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Combined kinetic mechanism describing activation and inhibition of muscle glycogen phosphorylase *b* by adenosine 5′-monophosphate

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Abstract

The kinetic analysis of the glycogen chain growth reaction catalyzed by glycogen phosphorylase *b* from rabbit skeletal muscle has been carried out over a wide range of concentrations of AMP under the saturation of the enzyme by glycogen. The applicability of 23 different variants of the kinetic model involving the interaction of AMP and glucose 1-phosphate binding sites in the dimeric enzyme molecule is considered. A kinetic model has been proposed which assumes: (i) the independent binding of one molecule of glucose 1-phosphate in the catalytic site on the one hand, and AMP in both allosteric effector sites and both nucleoside inhibitor sites of the dimeric enzyme molecule bound by glycogen on the other hand; (ii) the binding of AMP in one of the allosteric effector sites results in an increase in the affinity of other allosteric effector site to AMP; (iii) the independent binding of AMP to the nucleoside inhibitor sites of the dimeric enzyme molecule; (iv) the exclusive binding of the second molecule of glucose 1-phosphate in the catalytic site of glycogen phosphorylase *b* containing two molecules of AMP occupying both allosteric effector sites; and (v) the catalytic act occurs exclusively in the complex of the enzyme with glycogen, two molecules of AMP occupying both allosteric effector sites, and two molecules of glucose 1-phosphate occupying both catalytic sites.

Keywords: Glycogen phosphorylase *b*; Kinetic mechanism; Allosteric regulation

Abbreviations: Glucose-1-P, glucose 1-phosphate.

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1. Introduction

Glycogen phosphorylase (1,4-α-D-glucan:orthophosphate glycosyltransferase, EC 2.4.1.1) catalyzes the reversible phosphorolytic cleavage of the α-1,4-glycosidic bonds at the non-reducing ends of the side chains of glycogen, resulting in the formation of glucose-1-P. The dephosphorylated form of the enzyme (glycogen phosphorylase *b*) from rabbit skeletal muscles is a dimer consisting of two identical subunits. Glycogen phosphorylase *b* reveals a catalytic activity in the presence of the allosteric activator AMP [1,2]. The activatory action of AMP is due to nucleotide binding in the allosteric effector site located in the region of the contact of two subunits [3,4]. The increase in AMP concentration results to decreasing its activatory action due to the nucleotide binding in the nucleoside inhibitor site [5,6]. This site is located at the entrance to the channel to the catalytic site of the enzyme [3,4].

The activation of glycogen phosphorylase *b* does not follow the hyperbolic law: the dependence of the enzyme activity on AMP concentration is non-linear in the reciprocal plot [7]. The kinetic investigations of the enzyme activation induced by AMP showed that glycogen phosphorylase *b* reveals a catalytic activity exclusively due to the binding of two molecules of AMP and two molecules of glucose-1-P by the dimeric molecule of the enzyme under the conditions of the enzyme saturation by glycogen [8,9]. In the present paper, we study the steady-state kinetics of the reaction catalyzed by glycogen phosphorylase *b* from rabbit skeletal muscles in a wide range of AMP concentration under the conditions of the enzyme saturation by glycogen. Some kinetic models are used for quantitative analysis of the steady-state kinetics of glycogen synthesis catalyzed by the enzyme. The parameters of the rate equation for the enzymatic reaction were calculated by a non-linear regression method. The kinetic model of the reaction was chosen according to the following criteria: (i) the convergence under regressional analysis; (ii) the reliability of the values of the parameters of the model; and (iii) the minimum of the sum of the weighted squares of the difference between the experimental and calculated values of the reaction rate [10].

2. Materials and methods

2.1. Materials

Phosphorylase *b* was isolated from rabbit skeletal muscles according to Fisher and Krebs [11] with the use of β-mercaptoethanol instead of D-L-cystein. Four-fold-crystallized preparation of the enzyme was used over 2 weeks after isolation. AMP was removed from the enzyme solution by passage through a Norit A column according to Fisher and Krebs [11]. This solution was used for 1 day. The enzyme concentration was determined spectrophotometrically at 280 nm. The absorption coefficient and molecular mass of the phosphorylase *b* subunit were assumed to be 1.32 $(g/l)^{-1}cm^{-1}$ [12] and 97 500 Da [13], respectively.

Pig liver glycogen (Olina, Latvia) was purified by reprecipitation with ethanol according to Sutherland and Wosilait [14]. The average molecular mass of glycogen and proportion of non-reducing glucose residue were 5500 kDa [15] and 6.4%, respectively [16]. Disodium adenosine 5′-monophosphate and dipotassium glucose 1-phosphate was purchased from Reanal (Hungary). All other reagents (research and analytical grade) were purchased from Soyuzkhimreactiv (Russia).

2.2. Phosphorylase assay

The catalytic activity of phosphorylase *b* in the direction of glycogen synthesis was determined using the turbidimetric method [17] at 310 nm with a recording spectrophotometer (Cary-219, Varian) equipped with a thermojacketed cuvette holder. The initial steady-state rates of the enzymatic reaction were measured at 30°C in 0.05 M glycylglycine buffer, pH 6.8, containing 0.2 mM EDTA. The ionic strength of the solution was increased to 0.3 M using KCl. The glucose-1-P and AMP increments in the ionic strength of the solution were calculated using pK_a values equal to 6.01 [18] and 6.24 [19], respectively (at 30°C

and 0.3 M ionic strength). The enzymatic reaction was initiated by adding an aliquot of the enzyme solution to the reaction mixture. The special experiments showed that the value of the initial steady-state rate of the enzymatic reaction was independent of the order of the addition of the reaction components. All the experiments were carried out at fixed glycogen concentration of 1 g/l. The increase in glycogen concentration up to 5 g/l did not influence the value of the initial steady-state rate of the enzymatic reaction. The relative error in the measurement of the initial steady-state rate of the enzymatic reaction was determined to be 3.0%.

2.3. Computer calculations

The parameters of the equation for the initial steady-state rate of the enzymatic reaction were calculated using the non-linear regression method according to the Marquardt algorithm. We used a computer program proposed by Duggleby [20] with the following modifications: (i) the equation of the initial steady-state rate of the enzymatic reaction for the kinetic model was used as a goal function; (ii) the explicit calculation of the partial derivatives of the dependent variable on the regression parameters was used instead of the approximated calculation by the numerical method; (iii) the number of the regressional parameters was increased up to 38; and (iv) the data input was carried out with the use of a separate file of the experimental data. The calculations were carried out using an IBM-compatible personal computer.

