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KINETIC PROPERTIES OF α-GLUCAN PHOSPHORYLASE FROM *INDOCIBIUM GUTTATTAM*

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Summary

The kinetic properties of α-glucan phosphorylase *b* (α-D-1,4-glucan : orthophosphate α-D-glucosyltransferase, EC 2.4.1.1) purified from a deep-sea fish *Indocibium guttattam* were studied in the direction of glycogen synthesis. There was no homotropic site-site interaction between glucose 1-phosphate sites and between glycogen sites. However, heterotropic interaction was observed between substrate and activator sites.

The kinetic data obtained for the fish enzyme were consistent with the rapid equilibrium random mechanism reported for α-glucan phosphorylase from other sources. All the dissociation constants were 2—3 times higher for the fish enzyme than for the rabbit enzyme.

Although the fish enzyme exhibited a greater affinity for AMP at 30°C as compared to the rabbit enzyme, these sites were characterized by a lack of homotropic cooperativity. Heterotropic cooperativity was observed between AMP and glucose 1-phosphate sites.

Introduction

α-Glucan phosphorylase (α-D-1,4-glucan : orthophosphate α-D-glucosyltransferase, EC 2.4.1.1) has received particular attention because of the significance of the reaction it catalyses and also of the allosteric nature of its control. The present knowledge about this enzyme is mainly based on studies with phosphorylase from rabbit and other terrestrial animals. Information regarding the enzyme from acquatic fauna is scanty. Temperature effects and interconversions of the *a* and *b* forms also have been studied [1—3].

We have shown earlier that phosphorylase purified from the marine fish, *Indocibium guttattam* has allosteric properties distinctly different from those reported for the enzyme from other sources [4]. While the rabbit enzyme shows sigmoidal kinetics for its substrates and activator, the fish enzyme behaves like a desensitized enzyme. This difference prompted a more detailed

study of the enzyme from *I. guttattam* mainly for the purpose of comparison with phosphorylase from other sources. A rigorous comparison is, however, not possible at the moment with phosphorylase from acquatic sources because the available information is either inadequate or beyond the scope of this paper.

Materials and Methods

Phosphorylase *b* from *I. guttattam* was purified to apparent homogeneity as described earlier [5]. Glucose-1-*P* was obtained from V.P. Chest Institute, New Delhi, AMP was from E. Merck, Germany, glycogen from Centron Research Laboratories and sodium β-glycerophosphate and bovine serum albumin from Koch-Light Lab., London. All other chemicals were of analytical grade.

Initial velocities were measured in the direction of glycogen synthesis according to the procedure of Illingworth and Cori [6] using cysteine β-glycerophosphate buffer, pH 6.8. The liberated inorganic phosphate was estimated by the method of Fiske and Subbarow [7]. The enzyme solutions were incubated in the buffer for 30 min at 30°C before assay. The reactions were carried out at 30°C unless specified otherwise. Specific activities are expressed as μmol of inorganic phosphate liberated per min per mg of protein. The protein concentrations were determined using Folin reagent [8] according to Lowry et al. [9] with bovine serum albumin as standard.

Results and Discussion

The initial velocity data for the transfer of glucosyl residues to glycogen at varying levels of glycogen and glucose-1-*P* were analysed using double reciprocal plots. The plots were all linear in the range of glycogen (2.28—57.00 mM glucosyl residue) and glucose-1-*P* (8.0—32.0 mM) employed indicating lack of homotropic cooperativity between glycogen sites and between glucose-1-*P* sites. This is similar to the results with other phosphorylases [2,10—14].

The apparent K_m values for glycogen and glucose-1-*P* as a function of each other were evaluated from the reciprocal plots and are given in Table I. The results show that the apparent K_m values for glycogen decrease with increasing concentrations of glucose-1-*P* and vice versa illustrating heterotropic interaction

TABLE I

APPARENT K_m VALUES FOR GLUCOSE-1-*P* AND GLYCOGEN AT DIFFERENT CONCENTRATIONS OF EACH OTHER FOR PHOSPHORYLASE *b* FROM *I. GUTTATTAM*

Phosphorylase *b* was assayed in 15 mM cysteine/20 mM glycerophosphate, pH 6.8, at 30°C. The reaction mixtures contained 1 mM AMP.

