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Kinetic Mechanism of Activation of Muscle Glycogen Phosphorylase *b* by Adenosine 5′-Monophosphate

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The kinetic analysis of the enzymatic reaction catalyzed by glycogen phosphorylase *b* from the rabbit skeletal muscle has been carried out in the direction of glycogen synthesis under the conditions of the saturation of the enzyme by glycogen. The applicability of 12 different variants of the Monod–Wyman–Changeux model and 6 kinetic schemes involving the interaction of AMP- and glucose-1-phosphate-binding sites in the dimeric enzyme molecule is considered. The kinetic model has been proposed, which assumes (i) the independent binding of AMP and one molecule of glucose 1-phosphate with the enzyme saturated by glycogen, (ii) the exclusive binding of the second molecule of glucose 1-phosphate by the enzyme containing two molecules of AMP and one molecule of glucose 1-phosphate, and (iii) the exclusive ability of the complex of the enzyme with glycogen, two molecules of AMP, and two molecules of glucose 1-phosphate to undergo the catalytic transformation. The parameters of the equation of the initial steady-state rate of the enzymatic reaction are calculated by the nonlinear regression method. The proposed kinetic scheme is shown to satisfy to the following criteria: (i) the convergence under the regressional analysis, (ii) the reliability of the values of the parameters of the model, (iii) the minimality of the sum of the weighted squares of the differences between the experimental and calculated values of the reaction rate.

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 nonreducing ends of the side chains of glycogen, resulting in the formation of glucose-1-P.[2] The dephosphorylated form of enzyme (glycogen phosphorylase *b*) from rabbit skeletal muscles is a dimer of two identical subunits. Glycogen phosphorylase *b* reveals a catalytic activity in the presence of the allosteric activator AMP (1). AMP binds in the allosteric site located at a distance of 3.2 nm from the catalytic site (2). The activatory action of AMP does not follow the hyperbolic law: the dependence of the enzyme activity on AMP concentration is nonlinear in the reciprocal plot (3). The different variants of the Monod–Wyman–Changeux model (4), which assumes the existence of two conformational states (R and T) of the oligomeric enzyme, were used to describe the kinetics of glycogen phosphorylase *b* action. Helmreich and collaborators (5) supposed that the catalytic activities of R- and T-states were identical, but the affinities of these states to the ligands were different. Other authors (6, 7) suggested that R-state is active, while T-state is inactive and binds neither substrates nor AMP. Bresler and Firsov (8) suggested that T-state is inactive, but has affinity to AMP, while R-state becomes active when two molecules of AMP are bound. Lowry *et al.* (9) used a traditional approach to the kinetic study of the reaction catalyzed by glycogen phosphorylase *b*. They suggested that (i) the binding of one molecule of the ligand affects the binding of the second molecule of the ligand, (ii) the binding of one ligand affects the binding of other ligand, and (iii) the enzyme reveals catalytic power exclusively upon the binding with two molecules of AMP and two molecules of inorganic phosphate under the conditions of saturation by glycogen. It should be noted that these authors calculated the parameters of the equation of the enzymatic reaction rate with the use of the graphic methods without any information about a structure of the error of their experiments and without any statistical estimation of the obtained values.

An alternative approach to the kinetic analysis of the allosteric enzymes involves the use of the nonlinear regression method for the determination of the parameters of the enzymatic reaction rate equation (10, 11). Reich *et al.* (10) used this method to study the kinetics of the enzymatic reaction catalyzed by glycogen phosphorylase *b* from pig skeletal muscles. They found that the steady-

[1] To whom correspondence should be addressed.
[2] Abbreviation used: glucose-1-P, glucose 1-phosphate.

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state kinetics of this reaction were described satisfactorily by the Monod–Wyman–Changeux model involving the catalytically inactive T-state of the enzyme. Kasvinsky *et al.* (11) used the method of nonlinear regression for the analysis of the applicability of seven kinetic models for fitting the steady-state kinetics for the reaction of the lengthening of maltoheptaose saccharide chain catalyzed by glycogen phosphorylase *a* from rabbit skeletal muscles.

In the present paper we study the applicability of a number of kinetic models to the steady-state kinetics of glycogen synthesis catalyzed by glycogen phosphorylase *b* from the rabbit skeletal muscles under the conditions of saturation of the enzyme by glycogen. The variants of the Monod–Wyman–Changeux model used by us are chosen taking into account the variants of this model proposed by other authors. We analyzed some variants of the model involving the interaction of AMP- and glucose-1-P-binding sites. The parameters of the enzymatic reaction rate equation were calculated using the method of nonlinear regression. The kinetic model of the enzymatic reaction was chosen according to the following criteria (12): (i) the convergence under the regressional analysis, (ii) the reliability of the values of the parameters of the model, (iii) the minimality of the sum of the weighed squares of the differences between the experimental and calculated values of the reaction rate.

MATERIALS AND METHODS

Materials. Phosphorylase *b* was isolated from rabbit skeletal muscles according to Fischer and Krebs (13) with the modifications proposed by Lissovskaja *et al.* (14). Fourfold-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 Fischer and Krebs (13). This solution was used for 1 day. The enzyme concentration was determined spectrophotometrically at 280 nm. The absorption coefficient and the molecular mass of phosphorylase *b* monomer were assumed to be 1.32 (g/liter) $^{-1}$ cm $^{-1}$ (15) and 97,500 Da (16), respectively.