3. Results and discussion

The experimental dependence of the initial steady-state rate of the enzymatic reaction catalyzed by rabbit muscle phosphorylase *b* on the AMP concentration has a bell-shaped curve in semi-logarithmic coordinates (Fig. 1). The ascending branch of the bell-shaped curve results from the activatory action of AMP due to its binding in the allosteric effector site of the enzyme molecule. The descending branch of the curve results from the inhibitory action of AMP due to its binding in the nucleoside inhibitor site. To quantitatively describe the steady-state kinetics of the reaction, consider a model of the direct cooperativity [21] for the dimeric enzyme in the case that effector binds both in activatory sites and in inhibitory sites of the enzyme subunit. Assume that (i) a ligand (AMP or glucose-1-P) binding with one subunit of the dimeric enzyme molecule results in a change in the affinity of the other subunit to the ligand; (ii) the binding of any ligand influences the affinity of the enzyme to other ligand; and (iii) a ternary complex of the enzyme with glycogen and glucose-1-P is unable to the catalytic transformation if it does not contain AMP bound in the activatory site of the enzyme. A corresponding kinetic scheme under the conditions of enzyme saturation by glycogen is shown in Fig. 2. The following designations are used: E is the enzyme–glycogen complex, S is glucose-1-P; A is AMP; P is the product(s) of the enzymatic reaction; $EA_mS_nI_r$ is the enzyme–glycogen complex filled with m activatory, n catalytic and r inhibitory sites in the enzyme molecule; K_{amnr}, K_{imnr}, and K_{smnr} are the microscopic constants for dissociation of the enzyme species with the liberation of a corresponding ligand from the activatory, inhibitory or catalytic site, respectively; k_{fgh} is the catalytic constant for the enzyme–glycogen complex filled with f activatory, g catalytic and h inhibitory sites in the enzyme molecule; f, g, h, j, k, m, n, p, q and r are explicit numbers. The equation for the initial steady-state rate (v) of the enzymatic reaction may be written in the form:

$$v = \sum_{f=1}^{2} \sum_{g=1}^{2} \sum_{h=0}^{2} g k_{fgh} [EA_f S_g I_h], \quad (1)$$

where $[EA_fS_gI_h]$ is the concentration of the enzyme–glycogen complex filled with f activatory, g catalytic and h inhibitory sites in the enzyme molecule. We can write the equation for a material balance as follows:

$$[E]_0 = \sum_{p=0}^{2} \sum_{q=0}^{2} \sum_{r=0}^{2} [EA_p S_q I_r], \quad (2)$$

where $[E]_0$ is the total concentration of the enzyme. The microscopic constants for dissociation of the enzyme species may be written in the following form:

$$\tfrac{1}{2}K_{a1jk} = \frac{[\mathrm{ES}_j\mathrm{I}_k][A]_0}{[\mathrm{EAS}_j\mathrm{I}_k]}, \tag{3}$$

$$2K_{a2jk} = \frac{[EAS_jI_k][A]_0}{[EA_2S_jI_k]}, \tag{4}$$

$$\frac{1}{2}K_{sm1n} = \frac{[EA_mI_n][S]_0}{[EA_mSI_n]}, \tag{5}$$

$$2K_{sm2n} = \frac{[EA_mSI_n][S]_0}{[EA_mS_2I_n]}, \tag{6}$$

$$\frac{1}{2}K_{ipq1} = \frac{[EA_pS_q][A]_0}{[EA_pS_qI]}, \tag{7}$$

$$2K_{ipq2} = \frac{[EA_pS_qI][A]_0}{[EA_pS_qI_2]}, \tag{8}$$

where $[A]_0$ and $[S]_0$ are the total concentration of AMP or glucose-1-P, respectively. Using Eqs. (2)–(8) we write Eq. (1) as follows:

$$\begin{aligned}
v = \frac{V[A]_0[S]_0}{K_{a1}K_{s1}} \Bigg\{ & \left(\frac{\beta_{120}[S]_0}{\alpha_{s120}K_{s2}} + \beta_{110}\right)\frac{2}{\alpha_{s110}} \\
& + \left(\frac{[S]_0}{\alpha_{s220}K_{s2}} + \beta_{210}\right)\frac{[A]_0}{\alpha_{s210}K_{a2}} \\
& + \left(\frac{\beta_{221}[S]_0}{\alpha_{s221}K_{s2}} + \beta_{211}\right)\cdot\frac{2[A]_0^2}{\alpha_{s211}\alpha_{i201}K_{a2}K_{i1}} \\
& + \left(\frac{\beta_{121}[S]_0}{\alpha_{s121}K_{s2}} + \beta_{111}\right)\frac{4[S]_0}{\alpha_{s111}\alpha_{i101}K_{i1}} \\
& + \left(\frac{\beta_{222}[S]_0}{\alpha_{s222}K_{s2}} + \beta_{212}\right)\cdot\frac{[A]_0^3}{\alpha_{s212}\alpha_{i201}K_{a2}K_{i1}K_{i2}} \\
& + \left(\frac{\beta_{122}[S]_0}{\alpha_{s122}K_{s2}} + \beta_{112}\right)\frac{2[A]_0^2}{\alpha_{s112}\alpha_{i101}K_{i1}K_{i2}}\Bigg\} \\
/\Bigg\{ & 1 + \frac{[S]_0}{K_{s1}} \quad \left(2 + \frac{[S]_0}{K_{s2}}\right) \\
& + \frac{2[A]_0}{K_{a1}}\left[1 + \frac{[S]_0}{\alpha_{s110}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s120}K_{s2}}\right)\right] \\
& + \frac{[A]_0^2}{K_{a1}K_{a2}}\left[1 + \frac{[S]_0}{\alpha_{s210}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s220}K_{s2}}\right)\right] \\
& + \frac{2[A]_0}{K_{i1}}\left[1 + \frac{[S]_0}{\alpha_{s011}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s021}K_{s2}}\right)\right] \\
& + \frac{4[A]_0^2}{\alpha_{i101}K_{a1}K_{i1}}\left[1 + \frac{[S]_0}{\alpha_{s111}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s121}K_{s2}}\right)\right] \\
& + \frac{2[A]_0^3}{\alpha_{i201}K_{a1}K_{a2}K_{i1}}\left[1 + \frac{[S]_0}{\alpha_{s211}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s221}K_{s2}}\right)\right] \\
& + \frac{[A]_0^2}{K_{i1}K_{i2}}\left[1 + \frac{[S]_0}{\alpha_{s012}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s022}K_{s2}}\right)\right] \\
& + \frac{2[A]_0^3}{\alpha_{i101}\alpha_{i102}K_{a1}K_{i1}K_{i2}}\left[1 + \frac{[S]_0}{\alpha_{s112}K_{s1}}\right. \\
& \left.\times\left(2 + \frac{[S]_0}{\alpha_{s122}K_{s2}}\right)\right] + \frac{[A]_0^4}{\alpha_{i201}\alpha_{i202}K_{a1}K_{a2}K_{i1}K_{i2}} \\
& \times\left[1 + \frac{[S]_0}{\alpha_{s212}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s222}K_{s2}}\right)\right]\Bigg\},
\end{aligned} \tag{9}$$

where $V = 2k[E]_0$, $\beta_{fgh} = k_{fgh}/k_{220}$, $f = 1, 2$, $g = 1, 2$, $h = 0, 1, 2$, $k = k_{220}$, $K_{s1} = K_{s010}$, $K_{s2} = K_{s020}$, $K_{a1} = K_{a100}$, $K_{a2} = K_{a200}$, $K_{i1} = K_{i001}$, $K_{i2} = K_{i002}$, $\alpha_{sm1n} = K_{sm1n}/K_{s1}$, $\alpha_{sm2n} = K_{sm2n}/K_{s2}$, $\alpha_{a1jk} = K_{a1jk}/K_{a1}$, $\alpha_{a2jk} = K_{a2jk}/K_{a2}$, $\alpha_{ipq1} = K_{ipq1}/K_{i1}$, and $\alpha_{ipq2} = K_{ipq2}/K_{i2}$. Eq. (9) was found to provide no convergence under the regressional analysis. The β_{122} value was found to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 5×10^{-11} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with one activatory, two catalytic and two inhibitory sites is unable to a catalytic transformation.