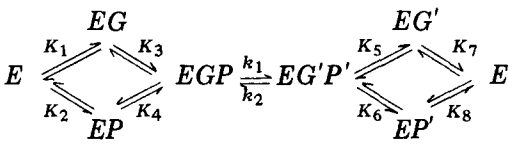
Glucose-1-*P* (mM)	K_m for glycogen (mM glucosyl residue)	Glycogen (mM glucosyl residue)	K_m for glucose-1-*P* (mM)
8.0	3.8	2.28	10.5
12.0	3.3	5.70	8.5
16.0	3.2	14.28	7.4
20.0	3.1	28.50	6.4
24.0	3.0	57.00	6.0

between substrate sites. Such heterotropic interactions have been reported for rabbit liver phosphorylase [10], rabbit muscle phosphorylase *b* and *a* [12,13] and lobster phosphorylase [1]. Thus the behaviour of the fish enzyme is qualitatively similar to that of phosphorylase from other sources.

The K_m values of the fish phosphorylase for glucose-1-*P* and glycogen were, respectively, 2-fold and 4-fold greater than those reported for the rabbit muscle enzyme [12]. Thus the fish enzyme has a relatively lower affinity for the substrates at 30°C.

The kinetic mechanism of α-glucan phosphorylase from rabbit liver [10] rabbit muscle [11], and *Escherichia coli* [14] has been shown to be rapid equilibrium random bi bi. The reciprocal plots for the substrates mentioned above for the fish enzyme are also consistent with rapid equilibrium random mechanism. The point of intersection of the lines in the reciprocal plot for glycogen at different levels of glucose-1-*P* corresponds to the reciprocal of the dissociation constants of glucose-1-*P*, and enzyme · AMP complex. This value (K_8 = 15.3 mM) is nearly twice as that for the rabbit muscle enzyme. The dissociation constant K_7 was evaluated similarly from the reciprocal plots for glycogen at different levels of glucose-1-*P*.

In phosphorylase-catalysed reaction, glycogen is the product as well as the reactant and hence its concentrations is unaffected during initial rate measurements. Also it has been shown that the dissociation constant is the same if initial rate is measured in the direction of glycogen synthesis or degradation [10,11]. Based on these Engers et al. [11] proposed a scheme for rabbit muscle enzyme. This is given in Scheme 1. An essentially similar mechanism is proposed for *E. coli* phosphorylase also [14].



Scheme 1. Proposed rapid equilibrium random bi bi mechanism for rabbit muscle phosphorylase *b* [11]. E, G, and P are enzyme · AMP complex, glycogen and orthophosphate, respectively. G′ is glycogen with one terminal glucose residue less and P′ is glucose-1-*P*. K_1—K_8 are dissociation constants as shown in the scheme; k_1 and k_2 are rate constants for the reaction in the direction of glycogen degradation and synthesis, respectively.

On the assumption that the affinity of the enzyme for glycogen for binding in the synthetic and degradation direction is the same, Engers et al. [11] derived the rate equation for Scheme 1 as below:

$$\frac{[E]_0}{v} = \frac{1}{k_1} + \frac{2K_3}{[P]k_1} + \frac{K_4}{[G]k_1} + \frac{K_1K_3}{[G][P]k_1} \tag{1}$$

which can be written in the general form of Dalziel [15] as:

$$\frac{[E]_0}{v} = \Phi_0 + \frac{\Phi_1}{[G]} + \frac{\Phi_2}{[P]} + \frac{\Phi_{1,2}}{[G][P]} \tag{2}$$

where $[E]_0$ = total enzyme concentration; v = initial velocity.

TABLE II

KINETIC COEFFICIENTS AND CONSTANTS FOR PHOSPHORYLASE *b* FROM *I. GUTTATTAM* [a] AND RABBIT MUSCLE [b] AT 30°C

		I. guttattam	Rabbit muscle
Kinetic coefficients			
Φ'_0	(mg of protein · min per μmol)	0.0163	0.0155
Φ'_1	(min · mg)	36.0	51.0
Φ'_2	(min · mg)	120.0	13.0
$\Phi'_{1,2}$	(mol · min per mg)	0.53	0.104
Kinetic constants			
k_2	(mol/mg per min)	61.4	64.0
K_5	(mM glucose-1-*P*)	3.6	1.5
K_6	(mM glycogen as glucosyl residue)	2.8	0.9
K_7	(mM glycogen)	9.0	4.4
K_8	(mM glucose-1-*P*)	15.3	7.4

[a] Values calculated from secondary plots of slopes and intercepts of reciprocal plots for glucose-1-*P* and glycogen (see text). Initial velocities were measured in the direction of glycogen synthesis i.e. for the reverse reaction shown in Scheme 1. Using the same notations of Scheme 1, the rate equation for glycogen synthesis may be written as:

$$\frac{[E]_0}{v} = \frac{1}{k_2} + \frac{2K_5}{k_2[P']} + \frac{K_6}{k_2[G']} + \frac{K_5K_7}{k_2[G'][P']} \quad (3)$$

$$\text{or } \frac{[E]_0}{v} = \phi'_0 + \frac{\phi'_1}{[G']} + \frac{\phi'_2}{[P']} + \frac{\phi'_{1,2}}{[G'][P']} \quad (4)$$

[b] Data from ref. 11.