Pig liver glycogen (Olina, Latvia) was purified by reprecipitation with ethanol according Sutherland and Wosilait (17). The average molecular glycogen mass and proportion of nonreducing glucose residue were 5,500,000 Da and 6.4%, respectively (18). Disodium adenosine 5'-monophosphate and dipotassium glucose-1-phosphate were purchased from Reanal (Hungary). All other reagents (research and analytical grade) were purchased from Soyuzkhimreactiv (Russia).

Phosphorylase assay. The catalytic activity of phosphorylase *b* in the direction of glycogen synthesis was determined using turbidimetric method (19) 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 0.3 M KCl and 0.2 mM EDTA. 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 a fixed glycogen concentration of 1 g/liter. Increase in glycogen concentration up to 5 g/liter did not influence on the value of the initial steady-state rate of the enzymatic reaction. Relative error in the measurement of the initial steady-state rate of the enzymatic reaction was determined to be 3.0%.

Computer calculations. The parameters of the equation for the initial steady-state rate of the enzymatic reaction were calculated using the nonlinear 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 regression parameters was increased up to 12, (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.

RESULTS AND DISCUSSION

The experimental dependences of the initial steady-state rate of the enzymatic reaction catalyzed by rabbit muscle phosphorylase *b* on the glucose-1-P concentration are nonlinear in the reciprocal coordinates (Fig. 1). These dependences were obtained at various fixed concentrations of AMP indicated in the legend to the figure. Analogous dependence of the initial steady-state rate of the enzymatic reaction on the glucose-1-P concentration obtained at 0.3 mM AMP is also nonlinear in the reciprocal coordinates (not shown in Fig. 1). The nonlinear dependences of the initial steady-state rate of the reaction catalyzed by rabbit muscle phosphorylase *b* on the glucose-1-P concentration in Scatchard coordinates were found by Helmreich with collaborators (5). The nonlinear character of these plots is indicative of nonhyperbolic kinetics of the phosphorylase *b* action. The computer analysis of the dependences shown in Fig. 1 using the empirical Hill equation indicates that the value of the Hill coefficient for glucose-1-P does not appear to depend on the chosen concentration of AMP. The average value of the Hill coefficient for glucose-1-P is 1.4 ± 0.1. We use these data also

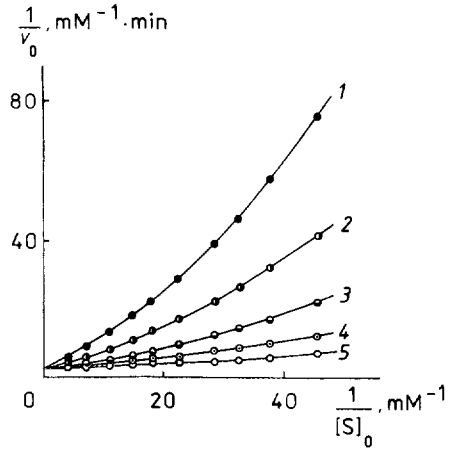

FIG. 1. The dependence of the initial steady-state rate of the reaction catalyzed by phosphorylase *b* on the glucose-1-P concentration in the presence of 1 g/liter glycogen at fixed concentrations of AMP (mM): 0.04 (*1*), 0.06 (*2*), 0.1 (*3*), 0.2 (*4*), 1.0 (*5*). Points are experimental data; solid lines are calculated according to Eq. [18] for the following values of parameters: k = 89 s^{-1}, K_{s1} = 0.22 mM, K_{s2} = 0.09 mM, K_{s1} = 4.4 mM, K_{s2} = 1.5 mM, $[E]_0$ = 59 nM.

to calculate the value of the Hill coefficient for AMP, which does not depend on the chosen concentration of glucose-1-P. The average value of the Hill coefficient for AMP is 1.6 ± 0.1. The values of the Hill coefficients for AMP and glucose-1-P calculated by us are practically identical to those determined by other authors (6, 21–23).

It is reasonable to start the analysis of the possible mechanisms resulting in the nonhyperbolic kinetics of the phosphorylase *b* action from the discussion of the generalized Monod–Wyman–Changeux model. According to this model the transition between two conformational states of the oligomeric enzyme occurs by the concerted manner. The ligand-binding sites are equivalent and noninteracting in any enzyme state. The conformational states of the enzyme differ in their affinity to ligand or/ and in the catalytic activity. The kinetic scheme of the generalized Monod–Wyman–Changeux model for dimeric unisubstrate enzyme in the presence of the allosteric activator is presented in Fig. 2. According to this scheme the equation of the initial steady-state rate of the enzymatic reaction may be written in the form

$$\upsilon_0 = \frac{V(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)[S]_0/K_s + V'L(1 + [A]_0/K'_a)^2(1 + [S]_0/K'_s)[S]_0/K'_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L(1 + [A]_0/K'_a)^2(1 + [S]_0/K'_s)^2}, \quad [1]$$

where υ_0 is the initial steady-state rate of the enzymatic reaction, V and V' are the maximal rates for R- and T-state respectively, $[S]_0$ and $[A]_0$ are substrate and activator concentrations, respectively, K_s and K'_s are the dissociation constants for the complexes between substrate and R- and T-state respectively, K_a and K'_a are the dissociation constants for the complexes between activator and R- and T-state respectively, L is the allosteric constant. This variant of the generalized Monod–Wyman–Changeux model is not applicable to fit our experimental data because the Eq. [1] does not give convergence.