Assume further that $\beta_{122} = 0$. No convergence was found in this case. The β_{112} value was found

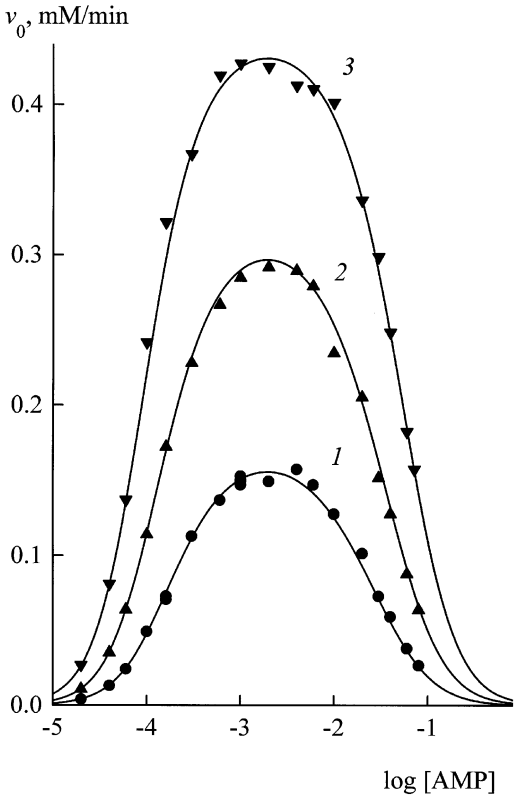

Fig. 1. The dependences of the initial steady-state rate of the enzymatic reaction catalyzed by rabbit muscle phosphorylase *b* in the presence of glycogen (1 g/l) on the AMP concentration at the following concentrations of glucose-1-P (mM): 2.0 (1), 4.0 (2), and 8.0 (3). The points are the experimental data, the curves are calculated according to Eq. (21) at the following parameter values: $K_{a1} = 0.22$ mM, $K_{a2} = 0.088$ mM, $K_{s1} = 4.4$ mM, $K_{s2} = 1.3$ mM, $K_i = 40$ mM, $k = 84$ s^{-1}, $[E]_0 = 62$ nM.

to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 9×10^{-10} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with two activatory, two catalytic and two inhibitory sites is unable to a catalytic transformation. Therefore, we further assume that $\beta_{112} = 0$. No convergence was found also in this case. The β_{121} value was found to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 2×10^{-9} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with one activatory, two catalytic and one inhibitory sites is unable to a catalytic transformation. Therefore, we assume further that $\beta_{121} = 0$. No convergence was found also in this case. The β_{111} value was found to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 2×10^{-10} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with one activatory, one catalytic and one inhibitory sites is unable to a catalytic transformation. Therefore, we further assume that $\beta_{111} = 0$. No convergence was

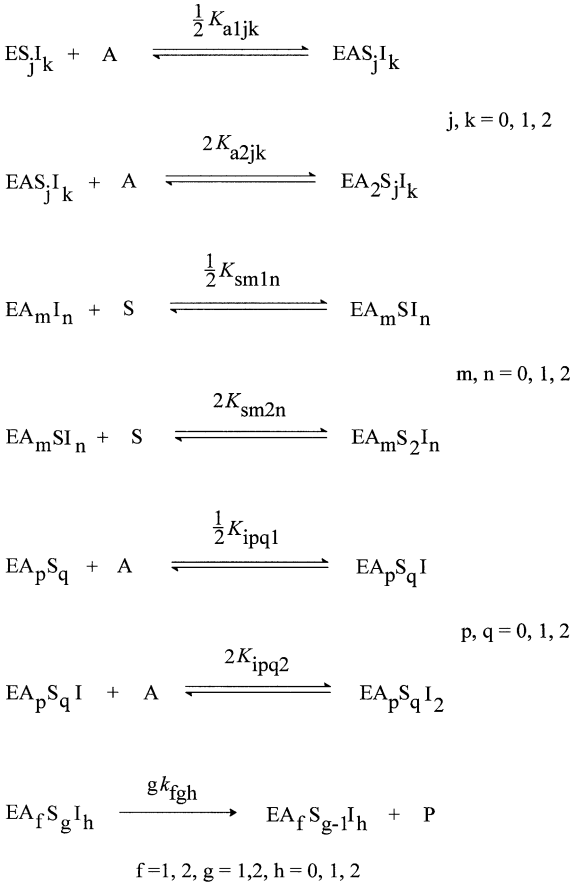

Fig. 2. The kinetic scheme for the dimeric bisubstrate enzyme with the interacting ligand-binding sites in the case of the binding of effector both in the activatory sites and in the inhibitory sites under the conditions of the enzyme saturation by one substrate.

found in this case either. The values of α_{s111}, α_{s121}, α_{s012}, α_{s022}, α_{s112}, α_{s122}, and α_{i102} exceed 3×10^{9}, 2×10^{16}, 1×10^{10}, 1×10^{14}, 9×10^{13}, 2×10^{8} and 9×10^{10}, respectively. This fact shows that one can ignore the formation of the following enzyme species: EASI, EAS_2I, ESI_2, ES_2I_2, $EASI_2$, EAS_2I_2, EAI_2. The corresponding equation for the reaction rate may be written in the form:

$$
\begin{aligned}
v = \frac{V[A]_0[S]_0}{K_{a1}K_{s1}} \Bigg\{ & \left(\frac{\beta_{120}[S]_0}{\alpha_{s120}K_{s2}} + \beta_{110}\right)\frac{2}{\alpha_{s110}} \\
& + \left(\frac{[S]_0}{\alpha_{s220}K_{s2}} + \beta_{210}\right)\frac{[A]_0}{\alpha_{s210}K_{a2}} \\
& + \left(\frac{\beta_{221}[S]_0}{\alpha_{s221}K_{s2}} + \beta_{211}\right)\cdot\frac{2[A]_0^2}{\alpha_{s211}\alpha_{i201}K_{a2}K_{i1}} \\
& + \left(\frac{\beta_{222}[S]_0}{\alpha_{s222}K_{s2}} + \beta_{212}\right)\frac{[A]_0^3}{\alpha_{s212}\alpha_{i201}K_{a2}K_{i1}K_{i2}}\Bigg\} \\
& \Big/\Bigg\{1 + \frac{[S]_0}{K_{s1}}\left(2 + \frac{[S]_0}{K_{s2}}\right) + \frac{2[A]_0}{K_{a1}} \\
& \times\left[1 + \frac{[S]_0}{\alpha_{s110}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s120}K_{s2}}\right)\right] \\
& + \frac{[A]_0^2}{K_{a1}K_{a2}}\left[1 + \frac{[S]_0}{\alpha_{s210}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s220}K_{s2}}\right)\right] \\
& + \frac{2[A]_0}{K_{i1}}\left[1 + \frac{[S]_0}{\alpha_{s011}K_{s1}}\times 2 + \frac{[S]_0}{\alpha_{s021}K_{s2}}\right] \\
& + \frac{4[A]_0^2}{\alpha_{i101}K_{a1}K_{i1}} + \frac{2[A]_0^3}{\alpha_{i201}K_{a1}K_{a2}K_{i1}} \\
& \times\left[1 + \frac{[S]_0}{\alpha_{s211}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s221}K_{s2}}\right)\right] + \frac{[A]_0^2}{K_{i1}K_{i2}} \\
& + \frac{[A]_0^4}{\alpha_{i201}\alpha_{i202}K_{a1}K_{a2}K_{i1}K_{i2}} \\
& \times\left[1 + \frac{[S]_0}{\alpha_{s212}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s222}K_{s2}}\right)\right]\Bigg\} \qquad (10)
\end{aligned}
$$

Eq. (10) was found to be not applicable to our experimental data because it did not give convergence under the regressional analysis. The β_{212} value was found to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 3×10^{-9} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with two activatory, one catalytic and two inhibitory sites is unable to a catalytic transformation.

