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

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Abstract—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 AMP concentration under the saturation of the enzyme by glycogen. Applicability of some 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 of the enzymatic reaction describing adequately the activation of the enzyme by AMP and inhibition at sufficiently high concentrations of AMP is proposed.

Key words: glycogen phosphorylase *b*, kinetic mechanism, allosteric regulation

Glycogen phosphorylase (1,4-α-D-glucan:orthophosphate glycosyltransferase, EC 2.4.1.1) catalyzes the reversible phosphorolytic splitting of the terminal glycosyl residues from polysaccharide chains of glycogen in the form of glucose-1-P [1]. The dephosphorylated form of the enzyme (glycogen phosphorylase *b*) from rabbit skeletal muscles consists of two identical subunits and reveals catalytic activity only in the presence of the allosteric activator AMP [2]. The activating action of AMP is due to nucleotide binding in the allosteric effector site located in the region of the contact of enzyme subunits [3, 4]. The decrease in activating action of AMP is observed when its concentration rises, because of nucleotide binding in the nucleoside inhibitor site [5, 6]. The latter is located at the entrance to the channel to the catalytic site of the enzyme [3, 4].

The activation of glycogen phosphorylase *b* induced by AMP is not described by the simple equation of hyperbolic form: the dependence of the rate of the enzymatic reaction on the activator concentration is not linearized in double reciprocal coordinates [7]. Kinetic investigations of the enzyme activation 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 conditions of enzyme saturation by glycogen [8-11]. In the present paper, the steady-state kinetics of the reaction catalyzed by glycogen phosphorylase *b* from rabbit skeletal muscles is studied over a wide range of AMP concentration under the conditions of enzyme saturation by glycogen. The kinetic model of the reaction was chosen according to the following criteria [12]: 1) convergence under regression analysis; 2) reliability of the values of the parameters of the equation for the initial rate of the reaction; 3) minimum of the sum of the weighted squares of the difference between the experimental and calculated values of the reaction rate.

MATERIALS AND METHODS

Glycogen phosphorylase *b* was isolated from rabbit skeletal muscles according to Fisher and Krebs [13] using β-mercaptoethanol instead of DL-cysteine. Fourfold-crystallized preparation of the enzyme was used over less than two weeks after isolation. AMP was removed from the enzyme solution by adsorption on Norit A activated charcoal [13]. The prepared solution of the enzyme was used over one day. Pig liver glycogen (Olaina Chemical Reagents Plant) was subjected to additional purification [14]. The average molecular mass of glycogen and proportion of the terminal glucose residues were 5,500 kD [15] and 6.4%, respectively [16]. The enzyme concentration was determined spectrophotometrically at 280 nm using the specific coefficient of absorption of 1.32

Abbreviation: glucose-1-P) glucose 1-phosphate.

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(g/liter)$^{-1}$·cm^{-1} [17]. The molecular mass of the enzyme subunit was assumed to be 97,500 daltons [18].

The catalytic activity of glycogen phosphorylase *b* was determined by the turbidimetric method [19] at 310 nm using a Cary-219 spectrophotometer (Varian, USA) at temperature 30°C. The reaction mixture contained glycogen (1 g/liter), 0.2 mM EDTA, and 0.05 M glycylglycine buffer, pH 6.8. The ionic strengths of the all solutions were raised to 0.3 M using KCl. The glucose-1-P and AMP increments in the ionic strength were calculated based on the values of pK_a = 6.01 [20] and 6.24 [21], respectively. The enzymatic reaction was initiated by adding the enzyme. It was shown specially that the change in the order of the addition of the components of the enzymatic reaction and the increase in glycogen concentration up to 5 g/liter had no influence on the initial steady-state rate. Relative error in the measurement of the initial steady-state rate of the enzymatic reaction was 3%.

The parameters of the equation for the initial steady-state rate of the enzymatic reaction were computed by the nonlinear regression method. The following modifications were incorporated into the program used [22]: 1) instead of the approximated calculation of the partial derivatives of the dependent variable with respect to the parameters of the equation of the regression by the numerical method, the explicit calculation of these values was used; 2) the number of parameters was increased to 38; 3) the experimental data were input using a separate file.