As for the variants of the generalized Monod–Wyman–Changeux model, they involve the additional statements about the properties of R- and T-states of the enzyme. In the case of K-system according Monod *et al.* (4) R- and T-states have the identical catalytic activities and the different affinities to the ligand(s). If the unisubstrate dimeric enzyme refers to K-system, then the equation of the initial steady-state rate of the enzymatic reaction is

$$\upsilon_0 = V\frac{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)[S]_0/K_s + L(1 + [A]_0/K'_a)^2(1 + [S]_0/K'_s)[S]_0/K'_a}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L(1 + [A]_0/K'_a)^2(1 + [S]_0/K'_s)^2}, \quad [2]$$

where V is the maximal rate for R- and T-state of the enzyme. This variant of the generalized Monod–Wyman–Changeux model is not applicable to our experimental data because Eq. [2] does not give convergence.

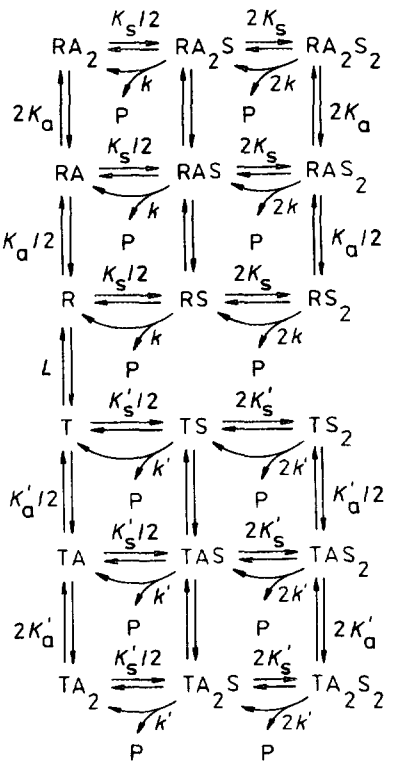

FIG. 2. The kinetic scheme of the generalized Monod–Wyman–Changeux model for dimeric unisubstrate enzyme in the presence of the allosteric activator. The following designations are used: R and T are different conformational states of the dimeric molecule of the enzyme, S is the substrate, A is the allosteric activator, P is the product(s) of the enzymatic reaction, K_s and K'_s are the dissociation constants for the complexes of the substrate with R- and T-state respectively, K_a and K'_a are the dissociation constants for the complexes of the activator with R- and T-state respectively, L is the allosteric constant, k and k' are the catalytic constants for R- and T-state, respectively.

To take into account that phosphorylase *b* from rabbit skeletal muscles is inactive in the absence of AMP, it is reasonable to discuss the variant of the generalized Monod–Wyman–Changeux model in which the complexes of substrate with R- and T-state are catalytically inactive except when they contain bound AMP. The equation of the initial steady-state rate of the enzymatic reaction may be written in the form

$$\upsilon_0 = \frac{V(2 + [A]_0/K_a)[A]_0/K_a(1 + [S]_0/K_s)[S]_0/K_s + V'L(2 + [A]_0/K'_a)[A]_0/K'_a(1 + [S]_0/K'_s)[S]_0/K'_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L(1 + [A]_0/K'_a)^2(1 + [S]_0/K'_s)^2}. \quad [3]$$

This variant of the generalized Monod–Wyman–Changeux model is not applicable to our experimental data

because Eq. [3] does not give convergence. Thus, the variants of the generalized Monod–Wyman–Changeux model, which assumes that T-state is catalytically active, are not applicable to our experimental data because the corresponding equations of the reaction rate do not give convergence.

Let us discuss the variants of the generalized Monod–Wyman–Changeux model in which T-state is catalytically inactive. If other additional statements are absent, then the equation of the initial steady-state rate of the enzymatic reaction can be written in the form

$$v_0 = \frac{V(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)[S]_0/K_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L(1 + [A]_0/K'_a)^2(1 + [S]_0/K'_s)^2} . \quad [4]$$

This variant of the generalized Monod–Wyman–Changeux model is not applicable to our experimental data because Eq. [4] does not give convergence.

Since AMP is absolutely required as activator, it is reasonable to discuss the variant of the generalized Monod–Wyman–Changeux model in which the complexes of substrate with R-state are catalytically inactive except the case when they contain bound AMP. We can write the equation of the initial steady-state rate of the enzymatic reaction as follows:

$$v_0 = \frac{V(2 + [A]_0/K_a)[A]_0/K_a(1 + [S]_0/K_s)[S]_0/K_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L(1 + [A]_0/K'_a)^2(1 + [S]_0/K'_s)^2} . \quad [5]$$

This variant of the generalized Monod–Wyman–Changeux model is not applicable to fit our experimental data because the Eq. [5] does not give convergence.