Assume further that $\beta_{212}=0$ in Eq. (10). No convergence was found in this case. The β_{222} value was found to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 4×10^{-10} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with one activatory, one catalytic and two inhibitory sites is unable to a catalytic transformation. Assume further that $\beta_{222}=0$ in Eq. (10). No convergence was found in this case either. The values of α_{s011} and α_{s021} exceed 1×10^{13} and 9×10^{10}, respectively. This fact shows that the formation of ESI and ES_2I can be neglected. The corresponding equation for the reaction rate may be written in the form:

$$
\begin{aligned}
v = \frac{V[A]_0[S]_0}{K_{a1}K_{s1}} \Bigg\{ & \left(\frac{\beta_{120}[S]_0}{\alpha_{s120}K_{s2}} + \beta_{110}\right)\frac{2}{\alpha_{s110}} \\
& + \left(\frac{[S]_0}{\alpha_{s220}K_{s2}} + \beta_{210}\right)\frac{[A]_0}{\alpha_{s210}K_{a2}} \\
& + \left(\frac{\beta_{221}[S]_0}{\alpha_{s221}K_{s2}} + \beta_{211}\right)\cdot\frac{2[A]_0^2}{\alpha_{s211}\alpha_{i201}K_{a2}K_{i1}}\Bigg\} \\
& \Big/\Bigg\{1 + \frac{[S]_0}{K_{s1}}\left(2 + \frac{[S]_0}{K_{s2}}\right) + \frac{2[A]_0}{K_{a1}} \\
& \times\left[1 + \frac{[S]_0}{\alpha_{s110}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s120}K_{s2}}\right)\right] + \frac{[A]_0^2}{K_{a1}K_{a2}} \\
& \times\left[1 + \frac{[S]_0}{\alpha_{s210}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s220}K_{s2}}\right)\right] + \frac{2[A]_0}{K_{i1}} \\
& + \frac{4[A]_0^2}{\alpha_{i101}K_{a1}K_{i1}} + \frac{2[A]_0^3}{\alpha_{i201}K_{a1}K_{a2}K_{i1}} \\
& \times\left[1 + \frac{[S]_0}{\alpha_{s211}K_{s1}}\left(2 + \frac{[S]_0}{\alpha_{s221}K_{s2}}\right)\right] + \frac{[A]_0^2}{K_{i1}K_{i2}}
\end{aligned}
$$

$$+\frac{[A]_0^4}{\alpha_{i201}\alpha_{i202}K_{a1}K_{a2}K_{i1}K_{i2}}\times\left[1+\frac{[S]_0}{\alpha_{s212}K_{s1}}\left(2+\frac{[S]_0}{\alpha_{s222}K_{s2}}\right)\right]\Bigg\}. \quad (11)$$

Eq. (11) was found to be not applicable to our experimental data because it did not give convergence under the regressional analysis. The β_{110} value was found to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 2×10^{-9} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with one activatory and one catalytic sites is unable to a catalytic transformation.

Assume further that $\beta_{110}=0$ in Eq. (11). No convergence was found also in this case. The value of α_{s222} exceeds 5×10^{14} at the moment of interrupting the program run, indicating that the enzyme–glycogen complex filled with two activatory, two catalytic and two inhibitory sites is not formed practically. The corresponding equation for the reaction rate may be written in the form:

$$\upsilon=\frac{V[A]_0[S]_0}{K_{a1}K_{s1}}\Bigg\{\frac{2\beta_{120}[S]_0}{\alpha_{s110}\alpha_{s120}K_{s2}}+\left(\frac{[S]_0}{\alpha_{s220}K_{s2}}+\beta_{210}\right)\frac{[A]_0}{\alpha_{s210}K_{a2}}+\left(\frac{\beta_{221}[S]_0}{\alpha_{s221}K_{s2}}+\beta_{211}\right)\cdot\frac{2[A]_0^2}{\alpha_{s211}\alpha_{i201}K_{a2}K_{i1}}\Bigg\}$$
$$\Big/\Bigg\{1+\frac{[S]_0}{K_{s1}}\left(2+\frac{[S]_0}{K_{s2}}\right)+\frac{2[A]_0}{K_{a1}}\left[1+\frac{[S]_0}{\alpha_{s110}K_{s1}}\left(2+\frac{[S]_0}{\alpha_{s120}K_{s2}}\right)\right]+\frac{[A]_0^2}{K_{a1}K_{a2}}\cdot\left[1+\frac{[S]_0}{\alpha_{s210}K_{s1}}\left(2+\frac{[S]_0}{\alpha_{s220}K_{s2}}\right)\right]+\frac{2[A]_0}{K_{i1}}+\frac{2[A]_0^3}{\alpha_{i201}K_{a1}K_{a2}K_{i1}}$$
$$\times\left[1+\frac{[S]_0}{\alpha_{s211}K_{s1}}\left(2+\frac{[S]_0}{\alpha_{s221}K_{s2}}\right)\right]+\frac{[A]_0^2}{K_{i1}K_{i2}}+\frac{[A]_0^4}{\alpha_{i201}\alpha_{i202}K_{a1}K_{a2}K_{i1}K_{i2}}\times\left(1+\frac{2[S]_0}{\alpha_{s212}K_{s1}}\right)\Bigg\}. \quad (13)$$

Eq. (13) was found to be not applicable to our experimental data because it did not give conver-

$$\upsilon=\frac{V[A]_0[S]_0}{K_{a1}K_{s1}}\Bigg\{\frac{2\beta_{120}[S]_0}{\alpha_{s110}\alpha_{s120}K_{s2}}+\left(\frac{[S]_0}{\alpha_{s220}K_{s2}}+\beta_{210}\right)\frac{[A]_0}{\alpha_{s210}K_{a2}}+\left(\frac{\beta_{221}[S]_0}{\alpha_{s221}K_{s2}}+\beta_{211}\right)\cdot\frac{2[A]_0^2}{\alpha_{s211}\alpha_{i201}K_{a2}K_{i1}}\Bigg\}$$
$$\Big/\Bigg\{1+\frac{[S]_0}{K_{s1}}\left(2+\frac{[S]_0}{K_{s2}}\right)+\frac{2[A]_0}{K_{a1}}\left[1+\frac{[S]_0}{\alpha_{s110}K_{s1}}\left(2+\frac{[S]_0}{\alpha_{s120}K_{s2}}\right)\right]+\frac{[A]_0^2}{K_{a1}K_{a2}}\cdot\left[1+\frac{[S]_0}{\alpha_{s210}K_{s1}}\left(2+\frac{[S]_0}{\alpha_{s220}K_{s2}}\right)\right]+\frac{2[A]_0}{K_{i1}}+\frac{4[A]_0^2}{\alpha_{i101}K_{a1}K_{i1}}+\frac{2[A]_0^3}{\alpha_{i201}K_{a1}K_{a2}K_{i1}}$$
$$\times\left[1+\frac{[S]_0}{\alpha_{s211}K_{s1}}\left(2+\frac{[S]_0}{\alpha_{s221}K_{s2}}\right)\right]+\frac{[A]_0^2}{K_{i1}K_{i2}}+\frac{[A]_0^4}{\alpha_{i201}\alpha_{i202}K_{a1}K_{a2}K_{i1}K_{i2}}\left(1+\frac{2[S]_0}{\alpha_{s212}K_{s1}}\right)\Bigg\}. \quad (12)$$