Disodium adenosine 5′-monophosphate and dipotassium glucose 1-phosphate (Reanal, Hungary) were used in the work. Other reagents were produced in Russia.

RESULTS AND DISCUSSION

The dependence of the initial rate of the enzymatic reaction on the AMP concentration is bell-shaped in semi-logarithmic coordinates (Fig. 1). The ascending branch of the bell-shaped curve characterizes the activating action of AMP caused by the binding of AMP in the allosteric effector site of the enzyme molecule. The descending branch reflects the inhibitory action of AMP caused by its binding in the nucleoside inhibitor site. For quantitative description of the kinetics observed, a model of direct cooperativity [23] for the dimeric enzyme was used with a condition that effector binds both in the activating sites and inhibitory sites of the enzyme subunit. This model assumes that the sites of the binding of a ligand (AMP or glucose-1-P) are interacting, e.g., the binding of the ligand with one subunit results in a change in the affinity of the other subunit to the ligand. We assumed also that the binding of one ligand influences the binding of the other ligand and a ternary complex of the enzyme with glycogen and glucose 1-P is unable to undergo the catalytic transformation if it does not contain AMP in the activating site of the enzyme. However, this model seems to be not applicable to the data obtained because the criterion of the convergence under regression analysis is not fulfilled in this case. The convergence of the regression process was not observed also for some other variants of this model that assumes a fewer number of the complexes of the enzymes with ligands. Some other variants of this model that assume that the binding of AMP in the inhibitory sites of the enzyme with unsaturated activating sites is negligible are satisfied to the criterion of convergence under regression analysis but do not reliably determine the parameters of the equation for the initial rate of the enzymatic reaction.

The only mechanism of the enzymatic reaction satisfying the criteria of convergence and reliability of the

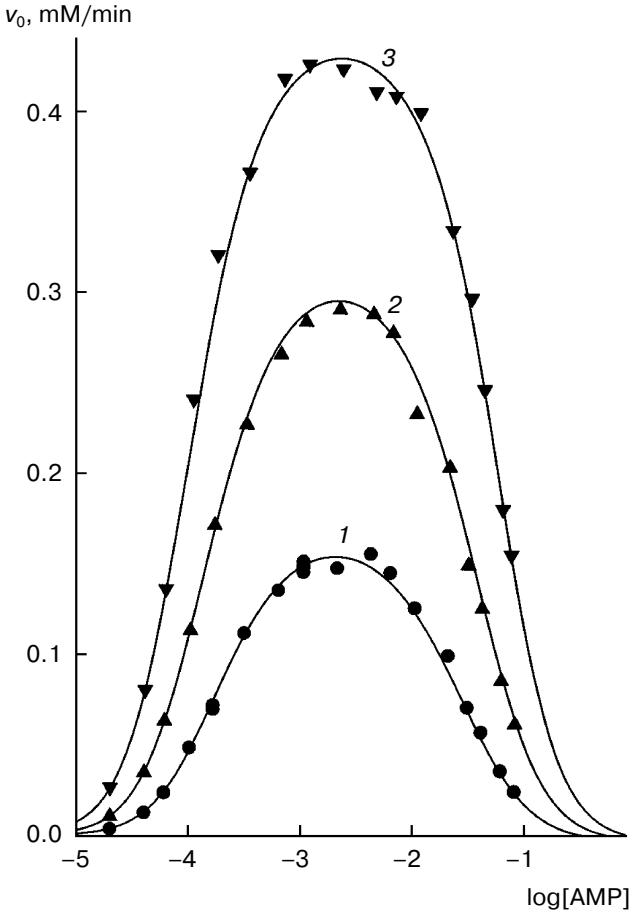

Fig. 1. Dependences of the initial steady-state rate of the enzymatic reaction catalyzed by glycogen phosphorylase *b* (62 nM) from rabbit skeletal muscle in the presence of glycogen (1 g/liter) on the AMP concentration at the following concentrations of glucose-1-P (mM): 2.0 (*1*), 4.0 (*2*), and 8.0 (*3*). The points are the experimental data; the curves are calculated according to the equation for the initial rate using of the parameter values given in the text.