According to Bresler and Firsov (8) R-state acquires the catalytically active conformation exclusively upon the binding of two molecule of AMP. Using this additional statement, we obtain the following equation of the initial steady-state rate of the enzymatic reaction:

$$v_0 = \frac{V[A]_0^2/K_a^2(1 + [S]_0/K_s)[S]_0/K_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L(1 + [A]_0/K'_a)^2(1 + [S]_0/K'_s)^2} . \quad [6]$$

This variant of the generalized Monod–Wyman–Changeux model is not applicable to our experimental data because Eq. [6] does not give convergence. Thus, the variants of the generalized Monod–Wyman–Changeux model which assume nonexclusive binding of both ligands are not applicable to fit our experimental data because the corresponding equations of the reaction rate do not give convergence.

Let us consider the variants of the generalized Monod–Wyman–Changeux model for the unisubstrate dimeric enzyme in the presence of allosteric activator for the case of the exclusive binding of the glucose-1-P with the R-state. Assuming that glucose-1-P is bound exclusively by R-state, we may write

$$v_0 = \frac{V(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)[S]_0/K_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L(1 + [A]_0/K'_a)^2} . \quad [7]$$

The mean values of the parameters of Eq. [7] were calculated by the nonlinear regression method: $V = 0.74 \pm 0.05$ mM/min, $K_a = 11 \pm 3$ μM, $K_s = 8.1 \pm 0.9$ mM, $K'_a = 1.6 \times 10^{10}$ M, $L = 350 \pm 140$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 9.8. However, the value of the dissociation constant for the complex of AMP with T-state is so large that the binding of AMP with T-state is negligible. This fact contradicts to an assumption that T-state is able to bind AMP (this assumption was made when deriving Eq. [7]). Thus, this variant of the concerted model is not applicable to fit our experimental data.

If both AMP and glucose-1-P are bound exclusively by R-state, then the equation of the initial steady-state rate of the enzymatic reaction is

$$v_0 = \frac{V(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)[S]_0/K_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L} . \quad [8]$$

The mean values of the parameters of Eq. [8] were calculated by the nonlinear regression method: $V = 0.74 \pm 0.05$ mM/min, $K_a = 11 \pm 3$ μM, $K_s = 8.1 \pm 0.9$ mM, $L = 350 \pm 140$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 9.6. This value exceeds the Fisher criterion value of 2.1 for the 95% confidence level. Thus, this variant of the concerted model does not fit adequately our experimental data.

To take into account that phosphorylase *b* from rabbit skeletal muscles is inactive in the absence of AMP, it is reasonable to discuss the variant of the generalized Monod–Wyman–Changeux model in which the complexes of substrate with R-state are catalytically inactive except when they contain bound AMP. The equation of the initial steady-state rate of the enzymatic reaction may be written in the following form:

$$v_0 = \frac{V(2 + [A]_0/K_a)[A]_0/K_a(1 + [S]_0/K_s)[S]_0/K_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L(1 + [A]_0/K'_a)^2} . \quad [9]$$

The mean values of the parameters of Eq. [9] were calculated using the nonlinear regression method: $V = 0.71 \pm 0.03$ mM/min, $K_a = 42 \pm 7$ μM, $K_s = 6.6 \pm 0.7$ mM, K'_a

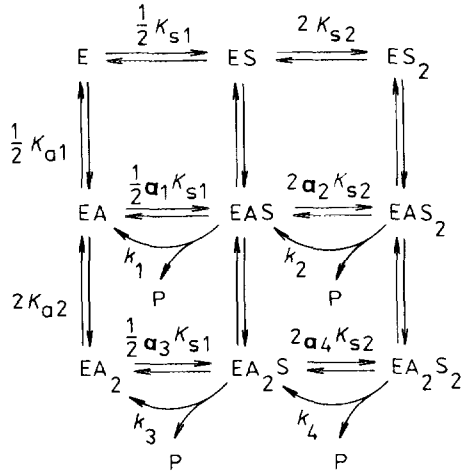

FIG. 3. The kinetic scheme for the dimeric bisubstrate enzyme with absolutely required activator in the case of saturation by one substrate. The following designations are used: E is the enzyme–glycogen complex; S is glucose-1-P; A is AMP; P is the product(s) of the enzymatic reaction; K_{s1} and K_{s2} are the dissociation constants for the complexes of the enzyme with glycogen and one or two molecules of glucose-1-P, respectively; K_{a1} and K_{a2} are the dissociation constants for the complexes of the enzyme with glycogen and one or two molecules of AMP, respectively; α_1, α_2, α_3, and α_4 are coefficients of the interaction between the binding sites for glucose-1-P and AMP; k_1, k_2, k_3, and k_4 are the constants of the rate of the catalytic transformation for the enzyme complexes.

$= 3.6 \times 10^{11}$ M, $L = 53 \pm 13$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 7.6. However, the value of the dissociation constant for the complex of AMP with T-state is so large that the binding of AMP with T-state is negligible. This fact contradicts to an assumption that T-state is able to bind AMP (this assumption was made when deriving Eq. [9]). Thus, this variant of the concerted model is not applicable to our experimental data.

Let to discuss the variant of the generalized Monod–Wyman–Changeux model proposed by Kurganov *et al.* (24). The following assumptions were used by these authors: (i) glucose-1-P and AMP are bound exclusively by R-state, and (ii) protomers containing both glucose-1-P and AMP are catalytically active. In this case we can write the equation of the initial steady-state rate of the enzymatic reaction as follows:

$$v_0 = \frac{V(2 + [A]_0/K_a)[A]_0/K_a(1 + [S]_0/K_s)[S]_0/K_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L}. \quad [10]$$

The mean values of the parameters of Eq. [10] were calculated by the nonlinear regression method: $V = 0.71 \pm 0.03$ mM/min, $K_a = 42 \pm 7$ μM, $K_s = 6.6 \pm 0.7$ mM, $L = 53 \pm 13$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 7.5. This value exceeds the Fisher criterion value of 2.1 for the 95% confidence level. Thus, this variant of the concerted model does not fit adequately our experimental data.