Eq. (12) was found to be not applicable to our experimental data because it did not give convergence under the regressional analysis. The value of α_{i101} exceeds 1×10^{17} at the moment of interrupting the program run, indicating that the enzyme–glycogen complex filled with one activatory, and one catalytic sites is not formed practically. The corresponding equation for the reaction rate may be written in the form:

gence under the regressional analysis. The β_{210} value was found to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 3×10^{-9} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with two activatory and one catalytic sites is unable to a catalytic transformation.

Assume further that $\beta_{210} = 0$ in Eq. (13). No convergence was found in this case either. The β_{221} value was found to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 3×10^{-9} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with two activatory, two catalytic and one inhibitory sites is unable to a catalytic transformation. Assume further that $\beta_{221} = 0$ in Eq. (10). Again, no convergence was found in this case. The value of α_{s221} exceeds 1×10^{10} at the moment of interrupting the program run, indicating that the enzyme–glycogen complex filled with two activatory, two catalytic and one inhibitory sites is not formed practically. The corresponding equation for the reaction rate may be written in the form:

$$
\begin{aligned}
v = {} & \frac{V[A]_0[S]_0}{K_{a1}K_{s1}} \left\{ \frac{2\beta_{120}[S]_0}{\alpha_{s110}\alpha_{s120}K_{s2}} \right. \\
& \left. + \frac{[A]_0[S]_0}{\alpha_{s210}\alpha_{s220}K_{a2}K_{s2}} + \frac{2\beta_{211}[A]_0^2}{\alpha_{s211}\alpha_{i201}K_{a2}K_{i1}} \right\} \\
& \Big/ \left\{ 1 + \frac{[S]_0}{K_{s1}} \left(2 + \frac{[S]_0}{K_{s2}} \right) \right. \\
& + \frac{2[A]_0}{K_{a1}} \left[1 + \frac{[S]_0}{\alpha_{s110}K_{s1}} \left(2 + \frac{[S]_0}{\alpha_{s120}K_{s2}} \right) \right] \\
& + \frac{[A]_0^2}{K_{a1}K_{a2}} \left[1 + \frac{[S]_0}{\alpha_{s210}K_{s1}} \left(2 + \frac{[S]_0}{\alpha_{s220}K_{s2}} \right) \right] \\
& + \frac{2[A]_0}{K_{i1}} + \frac{2[A]_0^3}{\alpha_{i201}K_{a1}K_{a2}K_{i1}} \left(1 + \frac{2[S]_0}{\alpha_{s211}K_{s1}} \right) \\
& + \frac{[A]_0^2}{K_{i1}K_{i2}} + \frac{[A]_0^4}{\alpha_{i201}\alpha_{i202}K_{a1}K_{a2}K_{i1}K_{i2}} \\
& \times \left. \left(1 + \frac{2[S]_0}{\alpha_{s212}K_{s1}} \right) \right\}. \qquad (14)
\end{aligned}
$$

Eq. (14) was found to be non-applicable to our experimental data because it did not give convergence under the regressional analysis. The β_{120} value was found to decrease constantly in the process of the evaluation of the regression parameters. Its value does not exceed 4×10^{-11} at the moment of interrupting the program run. This fact shows that the enzyme–glycogen complex filled with one activatory and two catalytic sites is unable to a catalytic transformation.

Assume further that $\beta_{120} = 0$ in Eq. (14). No convergence was found in this case either. The value of α_{s120} exceeds 4×10^{10} at the moment of interrupting the program run, indicating that the enzyme–glycogen complex filled with one activatory and two catalytic sites is not formed practically. The corresponding equation for the reaction rate may be written in the form:

$$
\begin{aligned}
v = {} & \frac{V[A]_0[S]_0}{K_{a1}K_{s1}} \\
& \left\{ \frac{[A]_0[S]_0}{\alpha_{s210}\alpha_{s220}K_{a2}K_{s2}} + \frac{2\beta_{211}[A]_0^2}{\alpha_{s211}\alpha_{i201}K_{a2}K_{i1}} \right\} \\
& \Big/ \left\{ 1 + \frac{[S]_0}{K_{s1}} \left(2 + \frac{[S]_0}{K_{s2}} \right) + \frac{2[A]_0}{K_{a1}} \right. \\
& \times \left(1 + \frac{2[S]_0}{\alpha_{s110}K_{s1}} \right) + \frac{[A]_0^2}{K_{a1}K_{a2}} \\
& \times \left[1 + \frac{[S]_0}{\alpha_{s210}K_{s1}} \left(2 + \frac{[S]_0}{\alpha_{s220}K_{s2}} \right) \right] + \frac{2[A]_0}{K_{i1}} \\
& + \frac{2[A]_0^3}{\alpha_{i201}K_{a1}K_{a2}K_{i1}} \left(1 + \frac{2[S]_0}{\alpha_{s211}K_{s1}} \right) \\
& + \frac{[A]_0^2}{K_{i1}K_{i2}} + \frac{[A]_0^4}{\alpha_{i201}\alpha_{i202}K_{a1}K_{a2}K_{i1}K_{i2}} \\
& \times \left. \left(1 + \frac{2[S]_0}{\alpha_{s212}K_{s1}} \right) \right\}. \qquad (15)
\end{aligned}
$$

Eq. (15) was found to be non-applicable to our experimental data because it did not give convergence under the regressional analysis. In the

process of the evaluation of the regression parameters, we observed the stable increase in the K_{i1} value which was compensated by the decrease in the values of α_{s211} and α_{i201} so that their product $\alpha_{s211}\alpha_{i201}\ K_{i1}$ was not changed practically. This variation of the regression parameters does not influence the value of sum of the weighted squares of the difference between the experimental and calculated values of the reaction rate. The K_{i1} value exceeds 1×10^9 at the moment of interrupting the program run, indicating that the enzyme–glycogen complex filled with inhibitory sites by AMP is not formed practically. By this we mean that the affinity of AMP to the activatory site of glycogen phosphorylase *b* is essentially higher than that to the inhibitory site of the enzyme. Thus, the AMP binding in the inhibitory sites occurs exclusively after a saturation of both activatory sites site of glycogen phosphorylase *b*.