When the slopes and the intercepts were replotted in secondary plots [15,16], linear lines were obtained. Moreover, internal consistencies for the rapid equilibrium random mechanism were found by common intercepts on the intercept plot and parallel lines on the slopes plot. Hence it is apparent that the kinetics of phosphorylase *b* from *I. guttattam* is also consistent with the random mechanism derived for phosphorylase from other sources.

Kinetic constants and coefficients for the fish enzyme in the synthetic direction were evaluated from secondary plots and compared with the corresponding values reported for rabbit muscle phosphorylase *b* in Table II.

The catalytic constant k_2 for the synthesis of glycogen catalysed by the fish enzyme (61.4 μmol of orthophosphate liberated per min per mg protein) is comparable to the value (64.0) reported for rabbit skeletal muscle phosphorylase *b* [11] and to the value (71.0) reported for lobster tail muscle phosphorylase *b* [1]. Both for the fish and rabbit enzymes the affinity of glucose-1-*P* is greater for the enzyme · AMP · glycogen complex than for the enzyme · AMP complex and the affinity of the glycogen is greater for the enzyme · AMP · glucose-1-*P* complex than for the enzyme · AMP complex. Moreover, there is a striking difference, i.e. all the dissociation constants (K_5, K_6, K_7 and K_8) are about two to three times greater for the fish enzyme as compared to the rabbit enzyme showing that the former has lower affinities for its substrates at 30°C.

Kinetics with respect to AMP

We have reported earlier that phosphorylase *b* from *I. guttattam* is distinctly different from rabbit muscle and other phosphorylases in its allosteric properties

TABLE III

K_m VALUES FOR AMP AND GLUCOSE-1-*P* AT DIFFERENT CONCENTRATIONS OF EACH OTHER FOR PHOSPHORYLASE *b* FROM *I. GUTTATTAM*

The values were obtained from double reciprocal plots. Phosphorylase *b* was assayed in 15 mM cysteine/20 mM glycerophosphate, pH 6.8, at 30°C. The assay mixtures contained 1% glycogen.

Glucose-1-*P* (mM)	K_m for AMP (M)	AMP (M)	K_m for glucose-1-*P* (mM)
12.0	$8.5 \cdot 10^{-5}$	$1.0 \cdot 10^{-5}$	30.0
16.0	$3.2 \cdot 10^{-5}$	$2.5 \cdot 10^{-5}$	19.0
20.0	$3.0 \cdot 10^{-5}$	$5.0 \cdot 10^{-5}$	13.3
24.0	$2.8 \cdot 10^{-5}$	$1.0 \cdot 10^{-4}$	8.5
32.0	$2.1 \cdot 10^{-5}$	$1.0 \cdot 10^{-3}$	7.0

[4]. The kinetics of glucose-1-*P* and AMP at different concentrations of each other was studied. The double reciprocal plots for AMP were linear in the range of AMP concentrations (10^{-5}—10^{-3} M) for different constant levels of glucose-1-*P* (8.0—32.0 mM) indicating lack of homotropic cooperativity between AMP sites. In this respect the fish enzyme is significantly different from rabbit muscle and other phosphorylase where the AMP sites show cooperativity. The variations of the apparent K_m values are presented in Table III. The table illustrates that the fish enzyme shows heterotropic cooperativity like the rabbit enzyme.

Influence of temperature on glucose-1-P kinetics

The glucose-1-*P* kinetics of the fish enzyme was analysed at three different temperatures (20, 30, and 37°C). The apparent K_m values for glucose-1-*P* did not vary considerably in this range. The Arrhenius' plot over this range of temperatures was linear and the energy of activation corresponded to 19.4 kcal. This value is comparable to 20.0 kcal reported for rabbit muscle phosphorylase *b*, but higher than the value (15.0) reported for lobster muscle phosphorylase *b* [1].

The results revealed many similarities and differences in the properties of phosphorylase *b* from this deep-sea fish and that of rabbit muscle phosphorylase *b*. Though the kinetic mechanism is the same the kinetic constants differ markedly. The fish enzyme is characterized by relatively higher values for the Michaelis constants for substrates and decreased heterotropic interactions as compared to the rabbit enzyme. The major difference between the two enzymes is in their response to the activator, AMP and the metabolic inhibitors like glucose, glucose-6-*P*, and ATP [4].

The results show that the role played by phosphorylase *b* in the regulation of glycogen degradation may be possibly very less significant in the fish, viz., *I. guttattam*. Better understanding of the glycogen metabolism in this fish requires a knowledge of the properties of phosphorylase *a*.

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