If (i) glucose-1-P is bound exclusively by R-state, (ii) AMP is bound by both states of the enzyme, and (iii) R-state acquires the catalytic activity exclusively upon binding of two molecules of AMP, then the equation of the initial steady-state rate of the enzymatic reaction may be written in the form

$$v_0 = \frac{V[A]_0^2/K_a^2(1 + [S]_0/K_s)[S]_0/K_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L(1 + [A]_0/K'_a)^2}. \quad [11]$$

The mean values of the parameters of Eq. [11] were calculated by the nonlinear regression method: $V = 0.70 \pm 0.13$ mM/min, $K_a = 22 \pm 2$ μM, $K_s = 3.5 \pm 0.3$ mM, $K'_a = 218 \pm 12$ μM, $L = 113 \pm 17$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 2.7. This value exceeds the Fisher criterion value of 2.1 for the 95% confidence level. Thus, this variant of the concerted model does not fit adequately our experimental data.

In the case of the exclusive binding of both glucose-1-P and AMP by R-state, which acquires catalytic activity exclusively upon binding of two molecules of AMP, the equation of the initial steady-state rate of the enzymatic reaction is

$$v_0 = \frac{V[A]_0^2/K_a^2(1 + [S]_0/K_s)[S]_0/K_s}{(1 + [A]_0/K_a)^2(1 + [S]_0/K_s)^2 + L}. \quad [12]$$

The mean values of the parameters of Eq. [12] were calculated by the nonlinear regression method: $V = 0.85 \pm 0.05$ mM/min, $K_a = 16 \pm 3$ μM, $K_s = 9.5 \pm 0.9$ mM, $L = 89 \pm 33$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 7.2. This value exceeds the Fisher criterion value of 2.1 for the 95% confidence level. Thus, this variant of the concerted model does not fit adequately our experimental data.

Let us discuss the kinetic model of the other kind. The model assumes that: (i) there are interactions between the binding sites for the ligands (glucose-1-P and AMP) (i.e., the binding of the ligand in one site results in the change in the affinity of the other site to this ligand), (ii) the binding of one ligand affects the binding of the other ligand, (iii) the complex of the enzyme, glycogen, and glucose-1-P does not undergo catalytic transformation except when it contains bound AMP. The kinetic scheme for this model under the conditions of the saturation of the enzyme by glycogen is presented in Fig. 3. The corresponding equation of the initial steady-state rate of the enzymatic reaction may be written in the form

$$v_0 = \frac{2[E]_0[A]_0[S]_0/K_{a1}K_{s1}(2k_1/\alpha_1 + k_2[S]_0/\alpha_1\alpha_3K_{s2} + k_3[A]_0/\alpha_2K_{a2} + k_4[A]_0[S]_0/\alpha_2\alpha_4K_{a2}K_{s2})}{(1 + 2[S]_0/K_{s1} + [S]_0^2/K_{s1}K_{s2} + 2[A]_0/K_{a1} + [A]_0^2/K_{a1}K_{a2} + 4[A]_0[S]_0/\alpha_1K_{a1}K_{s1} + 2[A]_0[S]_0^2/\alpha_1\alpha_3K_{a1}K_{s1}K_{s2} + 2[A]_0^2[S]_0/\alpha_2K_{a1}K_{a2}K_{s1} + [A]_0^2[S]_0^2/\alpha_2\alpha_4K_{a1}K_{a2}K_{s1}K_{s2})} \quad [13]$$

where v_0 is the initial steady-state rate of the enzymatic reaction, $[E]_0$ is the total concentration of the enzyme–glycogen complex, $[S]_0$ and $[A]_0$ are substrate and activator concentrations respectively, k_1 is the rate constant for the catalytic transformation of the enzyme–glycogen complex containing one molecule of glucose-1-P and one molecule of AMP, k_2 is the rate constant for the catalytic transformation of the enzyme–glycogen complex containing two molecules of glucose-1-P and one molecule of AMP, k_3 is the rate constant for the catalytic transformation of the enzyme–glycogen complex containing one molecule of glucose-1-P and two molecules of AMP, k_4 is the rate constant for the catalytic transformation of the enzyme–glycogen complex containing two molecules of glucose-1-P and two molecules of AMP, K_{s1} and K_{s2} are the dissociation constants for the enzyme–glycogen complex containing one or two molecules of glucose-1-P, respectively, K_{a1} and K_{a2} are the dissociation constants for the enzyme–glycogen complex containing one or two molecules of AMP, respectively, α_1 is a coefficient of the interaction between the binding sites for the first molecule of glucose-1-P and the first molecule of AMP, α_2 is a coefficient of the interaction between the binding sites for the second molecule of glucose-1-P and the first molecule of AMP, α_3 is a coefficient of the interaction between the binding sites for the first molecule of glucose-1-P and the second molecule of AMP, and α_4 is a coefficient of the interaction between the binding sites for the second molecule of glucose-1-P and the second molecule of AMP. This variant of the model of interacting ligand-binding sites is not applicable to our experimental data because Eq. [13] does not give convergence. The k_1 value was found to decrease constantly in the process of the evaluation of the regression parameters. This rate constant does not exceed 1×10^{-13} s^{-1} at the moment of the interrupting of the program run.