Assume further that the binding of AMP in the inhibitory sites of the enzyme with the vacant activatory sites is negligible. The corresponding kinetic scheme of the enzymatic reaction is shown in Fig. 3. The following designations are used: E is the enzyme–glycogen complex; S is glucose-1-P; A is AMP; P is the product(s) of the enzymatic reaction; EA_mS_n is the enzyme–glycogen complex containing m molecules of AMP and n molecules of glucose-1-P, K_{s1} and K_{s2} are the microscopic constants for dissociation of the enzyme species containing glucose-1-P with the liberation of glucose-1-P from the one or two catalytic sites, respectively, K_{a1} and K_{a2} are the microscopic constants for dissociation of the enzyme species containing AMP with the liberation of AMP from one or two activatory sites, respectively; K_{i1} and K_{i2} are the microscopic constants for dissociation of the enzyme species containing AMP with the liberation of AMP from one or two inhibitory sites, respectively; k is the catalytic constant; and α_1, α_2, α_3, α_4, α_5, and β are coefficients. The equation for the initial steady-state rate (v) of the enzymatic reaction may be written in the form:

$$v = \frac{V[A]_0[S]_0}{K_{a1}K_{s1}}\left\{\frac{[A]_0[S]_0}{\alpha_2\alpha_3K_{a2}K_{s2}} + \frac{2\beta[A]_0^2}{\alpha_4K_{a2}K_{i1}}\right\} \Big/ \left\{1 + \frac{[S]_0}{K_{s1}}\left(2 + \frac{[S]_0}{K_{s2}}\right) + \frac{2[A]_0}{K_{a1}}\left(1 + \frac{2[S]_0}{\alpha_1K_{s1}}\right) + \frac{[A]_0^2}{K_{a1}K_{a2}}\left[1 + \frac{[S]_0}{\alpha_2K_{s1}}\left(2 + \frac{[S]_0}{\alpha_3K_{s2}}\right)\right]\right\}$$

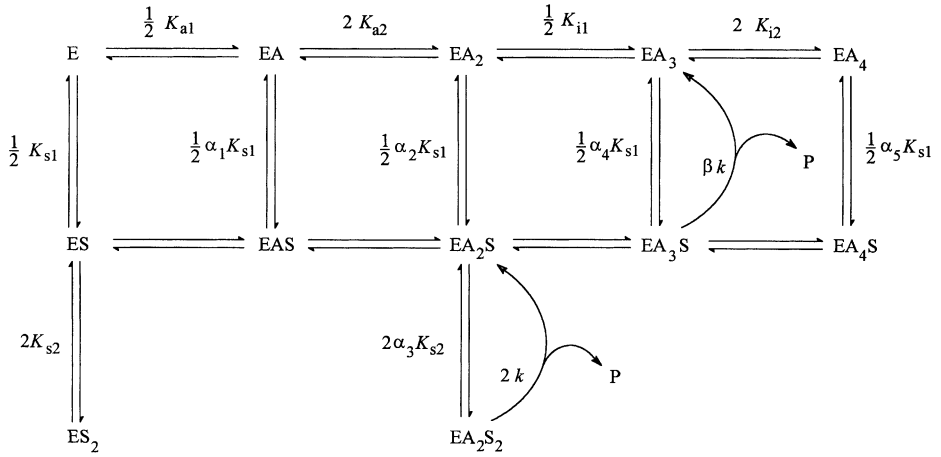

Fig. 3. The kinetic scheme for the dimeric bisubstrate enzyme with the interacting ligand-binding sites in the case of (i) the effector binding both in the high affinity activatory and in the low affinity inhibitory sites and (ii) the binding of the second molecule of one substrate with the species having vacant effector sites or with that having occupied activatory sites under the conditions of the enzyme saturation by other substrate.

$$+\frac{2[A]_0^3}{K_{a1}K_{a2}K_{i1}}\left(1+\frac{2[S]_0}{\alpha_4 K_{s1}}\right)+\frac{[A]_0^4}{K_{a1}K_{a2}K_{i1}K_{i2}}\left(1+\frac{2[S]_0}{\alpha_5 K_{s1}}\right)\Bigg\}, \quad (16)$$

where $\alpha_1=\alpha_{s110}$, $\alpha_2=\alpha_{s210}$, $\alpha_3=\alpha_{s220}$, $\alpha_4=\alpha_{s211}$, $\alpha_5=\alpha_{s212}$, $\beta=\beta_{211}$, $K_{i1}=2[EA_2][A]_0/[EA_2I]$, $K_{i2}=0.5[EA_2I][A]_0/[EA_2I_2]$, other parameters are above mentioned. The mean value of the parameters for Eq. (16) were determined by the non-linear regression method: $k=87\pm6\ s^{-1}$, $K_{a1}=0.13\pm0.04$ mM, $K_{a2}=0.16\pm0.06$ mM, $K_{s1}=3.5\pm0.8$ mM, $K_{s2}=1440\pm19\,400$ M, $K_{i1}=40\pm24$ mM, $K_{i2}=7.4\pm5.5$ mM, $\alpha_1=3.4\pm2.6$, $\alpha_2=0.89\pm0.41$, $\alpha_3=(0.11\pm1.49)\times10^{-5}$, $\alpha_4=0.9\pm0.7$, $\alpha_5=11\pm15$, $\beta=0.26\pm0.17$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 1.18. However, the value of K_{s2} is so great that the enzyme–glycogen complex containing two molecules of glucose-1-P is not formed. This fact indicates that the binding of the second molecule of glucose-1-P occurs exclusively after a saturation both activatory sites of the dimeric enzyme molecule.

Assume further that the binding of the second molecule of glucose-1-P with the dimeric enzyme molecule is negligible in the absence of bound AMP. The corresponding kinetic scheme of the enzymatic reaction is shown in Fig. 4. The following designations are used: E is the enzyme–glycogen complex; S is glucose-1-P; A is AMP; P is the product(s) of the enzymatic reaction; EA_mS_n is the enzyme-glycogen complex containing m molecules of AMP and n molecules of glucose-1-P; K_{s1} and K_{s2} are the microscopic constants for dissociation of the enzyme species containing glucose-1-P with the liberation of glucose-1-P from one or two catalytic sites, respectively; K_{a1} and K_{a2} are the microscopic constants for dissociation of the enzyme species containing AMP with the liberation of AMP from one or two activatory sites, respectively; K_{i1} and K_{i2} are the microscopic constants for dissociation of the enzyme species containing AMP with the liberation of AMP from one or two inhibitory sites, respectively; k is the catalytic constant, α_1, α_2, α_3, α_4, and β are coefficients. The equation for the initial steady-state rate (v) of the enzymatic reaction may be written in the form:

$$v=\frac{V[A]_0[S]_0}{K_{a1}K_{s1}}\left\{\frac{[A]_0[S]_0}{\alpha_2K_{a2}K_{s2}}+\frac{2\beta[A]_0^2}{\alpha_3K_{a2}K_{i1}}\right\}\Big/\left\{\left(1+\frac{2[S]_0}{K_{s1}}\right)\left(1+\frac{2[A]_0}{\alpha_1K_{a1}}+\frac{[A]_0^2}{\alpha_2K_{a1}K_{a2}}+\frac{2[A]_0^3}{\alpha_3K_{a1}K_{a2}K_{i1}}+\frac{[A]_0^4}{\alpha_4K_{a1}K_{a2}K_{i1}K_{i2}}\right)+\frac{[A]_0^2[S]_0^2}{\alpha_2K_{a1}K_{a2}K_{s1}K_{s2}}\right\}, \quad (17)$$