regression parameters is based on the following assumptions: 1) independent binding of the first molecule of glucose-1-P, on one hand, and AMP, on the other hand, in the allosteric effector and nucleoside inhibitor sites of the dimeric enzyme molecule bound by glycogen; 2) binding of AMP in one of the allosteric effector sites results in increase in the affinity of other allosteric effector site to AMP; 3) independent binding of AMP in the nucleoside inhibitor sites of the dimeric enzyme molecule; 4) exclusive binding of the second molecule of glucose-1-P in the catalytic site of the dimeric molecule containing two molecules of AMP in both allosteric effector sites; 5) the catalytic act occurs exclusively in the complex of the dimeric enzyme molecule with glycogen, two molecules of AMP occupying the two allosteric effector sites and two molecules of glucose-1-P bound in the both catalytic sites. The corresponding kinetic scheme 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, K_{s1} and K_{s2} are the microscopic dissociation constants describing the glucose-1-P addition to the enzyme–glycogen complex in the first and second catalytic sites, respectively, K_{a1} and K_{a2} are the analogous microscopic dissociation constants for complexes with AMP, K_i is the dissociation constant describing AMP addition to the inhibitory site of the enzyme, k is the rate constant for the chemical transformation. The corresponding equation for the initial steady-state rate (v) of the reaction can be written in the following form:

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

$$+\frac{[A]_0^2}{K_{a1}K_{a2}}(1+\frac{[A]_0}{K_i})^2] + \frac{[A]_0^2[S]_0^2}{K_{a1}K_{a2}K_{s1}K_{s2}}\},$$

where $[E]_0$, $[A]_0$, and $[S]_0$ are total concentrations of the enzyme, AMP, and glucose-1-P, respectively. The mean values of the parameters for this equation were determined by the nonlinear regression: $k = 84 \pm 3$ sec^{-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 1.35. This value is less than the Fisher criterion of 2.1 for the 95% confidence level. Thus, this equation describes adequately the obtained dependence of the initial steady-state rate for the reaction catalyzed by glycogen phosphorylase *b* on glucose-1-P and AMP concentrations under the conditions of the saturation of the enzyme by glycogen (Fig. 1).

Consider the possible conformational changes in the molecule of glycogen phosphorylase *b* in accordance with the proposed kinetic scheme. The circumstance that the binding of the first molecule of AMP in the allosteric effector site results in the increase in the affinity of the second allosteric effector site to AMP is indicative of the existence of the conformational changes in the dimeric enzyme molecule at the binding of the first molecule of AMP. AMP does not influence the binding of the first molecule of glucose-1-P in the catalytic site. This allows us to suggest that the conformational changes induced by the first molecule of AMP do not influence the locus of the binding of glucose-1-P in the catalytic site. The binding of the first molecule of glucose-1-P by the enzyme–glycogen complex containing two molecules of AMP results in an increase in the affinity to the second molecule of glucose-1-P. This fact indicates that the change of the enzyme conformation is induced by substrates and AMP together. According to the proposed kinetic scheme, the dimeric enzyme molecule saturated by glycogen acquires the catalytically active conformation exclusively upon the binding of both two molecules of AMP and two molecules of glucose-1-P.

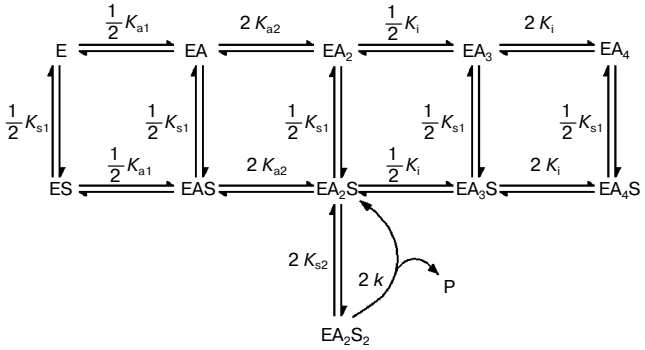

Fig. 2. Kinetic scheme for the allosteric regulation of glycogen phosphorylase *b* by AMP.

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