Let us discuss the variant of the model of interacting ligand-binding sites in which the enzyme–glycogen complex does not undergo the catalytic transformation when it contains one molecule of glucose-1-P and one molecule of AMP. In this case the equation of the initial steady-state rate of the enzymatic reaction is as follows:

$$v_0 = \frac{2[E]_0[A]_0[S]_0/K_{a1}K_{s1}(k_2[S]_0/\alpha_1\alpha_3K_{s2} + k_3[A]_0/\alpha_2K_{a2} + k_4[A]_0[S]_0/\alpha_2\alpha_4K_{a2}K_{s2})}{(1 + 2[S]_0/K_{s1} + [S]_0^2/K_{s1}K_{s2} + 2[A]_0/K_{a1} + [A]_0^2/K_{a1}K_{a2} + 4[A]_0[S]_0/\alpha_1K_{a1}K_{s1} + 2[A]_0[S]_0^2/\alpha_1\alpha_3K_{a1}K_{s1}K_{s2} + 2[A]_0^2[S]_0/\alpha_2K_{a1}K_{a2}K_{s1} + [A]_0^2[S]_0^2/\alpha_2\alpha_4K_{a1}K_{a2}K_{s1}K_{s2})} \quad [14]$$

This variant of the model of interacting ligand-binding sites is not applicable to experimental data because Eq. [14] does not give convergence. The k_2 value was found to decrease constantly in the process of the evaluation of the regression parameters. This rate constant does not exceed 1×10^{-6} s^{-1} at the moment of the interrupting of the program run.

Let us discuss the variant of the model of interacting ligand-binding sites in which the enzyme–glycogen complex does not undergo the catalytic transformation when it contains one molecule of AMP. In this case the equation of the initial steady-state rate of the enzymatic reaction may be written in the form

$$v_0 = \frac{2[E]_0[A]_0[S]_0/K_{a1}K_{s1}(k_3[A]_0/\alpha_2K_{a2} + k_4[A]_0[S]_0/\alpha_2\alpha_4K_{a2}K_{s2})}{(1 + 2[S]_0/K_{s1} + [S]_0^2/K_{s1}K_{s2} + 2[A]_0/K_{a1} + [A]_0^2/K_{a1}K_{a2} + 4[A]_0[S]_0/\alpha_1K_{a1}K_{s1} + [A]_0[S]_0^2/\alpha_1\alpha_3K_{a1}K_{s1}K_{s2} + 2[A]_0^2[S]_0/\alpha_2K_{a1}K_{a2}K_{s1} + [A]_0^2[S]_0^2/\alpha_2\alpha_4K_{a1}K_{a2}K_{s1}K_{s2})} \quad [15]$$

This variant of the model of interacting ligand-binding sites is not applicable our experimental data because Eq. [15] does not give convergence. At the moment of the interrupting of the program run α_2 value exceeds 4×10^{13}, indicating that the complex of the enzyme with glycogen, one molecule of AMP and two molecules of glucose-1-P, is not formed.

Let us discuss the variant of the model of interacting ligand-binding sites in which (i) the complex of the enzyme with glycogen, one molecule of AMP and two molecules of glucose-1-P, is not formed; (ii) the enzyme–glycogen complex does not undergo catalytic transformation when it contains one molecule of AMP and one molecule of glucose-1-P. In this case we can write the equation of the initial steady-state rate of the enzymatic reaction as follows:

$$v_0 = \frac{2[E]_0[A]_0[S]_0/K_{a1}K_{s1}(k_3[A]_0/\alpha_2K_{a2} + k_4[A]_0[S]_0/\alpha_2\alpha_4K_{a2}K_{s2})}{(1 + 2[S]_0/K_{s1} + [S]_0^2/K_{s1}K_{s2} + 2[A]_0/K_{a1} + [A]_0^2/K_{a1}K_{a2} + 4[A]_0[S]_0/\alpha_1K_{a1}K_{s1} + 2[A]_0^2[S]_0/\alpha_2K_{a1}K_{a2}K_{s1} + [A]_0^2[S]_0^2/\alpha_2\alpha_4K_{a1}K_{a2}K_{s1}K_{s2})} \quad [16]$$

The mean values of the parameters of Eq. [16] were calculated by the nonlinear regression method: $k_3 = 12 \pm 15$

s^{-1}, $k_4 = 82 \pm 5\ s^{-1}$, $K_{a1} = 0.28 \pm 0.16$ mM, $K_{a2} = 76 \pm 41$ μM, $K_{s1} = 8.9 \pm 7.4$ mM, $K_{s2} = 69 \pm 140$ mM, $\alpha_1 = 0.57 \pm 0.55$, $\alpha_3 = 0.64 \pm 0.34$, $\alpha_4 = 0.024 \pm 0.049$. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 1.8. However, eight parameters of Eq. [16] are not reliable according to the Student criterion for 95% confidence level. The nonreliability of the parameters of Eq. [16] makes impossible the use of this variant of the model to our experimental data.