where $K_{s2}=0.5[EA_2S][S]_0/[EA_2S_2]$, other parameters are above mentioned. The mean value of the parameters for Eq. (17) were determined by the non-linear regression method: $k=87\pm6\ s^{-1}$, $K_{a1}=0.130\pm04$ mM, $K_{a2}=0.16\pm0.06$ mM, $K_{s1}=3.5\pm0.8$ mM, $K_{s2}=1.57\pm0.44$ mM, $K_{i1}=40\pm24$ mM, $K_{i2}=7.4\pm5.4$ mM, $\alpha_1=3.4\pm2.6$, $\alpha_2=0.89\pm0.41$, $\alpha_3=0.9\pm0.7$, $\alpha_4=11\pm15$ and $\beta=0.26\pm0.17$. The ratio of the dispersions of the adequacy and reproducibility was calculated to be 1.17. However, seven parameters of Eq. (17) are not reliable according to Student's criterion for 95% confidence level. The non-reliability of the parameters of Eq. (17) makes impossible its using in the fitting procedure. Since the mean value of β is indistinguishable from zero, assume further that $\beta=0$. The equation for the initial steady-state rate (v) of the enzymatic reaction may be written in the form:

$$v=\frac{V[A]_0^2[S]_0^2}{\alpha_2K_{a1}K_{a2}K_{s1}K_{s2}}\Big/\left\{\left(1+\frac{2[S]_0}{K_{s1}}\right)\times\left(1+\frac{2[A]_0}{\alpha_1K_{a1}}+\frac{[A]_0^2}{\alpha_2K_{a1}K_{a2}}+\frac{2[A]_0^3}{\alpha_3K_{a1}K_{a2}K_{i1}}+\frac{[A]_0^4}{\alpha_4K_{a1}K_{a2}K_{i1}K_{i2}}\right)+\frac{[A]_0^2[S]_0^2}{\alpha_2K_{a1}K_{a2}K_{s1}K_{s2}}\right\}. \quad (18)$$

The parameters of Eq. (18) are identical to those of Eq. (17). The mean value of the parameters for

Eq. (18) were determined by the non-linear regression method: $k = 90 \pm 7\ s^{-1}$, $K_{a1} = 0.12 \pm 0.04$ mM, $K_{a2} = 0.19 \pm 0.08$ mM, $K_{s1} = 3.4 \pm 0.8$ mM, $K_{s2} = 1.8 \pm 0.5$ mM, $K_{i1} = 34 \pm 19$ mM, $K_{i2} = 34 \pm 29$ mM, $\alpha_1 = 3.7 \pm 2.8$, $\alpha_2 = 0.71 \pm 0.34$, $\alpha_3 = 1.0 \pm 0.8$, $\alpha_4 = 1.3 \pm 1.0$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 1.22. However, six parameters of Eq. (18) are not reliable according to the Student criterion for 95% confidence level. The non-reliability of the parameters of Eq. (18) makes impossible its using in the fitting procedure. Since the mean values of α_3 and α_4 are indistinguishable from unity, assume further that $\alpha_3 = 1$ and $\alpha_4 = 1$. The equation for the initial steady-state rate (v) of the enzymatic reaction may be written in the form:

$$v = \frac{V[A]_0^2[S]_0^2}{\alpha_2 K_{a1} K_{a2} K_{s1} K_{s2}} \Big/ \left\{ \left(1 + \frac{2[S]_0}{K_{s1}}\right) \times \left(1 + \frac{2[A]_0}{\alpha_1 K_{a1}} + \frac{[A]_0^2}{\alpha_2 K_{a1} K_{a2}} + \frac{2[A]_0^3}{K_{a1} K_{a2} K_{i1}} + \frac{[A]_0^4}{K_{a1} K_{a2} K_{i1} K_{i2}}\right) + \frac{[A]_0^2[S]_0^2}{\alpha_2 K_{a1} K_{a2} K_{s1} K_{s2}} \right\} \quad (19)$$

The parameters of Eq. (19) are identical to those for Eq. (17). The mean value of the parameters for Eq. (19) were determined by the non-linear regression method: $k = 90 \pm 7\ s^{-1}$, $K_{a1} = 0.13 \pm 0.02$ mM, $K_{a2} = 0.18 \pm 0.05$ mM, $K_{s1} = 3.7 \pm 0.4$ mM, $K_{s2} = 1.78 \pm 0.45$ mM, $K_{i1} = 34 \pm 9$ mM, $K_{i2} = 39 \pm 6$ mM, $\alpha_1 = 3.0 \pm 1.1$, $\alpha_2 = 0.67 \pm 0.30$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 1.17. However, the mean values of K_{i1} and K_{i2} are indistinguishable from one another according to Student's criterion for 95% confidence level. Furthermore, the mean value of α_3 and α_4 is indistinguishable from unity. The non-reliability of the parameters of Eq. (19) makes its use in the fitting procedure impossible. Since the mean values of K_{i1} and K_{i2} are indistinguishable from one another, assume further that $K_{i1} = K_i$ and $K_{i2} = K_i$. The equation for the initial steady-state rate (v) of the enzymatic reaction may be written in the form:

$$v = \frac{V[A]_0^2[S]_0^2}{\alpha_2 K_{a1} K_{a2} K_{s1} K_{s2}} \Big/ \left\{ \left(1 + \frac{2[S]_0}{K_{s1}}\right) \times \left[1 + \frac{2[A]_0}{\alpha_1 K_{a1}} + \frac{[A]_0^2}{\alpha_2 K_{a1} K_{a2}} + \frac{[A]_0^3}{K_{a1} K_{a2} K_i} \times \left(2 + \frac{[A]_0}{K_i}\right)\right] + \frac{[A]_0^2[S]_0^2}{\alpha_2 K_{a1} K_{a2} K_{s1} K_{s2}} \right\}. \quad (20)$$

The parameters of Eq. (20) are identical to those of Eq. (17). The mean value of the parameters for Eq. (20) were determined by the non-linear regression method: $k = 91 \pm 7\ s^{-1}$, $K_{a1} = 0.14 \pm 0.02$ mM, $K_{a2} = 0.18 \pm 0.05$ mM, $K_{s1} = 3.7 \pm 0.4$ mM, $K_{s2} = 1.82 \pm 0.45$ mM, $K_i = 35 \pm 9$ mM, $\alpha_1 = 3.0 \pm 1.1$, $\alpha_2 = 0.65 \pm 0.28$. The ratio of the dispersions of the adequacy and reproducibility was calculated to be 1.16. However, the mean values of α_1 and α_2 were indistinguishable from unity. The non-reliability of the parameters of Eq. (19) makes impossible its using in the fitting procedure. Assume further that $\alpha_1 = 1$ and $\alpha_2 = 1$. The corresponding kinetic scheme of the enzymatic reaction is shown in Fig. 5. The following designations are used: E is the enzyme–glycogen complex; S is glucose-1-P; A is AMP; P is the product(s) of the enzymatic reaction; EA_mS_n is the enzyme–glycogen complex containing m molecules of AMP and n molecules of glucose-1-P; K_{s1} and K_{s2} are the microscopic constants for dissociation of the enzyme species containing glucose-1-P with the liberation of glucose-1-P from the one or two catalytic sites, respectively; K_{a1} and K_{a2} are the microscopic constants for dissociation of the enzyme species containing AMP with the liberation of AMP from one or two activatory sites, respectively; K_i is the microscopic constant for dissociation of the enzyme species containing AMP with the liberation of AMP from the inhibitory site; k is the catalytic constant. The equation for the initial steady-state rate (v) of the enzymatic reaction may be written in the form:

$$v = \frac{V[A]_0^2[S]_0^2}{K_{a1}K_{a2}K_{s1}K_{s2}} \Big/ \left\{ \left(1 + \frac{2[S]_0}{K_{s1}}\right) \times \left[1 + \frac{2[A]_0}{K_{a1}} + \frac{[A]_0^2}{K_{a1}K_{a2}}\left(1 + \frac{[A]_0}{K_i}\right)^2\right] + \frac{[A]_0^2[S]_0^2}{K_{a1}K_{a2}K_{s1}K_{s2}} \right\} \quad (21)$$

