

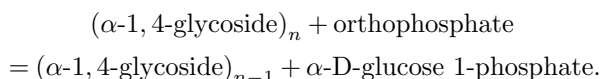
KINETIC MECHANISM OF ALLOSTERIC REGULATION OF MUSCLE GLYCOGEN PHOSPHORYLASEB BY ADENOSINE 5'-MONOPHOSPHATE

S. V. Klinov and B. I. Kurganov

The steady-state kinetics of the reaction catalyzed by glycogen phosphorylase *b* from rabbit skeletal muscles has been studied in a wide range of the concentrations of adenosine 5'-monophosphate in the direction of glycogen synthesis under the conditions of saturation of the enzyme by glycogen. The kinetic model has been proposed to describe the bell-shaped dependence of the initial rate of the enzymatic reaction on the concentration of adenosine 5'-monophosphate. The parameters of the equation for the initial rate of the enzymatic reaction are calculated by the nonlinear regression method. The conformational transitions in the glycogen phosphorylase *b* molecule compatible with the proposed kinetic mechanism are discussed.

A distinguishing feature of the allosteric enzymes is an existence of the distinct spatially separated site, in which the binding of the allosteric effector occurs. Allosteric enzymes, as a rule, consist of two or more subunits and have at least two catalytic and two allosteric sites [1]. One of the approaches to elucidation of the mechanism of the site interaction in the enzyme molecule is the study of the kinetic mechanism of allosteric regulation of the enzymatic activity.

Glycogen phosphorylase (1,4- α -D-glucan: orthophosphate glycosyltransferase, EC 2.4.1.1) catalyzes the reversible phosphorolytic splitting off the terminal glucose residues of the polysaccharide chains of glycogen in the form of α -D-glucose 1-phosphate:



The equilibrium of this reaction is shifted to left: the ratio of the equilibrium concentrations of α -D-glucose 1-phosphate and orthophosphate is equal to 0.28 at pH 6.8. However in the living cell the enzymatic reaction proceeds in the direction of glycogen degradation, because the intracellular concentration of orthophosphate is substantially higher the corresponding value for α -D-glucose 1-phosphate. α -D-Glucose 1-phosphate formed *in vivo* is utilized by phosphoglucomutase and the other enzymes.

Among mammalian glycogen phosphorylases the enzyme from the rabbit skeletal muscles is best studied [2]. In the muscle cell glycogen phosphorylase is adsorbed to glycogen particles. The enzyme exists in the two basic forms: (1) dephosphorylated form (glycogen phosphorylase *b*), which does not reveal a catalytic activity in the absence of allosteric activator adenosine 5'-monophosphate (AMP), and (2) phosphorylated form (glycogen phosphorylase *a*), which catalyzes the glycogen phosphorolysis in the absence of AMP. The transition from the one form to the other is exerted by phosphorylase kinase and phosphorylase phosphatase.

The polypeptide chain of glycogen phosphorylase from rabbit skeletal muscles contains 842 amino acid residues, forming a relatively compact subunit with the radius of about 3.0 nm. The main oligomeric form of the enzyme is a dimer, consisting of the identical subunits. Each subunit of glycogen phosphorylase involves two domains, consisting of the β -structural core surrounded by α -helices. Crystallographic studies revealed four ligand-binding sites: catalytic site, allosteric effector site, glycogen storage site and nucleoside inhibitor site [3-4]. The catalytic site is located between two domains in the center of the subunit. The catalytic site includes pyridoxal 5'-phosphate, aldehyde group of which forms Schiff's base with the ϵ -amino group of Lys 680. The diffusion of the substrates into the catalytic site occurs through a narrow channel which is 1.2 nm long. The access into the catalytic site of the enzyme is restricted by the part of the polypeptide chain involving the residues from 282 to 286. Other sites are located on the surface of the protein molecule. The allosteric effector site is at a distance of 3.2 nm from the catalytic site and situated in the region of the subunit contact. The glycogen storage site is 3.0 nm apart from the catalytic site and 4.0 nm apart from allosteric effector site. The nucleoside inhibitor site is located between the domains at the entrance to the catalytic site channel.

In the present paper the steady-state kinetics of the reaction catalyzed by glycogen phosphorylase *b* from the rabbit skeletal muscles has been studied in a wide range of the concentrations of the allosteric activator AMP in the direction of glycogen synthesis under the conditions of saturation of the enzyme by glycogen. Some kinetic models were used for the quantitative analysis of the steady-state kinetics of the enzymatic reaction. The parameters of the equation for the initial rate of the enzymatic reaction were calculated by the nonlinear regression method. The kinetic model of the enzymatic reaction was chosen according to the following criteria [5]: (1) the convergence under the regression analysis, (2) the reliability of the parameter values of the equation for the initial reaction rate, and (3) the

minimum value of the sum of the weighted squares of the differences between the experimental and calculated values of the initial reaction rate.