It should be noted that the mean values of parameters α_1 and α_3 do not significantly differ from unity according the Student criterion for 95% confidence level. So it is reasonable to discuss the variant of the model of interacting ligand-binding sites in which (i) the complex of the enzyme with glycogen, one molecule of AMP and two molecules of glucose-1-P is not formed, (ii) the affinity of the enzyme–glycogen complexes to the first molecule of glucose-1-P is not changed upon binding of AMP, and (iii) the enzyme–glycogen complex does not undergo the catalytic transformation when it contains one molecule of AMP and one molecule of glucose-1-P. In this case the equation of the initial steady-state rate of the enzymatic reaction can be written in the form

$$v_0 = \frac{2[E]_0[A]_0[S]_0/K_{a1}K_{s1}(k_3[A]_0/K_{a2} + k_4[A]_0[S]_0/\alpha_4 K_{a2}K_{s2})}{(1 + 2[S]_0/K_{s1} + [S]_0^2/K_{s1}K_{s2} + 2[A]_0/K_{a1} + [A]_0^2/K_{a1}K_{a2} + 4[A]_0[S]_0/K_{a1}K_{s1} + 2[A]_0^2[S]_0/K_{a1}K_{a2}K_{s1} + [A]_0^2[S]_0^2/\alpha_4 K_{a1}K_{a2}K_{s1}K_{s2})} \quad [17]$$

This variant of the model of interacting ligand-binding sites is not applicable to our experimental data because Eq. [17] does not give convergence. The K_{s2} value was found to increase constantly in the process of the evaluation of the regression parameters, while the $\alpha_4 K_{s2}$ value was not practically changed due to an decrease in the α_4 value. The variation of K_{s2} and α_4 values does not influence the value of a sum of the weighted squares of the difference between the experimental and calculated values of the reaction rate. At the moment of interrupting the program run K_{s2} exceeds 4×10^{10} M, indicating that the concentration of the complex of the enzyme with glycogen and two molecules of glucose-1-P is negligible.

Let us discuss the variant of the model of interacting ligand-binding sites in which (i) the complex of the enzyme with glycogen and two molecules of glucose-1-P is not formed, (ii) the complex of the enzyme with glycogen, one molecule of AMP and two molecules of glucose-1-P, is not formed, (iii) an affinity of the enzyme–glycogen complexes to the first molecule of glucose-1-P is not changed upon binding of AMP, and (iv) the enzyme–glycogen complex does not undergo the catalytic transformation when it contains one molecule of AMP and one molecule of glucose-1-P. In this case the equation of the initial steady-state rate of the enzymatic reaction may be written in the form

$$v_0 = \frac{2k[E]_0[A]_0^2[S]_0^2/K_{a1}K_{a2}K_{s1}K_{s2}}{(1 + 2[S]_0/K_{s1} + 2[A]_0/K_{a1} + [A]_0^2/K_{a1}K_{a2} + 4[A]_0[S]_0/K_{a1}K_{s1} + 2[A]_0^2[S]_0/K_{a1}K_{a2}K_{s1} + [A]_0^2[S]_0^2/K_{a1}K_{a2}K_{s1}K_{s2})} \quad [18]$$

where k is the rate constant for the catalytic transformation of the enzyme–glycogen complex containing two molecules of glucose-1-P and two molecules of AMP. The mean values of the parameters of Eq. [18] were calculated by the nonlinear regression method: $k = 89 \pm 3\ s^{-1}$, $K_{a1} = 0.22 \pm 0.02$ mM, $K_{a2} = 0.09 \pm 0.01$ mM, $K_{s1} = 4.4 \pm 0.4$ mM, $K_{s2} = 1.5 \pm 0.1$ mM. The ratio of the dispersions of the adequacy and reproducibility is calculated to be 1.7. This value is less than the Fisher criterion value of 2.1 for the 95% confidence level. Thus, this variant of the model of interacting ligand-binding sites fits adequately the dependence of the initial steady-state rate on glucose-1-P and AMP concentrations for the reaction catalyzed by glycogen phosphorylase *b* from the rabbit skeletal muscles under the conditions of the saturation of the enzyme by glycogen.

The analysis of the kinetic mechanisms of phosphorylase *b* reaction indicates that the variant of the model of interacting ligand-binding sites corresponding to Eq. [18] gives adequate description of our experimental data in accordance with the criteria by Bartfai and Mannervik (12). The kinetic mechanism under discussion is presented in Fig. 4. Let us discuss the possible conformational changes in phosphorylase *b* molecule that are compatible with the proposed kinetic model. The fact that the binding of the first molecule of AMP results in a decrease in the dissociation constant for the binding of the second molecule of AMP is indicative of the conformational change in the dimeric enzyme molecule induced by AMP. AMP does not affect the binding of the first molecule of glucose-1-P, suggesting that the conformational change in the enzyme induced by AMP does not extend on the locus of glucose-1-P binding. The fact that the binding of the first molecule of glucose-1-P by the enzyme–glycogen complex containing two molecules of AMP results in a decrease in the dissociation constant for the second molecule of glucose-1-P is indicative of the change in the enzyme conformation induced by substrates and AMP. According to the kinetic scheme presented in Fig. 4 the dimeric enzyme molecule saturated by glycogen acquires catalytic activity exclusively upon binding of two molecules of AMP and two molecules of glucose-1-P. This fact indicates that phosphorylase *b* is transformed into the catalytically active conformation exclusively upon binding of two molecules of AMP and two molecules of glucose-1-P under the conditions of saturation by glycogen.