The parameters of Eq. (21) are identical to those of Eq. (17). The mean value of the parameters for Eq. (21) were determined by the non-linear regression method: $k = 84 \pm 3\ \mathrm{s}^{-1}$, $K_{a1} = 0.22 \pm 0.02$ mM, $K_{a2} = 0.088 \pm 0.06$ mM, $K_{s1} = 4.4 \pm 0.4$ mM, $K_{s2} = 1.3 \pm 0.1$ mM, $K_i = 40 \pm 2$ mM. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 1.35. This value is less than the Fisher criterion value of 2.1 for the 95% confidence level. Thus, Eq. (21) adequately fits the dependence of the initial steady-state rate on glucose-1-P and AMP concentrations for the reaction catalyzed by glycogen phosphorylase *b* from rabbit skeletal muscle in the wide range of AMP concentration under the conditions of the saturation of the enzyme by glycogen.

Consideration of all the above mentioned kinetic equations indicates that, in an accordance with the accepted criteria, preference is given to the kinetic mechanism based on the following assumptions: (i) the independent binding of one molecule of glucose-1-P in the catalytic site, on the one hand, and AMP in both allosteric effector and both nucleoside inhibitor sites of the dimeric enzyme molecule bound by glycogen, on the other hand; (ii) the binding of AMP in one of the allosteric effector sites results in the increase in the affinity of other allosteric effector site to AMP; (iii) the independent binding of AMP in the nucleoside inhibitor sites of the dimeric enzyme molecule; (iv) the exclusive binding of the second molecule of glucose-1-P in the catalytic site of glycogen phosphorylase *b* containing two molecules of AMP occupying both allosteric effector sites; and (v) the catalytic act occurs exclusively in the complex of the enzyme with glycogen, two molecules of AMP occupying both allosteric effector sites and two molecules of glucose-1-P occupying both catalytic sites.

Consider the possible conformational changes in the molecule of glycogen phosphorylase *b* compatible with the proposed kinetic scheme. The

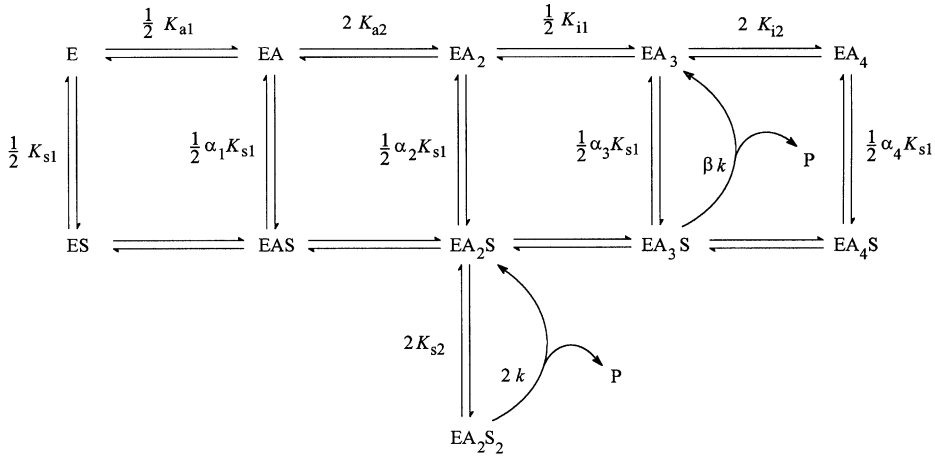

Fig. 4. The kinetic scheme for the dimeric bisubstrate enzyme with the interacting ligand-binding sites in the case of (i) the binding of effector both in the high affinity activatory sites and in the low affinity inhibitory sites and (ii) the binding of the second molecule of one substrate exclusively after the saturation both activatory sites under the conditions of the enzyme saturation by other substrate.

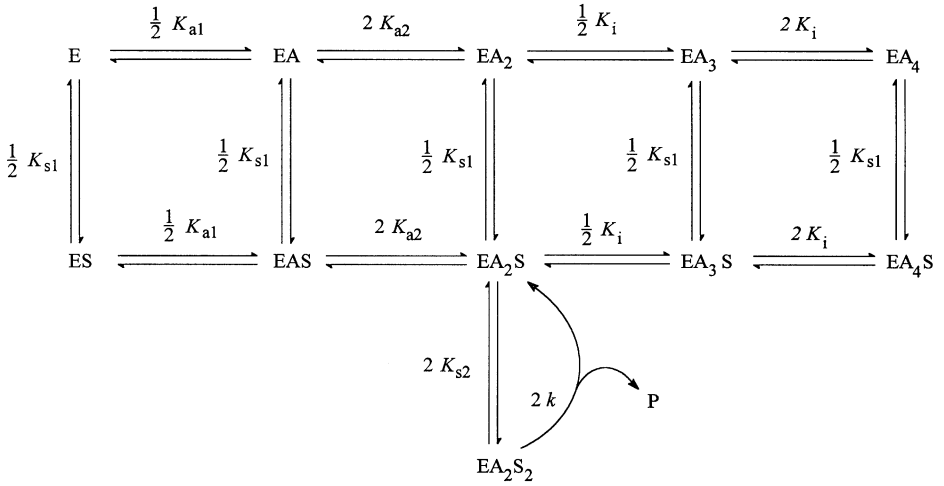

Fig. 5. The kinetic scheme for the allosteric regulation of glycogen phosphorylase *b* by AMP.

binding of the first molecule of AMP in the allosteric effector site of the dimeric enzyme species accompanies the increase in the affinity of the second allosteric effector site to AMP. The reason is probably that the conformational changes in the dimeric enzyme molecule occur upon the binding of the first molecule of AMP. The binding of AMP in sites of any kind does not influence the binding of the first molecule of glucose-1-P in the catalytic site of the enzyme. This fact suggests that the conformational changes induced by AMP do not influence the locus of the binding of glucose-1-P in the catalytic site of the enzyme. The binding of the first molecule of glucose-1-P by the enzyme species containing two molecules of AMP results in the increase in its affinity to the second molecule of glucose-1-P, possibly as a consequence of the conformational changes of the dimeric enzyme molecule induced by glucose-1-P and AMP together. According to the proposed kinetic scheme, the dimeric enzyme molecule bound by glycogen reveals catalytic activity exclusively upon the binding of both two molecules of AMP and two molecules of glucose-1-P. This means that the transition of the enzyme into the catalytically active conformation occurs upon the binding of both two molecules of AMP and two molecules of glucose-1-P.

Acknowledgements

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