Glycogen phosphorylase *b* was isolated from rabbit skeletal muscles according to the method of Fischer and Krebs [6]. The enzyme concentration was determined spectrophotometrically at 280 nm using the specific extinction coefficient equal to $1.32 \text{ (g/liter)}^{-1} \cdot \text{cm}^{-1}$ [7]. The molecular mass of the enzyme subunit was assumed to be 97500 Da [8]. The catalytic activity of glycogen phosphorylase *b* was determined by the turbidimetric method [9] at 30°C and 310 nm in the presence of 1.0 g/l glycogen, 0.2 mM EDTA and 0.3 M KCl in 0.05 M glycylglycine buffer, pH 6.8, using a spectrophotometer Cary-219 (Varian). It was shown that the increase in glycogen concentration up to 5 g/l did not influence the initial steady-state rate of the enzymatic reaction. The evaluation of the parameters of the equation for initial rate was carried out by the nonlinear regression method according to Marquardt algorithm with the computer program [10].

The dependence of the initial rate of the enzymatic reaction on the concentration of AMP has a shape of a bell in semi-logarithmic coordinates (Fig. 1). The ascending branch of the bell-shaped curve characterizes the activating action of AMP arising from the binding of AMP in the allosteric effector site of the enzyme molecule. The descending branch is connected with the inhibiting action of AMP due to its binding in the nucleoside inhibitor site of the enzyme. A number of the kinetic models suggesting the interaction between ligand-binding sites in the dimeric enzyme molecule was used to describe quantitatively the bell-shaped dependence of the initial rate of the enzymatic reaction on AMP concentration. The best fit with the above-mentioned criteria was obtained for the kinetic model with the following assumptions: (1) the independent binding of the first molecule of α -D-glucose 1-phosphate in the catalytic site and four molecules of AMP in the allosteric effector and nucleoside inhibitor sites of the dimeric enzyme molecule bound by glycogen, (2) the exclusive binding of the second molecule of α -D-glucose 1-phosphate in the catalytic site of the dimeric enzyme molecule containing two AMP molecules occupying both allosteric effector sites and one molecule of α -D-glucose 1-phosphate bound in the catalytic site of glycogen phosphorylase *b* adsorbed by glycogen, and (3) the catalytic act occurs exclusively in the complex of the dimeric enzyme molecule with glycogen, two AMP molecules occupying both allosteric effector sites and two molecules of α -D-glucose 1-phosphate bound in both catalytic sites. The proposed kinetic scheme is presented in Fig. 2. The values of the microscopic constants for the dissociation of the complexes of the glycogen-bound enzyme molecule with one and two molecules of AMP occupying one and two allosteric effector sites were found to be 0.22 ± 0.02 and 0.089 ± 0.006 mM respectively. The corresponding values for the dissociation constants of the complexes of the glycogen-bound enzyme molecule with three and four molecules of AMP occupying one and two nucleoside inhibitory sites respectively were estimated to be equal to one another and to be 40 ± 1 mM. The values of the microscopic constants for the dissociation of the

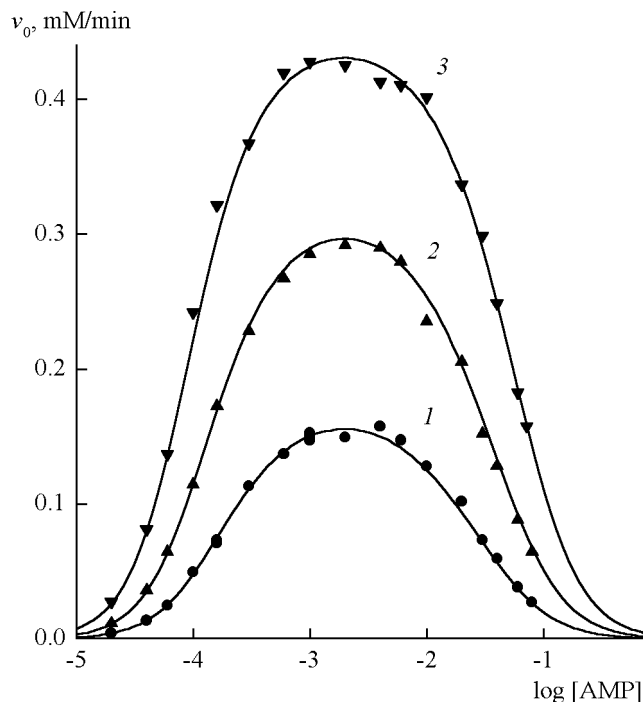


Fig. 1. The dependences of the initial steady-state rate for the reaction catalyzed by glycogen phosphorylase *b* from rabbit skeletal muscles in the presence of 1 g/l glycogen on the molar concentration of AMP at the following concentration of α -D-glucose 1-phosphate (mM): 2.0 (1), 4.0 (2), and 8.0 (3). The points are experimental data; solid lines were calculated in accordance with scheme presented in Fig. 2, using the following values of parameters: $K_{a1} = 0.22$ mM, $K_{a2} = 0.089$ mM, $K_{s1} = 4.5$ mM, $K_{s2} = 1.2$ mM, $K_i = 40$ mM, and $k = 85 \text{ s}^{-1}$.