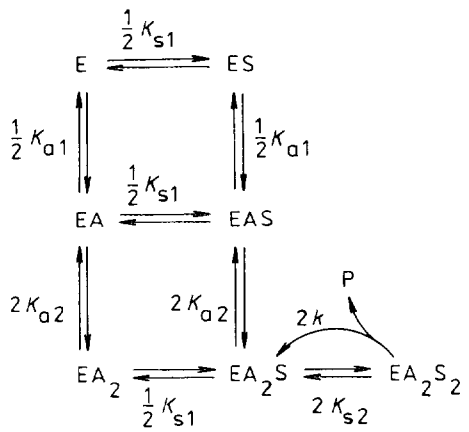

FIG. 4. The kinetic scheme for the reaction glycogen synthesis catalyzed by glycogen phosphorylase *b* at the saturation by glycogen. The following designations are used: E is the enzyme–glycogen complex; S is glucose-1-P; A is AMP; P is the product(s) of the enzymatic reaction; K_{s1} is the dissociation constant for the complex of the enzyme with glycogen and one molecule of glucose-1-P; K_{a1} and K_{a2} are the dissociation constants for the complexes of the enzyme with glycogen and one or two molecules of AMP, respectively; K_{s2} is the dissociation constant for the release of one molecule of glucose-1-P from the complex of the enzyme with glycogen, two molecules of AMP and two molecules of glucose-1-P.

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REFERENCES

1. Graves, D. J., and Wang, J. H. (1972) *in* The Enzymes (Boyer, P. D., Ed.), Vol. 7, pp. 435–482, Academic Press, New York.
2. Oikonomakos, N. G., Acharya, K. R., and Johnson, L. N. (1992) *in* Post-Translational Modifications of Proteins (Harding, J. J., and Crabbe, M. J. C., Eds.), pp. 81–151, CRC Press, Boca Raton, FL.
3. Helmreich, E., and Cori, C. F. (1964) *Proc. Natl. Acad. Sci. USA* **51,** 131–138.
4. Monod, J., Wyman, J., and Changeux, J.-P. (1965) *J. Mol. Biol.* **12,** 88–118.
5. Kastenschmidt, L. L., Kastenschmidt, J., and Helmreich, E. (1968) *Biochemistry* **7,** 4543–4556.
6. Madsen, N. B., and Shechosky, S. (1967) *J. Biol. Chem.* **242,** 3301–3307.
7. Buc, M. H., and Buc, H. (1968) *in* The Regulation of Enzyme Activity and Allosteric Interactions (Kvamme, E., and Pihl, A., Eds.), pp. 109–130, Universitetsforlaget, Oslo.
8. Bresler, S., and Firsov, L. (1971) *Biopolymers* **10,** 1187–1205.
9. Lowry, O. H., Schulz, D. W., and Passonneau, J. V. (1967) *J. Biol. Chem.* **242,** 271–280.
10. Reich, J. G., Wangermann, G., Falck, M., and Rohde, K. (1972) *Eur. J. Biochem.* **26,** 368–379.
11. Kasvinsky, P. J., Madsen, N. B., Fletterick, R. J., and Sygusch, J. (1978) *J. Biol. Chem.* **253,** 1290–1296.
12. Bartfai, T., and Mannervik, B. (1972) *FEBS Lett.* **26,** 252–256.
13. Fischer, E. H., and Krebs, E. G. (1958) *J. Biol. Chem.* **231,** 65–71.
14. Lissovskaja, N. P., Livanova, N. B., and Silonova, G. V. (1964) *Biokhimiya* **29,** 1012–1019. (in Russian)
15. Buc, M. H., Ulmann, A., Goldberg, M., and Buc, H. (1971) *Biochimie* **53,** 283–289.
16. Titani, K., Koide, A., Hermann, J., Ericsson, L. H., Kumar, S., Wade, R. D., Walsh, K. A., Neurath, H., and Fischer, E. H. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4762–4766.
17. Sutherland, E. W., and Wosilait, W. D. (1956) *J. Biol. Chem.* **218,** 459–468.
18. Klinov, S. V., Chebotareva, N. A., Lissovskaya, N. P., Davydov, D. R., and Kurganov, B. I. (1982) *Biochim. Biophys. Acta.* **709,** 91–98.
19. Sugrobova, N. P., Lissovskaja, N. P., and Kurganov, B. I. (1983) *J. Biochem. Biophys. Methods* **8,** 299–306.
20. Duggleby, R. G. (1984) *Comput. Biol. Med.* **14,** 447–455.
21. Sealock, R. W., and Graves, D. J. (1967) *Biochemistry* **6,** 201–207.
22. Black, W. J., and Wang, J. H. (1968) *J. Biol. Chem.* **243,** 5892–5898.
23. Oikonomakos, N. G., Sotiroudis, T. G., and Evangelopoulos, A. E. (1979) *Biochem. J.* **181,** 309–320.
24. Silonova, G. V., Livanova, N. B., and Kurganov, B. I. (1969) *Molekulyarnaya Biologiya* **3,** 768–784. (in Russian)