}{K_{IVi}}\right)} \quad (A10)$$

No. 11. (V_a type, pseudoactivation)

$$v_{Va} = \frac{V^0 \cdot \frac{1}{\left(1+\frac{a}{K_{IIIi}}\right)}}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{a}{K_{IVa}}\right)}} \quad (A11)$$

No. 12. (IV_a type, associative activation)

$$v_{IVa} = \frac{V^0}{1+\frac{K_m^0}{S}\cdot\frac{1}{\left(1+\frac{a}{K_{IVa}}\right)}} \quad (A12)$$

No. 13. (III_a type, catalytic activation)

$$v_{IIIa} = \frac{V^0 \cdot \left(1+\frac{a}{K_{IIIa}}\right)}{1+\frac{K_m^0}{S}} \quad (A13)$$

No. 14. (II_a type, unassociative activation)

$$v_{IIa} = \frac{V^0 \cdot \left(1 + \frac{a}{K_{IIIa}}\right)}{1 + \frac{K_m^0}{S} \cdot \left(1 + \frac{a}{K_{IVi}}\right)} \quad (A14)$$

No. 15. (I_a type, biparametrically coordinated activation)

$$v_{Ia} = \frac{V^0 \cdot \left(1 + \frac{a}{K_{IIIa}}\right)}{1 + \frac{K_m^0}{S} \cdot \frac{1}{\left(1 + \frac{a}{K_{IVa}}\right)}} \quad (A15)$$

APPENDIX 2

The required manipulations for experimental data processing of pNPP cleavage by porcine alkaline phosphatase (Table 2 and Fig. 1) for determination of the Hill coefficient (h) by use of the program Sigma Plot 2000 (USA).

Routine computing: Open the program Sigma Plot 2000 Fill in two digit columns: 1st column ($S \cdot 10^{-4}$M), 2nd column (v_o·μmol/min·μg protein) in Table 2.

File
Save as: (graph1)
Save
Graph
<Press↩on mouse>
Create Graph
<Press↩on mouse>
Scatter Plot
<next>
Simple Scatter
<next>
XY Pairs

Table 2: Dependence of a course of change in the initial rates (v_0) upon the concentration of pNPP

Column 1	Column 2
0	0.00
0.294	3.13
0.327	3.60
0.392	3.78
0.490	4.53
0.654	5.21
0.98	5.95
1.96	7.44

<next> (select: X: Col 1
Y: Col 2)
<finish> (the dotted graph of the Y (i.e., v_o) dependence upon X (i.e., S) on the display)
Statistics
<Press ↩ on mouse>
<Regression Wizard ... F5>
<Press ↩ on mouse> (the equation: $y = \frac{a \cdot x^b}{c^b + x^b}$ on the display

(Hill 3 Parameter)
<next> (the Eq. $y = \frac{a \cdot x^b}{c^b + x^b}$ with c^b and x^b circled in red on the display)
select the columns: x: Col 1
y: Col 2
<next> (preliminary solution of Eq. on the display) as:
a 9.347, i.e., (V^0 = 9.347 μmol min^{-1}·μg protein.
See dimension of v_0 in Table 2)
b 1.042e+0 i.e., (h = 1.042)
c 5.45e-1 i.e., ($K_m^0 = 0.545 \cdot 10^{-4}$ M. See dimension of S in Table 2)
<finish> (graph in curve line and dotted appears on the display)
Remove the graph
<Press [x] on the display> (the large Table Nonlinear Regression of digits as a solution of the Hill equation on the display)
<Put the cursor down> along this Table and get the final results as:
$R^2 = 0.9989$

	Coefficient	Std. Err.
a	9.3467	0.7516
b	1.0417	0.1293
c	0.5415	0.0928

R^2-coefficient of correlation, Std. Err-root mean square deviation of the parameters of Hill equation where (a = V^0), (b = h) and (c = K_m^0)
write down the a, b and c parameters in a laboratory note-book as:
V^0 = (9.35 ± 0.75)·μmol min^{-1} μg protein
h = (1.042 ± 0.13) (non dimensional coefficient)
K_m^0 = (0.5415 ± 0.0928)·10^{-4}M.
<Save>.

For progress of the graph to the form as in Fig. 1 (see the text):

Remove the large Table of digits from the display as
<Press [x] on display>
Remove data of Table 2
<Press [x] on display>
<Output> on the display the content of the file (graph1.JNB):
Graph Page 2
<Press ↵ on mouse>
<Open> Process graph 1 to the form given in the text (Fig. 1)
<Save>
Switch-of the computer.

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