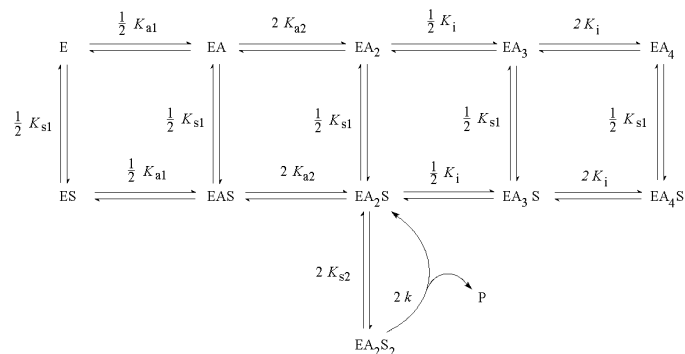


Fig. 2. The kinetic scheme for the allosteric regulation of glycogen phosphorylase *b* by AMP. E is the enzyme adsorbed on glycogen, A is AMP, S is α -D-glucose 1-phosphate, and P is the reaction product.

complexes of the glycogen-bound enzyme molecule with one and two molecules of α -D-glucose 1-phosphate occupying one and two catalytic sites were found to be 4.5 ± 0.4 and 1.2 ± 0.1 mM respectively. The value of the microscopic catalytic constant is equal to $85 \pm 4 \text{ s}^{-1}$. The proposed kinetic model allows to describe adequately the experimental dependences of the initial steady-state rate on α -D-glucose 1-phosphate and AMP concentrations for the reaction catalyzed by glycogen phosphorylase *b* from the rabbit skeletal muscles under the conditions of saturation of the enzyme by glycogen (Fig. 1).

Consider the possible conformational changes in glycogen phosphorylase *b* molecule in accordance with the pro-

posed kinetic model. The fact that the binding of the first molecule of AMP in an allosteric effector site results in the increase in the affinity of the second allosteric effector site to AMP is indicative of the conformational transition of the dimeric enzyme molecule upon the binding of the first molecule of AMP. However AMP does not affect the binding of the first molecule of α -D-glucose 1-phosphate, allowing to suggest that the conformational change in the enzyme induced by the first molecule of AMP does not extend on the locus of α -D-glucose 1-phosphate in the catalytic site. The fact that the binding of the first molecule of α -D-glucose 1-phosphate to the enzyme-glycogen complex containing two molecules of AMP results in an increase in the affinity to the second molecule of α -D-glucose 1-phosphate is indicative of the change in the enzyme conformation induced by substrates and AMP together. According to the proposed kinetic scheme, the dimeric enzyme molecule saturated by glycogen acquires catalytic activity exclusively upon binding of two molecules of AMP and two molecules of α -D-glucose 1-phosphate. This fact indicates that glycogen phosphorylase *b* passes into the catalytically active conformation exclusively upon binding of two molecules of AMP and two molecules of α -D-glucose 1-phosphate under the conditions of saturation by glycogen.

This work was supported by the Russian Foundation for Basic Research (Grants 990448639 and 001597787).

References

1. Kurganov B.I. *Allosteric Enzymes. Kinetic Behaviour*. Chichester: John Wiley and Sons. 1982. P. 4–8.
2. Kurganov B.I. In: *Peptides and Proteins*. Eds. V.T. Ivanov and V.M. Lipkin. Moscow: Nauka. 1995. **1**. P. 109–117.
3. Johnson, L.N., Hajdu, J., Acharya, K.R., Stuart, D.I., McLaughlin, P.J., Oikonomakos, N.G., and Barford D. In: *Allosteric Enzymes*. Ed. Herve G. Boca Raton: CRC Press. 1992. P. 81–127.
4. Oikonomakos, N.G., Acharya, K.R., and Johnson L.N. In: *Post-Translational Modifications of Proteins*. Eds. Harding J.J. and Crabbe M.J.C. Boca Raton: CRC Press. 1992. P. 81–151.
5. Bartfai, T. and Mannervik, B. *FEBS Lett.* 1972. **26**. P. 252–256.
6. Fischer E.H. and Krebs E.G. *J. Biol. Chem.* 1958. **231**. P. 65–71.
7. Buc M.H., Ulmann A., Goldberg M., and Buc H. *Biochimie*. 1971. **53**. P. 283–289.
8. Titani K., Koide A., Hermann J., Ericsson L.H., Kumar S., Wade R.D., Walsh K.A., Neurath H., and Fischer E.H. *Proc. Natl. Acad. Sci. USA*. 1977. **74**. P. 4762–4766.
9. Sugrobova N.P., Lisovskaja N.P., and Kurganov B.I. *J. Biochem. Biophys. Methods*, 1983. **8**. P. 299–306.
10. Duggleby R.G. *Comput. Biol. Med.* 1984. **14**. P. 447–455.