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α-GLUCAN PHOSPHORYLASE *b* FROM *INDOCIBIUM GUTTATTAM*: A KINETICALLY DIFFERENT PHOSPHORYLASE

G. SOMAN and GEORGE PHILIP

Department of Biochemistry, University of Kerala, Trivandrum, Kerala 695001 (India)

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Summary

α-Glucan phosphorylase *b* (1,4-α-D-glucan:orthophosphate α-glucosyltransferase, EC 2.4.1.1) isolated and purified from a deep-sea fish, *Indocibium guttattam* (a variety of butter fish), showed lack of cooperativity between glucose 1-phosphate sites in the presence of allosteric inhibitors such as glucose, glucose 6-phosphate and ATP. These inhibitors exhibited competitive kinetics for glucose 1-phosphate. Cooperativity of AMP sites was not induced in the presence of glucose but slightly induced in the presence of glucose 6-phosphate. In all these respects this enzyme differs from phosphorylase from other sources. The results indicate that the allosteric properties of phosphorylase from all animal sources need not conform to a general pattern.

α-Glucan phosphorylase *b* (1,4-α-D-glucan:orthophosphate α-glucosyltransferase, EC 2.4.1.1) isolated from frog muscle [1], rat muscle [2] and lobster [3] have kinetic and allosteric properties comparable to those of the enzyme from rabbit muscle [4]. The resemblances in properties of the enzyme from different animal sources have been taken to represent the allosteric behavior of phosphorylase in all animal species.

During a search for a phosphorylase *b* with properties different from those typified by the rabbit muscle enzyme in order to use it for hybridization with the latter, we have come across phosphorylase *b* from a deep-sea fish, *Indocibium guttattam* (a variety of butter fish) showing distinctly different allosteric properties. Here we report some of the properties of this enzyme which make it different from phosphorylase of other sources and its significance in the study of the allosteric mechanism.

α-Glucan phosphorylase was isolated and purified from *Indocibium guttattam* in several steps including ammonium sulphate fractionations, adsorp tion on alumina C_γ gel and chromatography on DEAE-cellulose column (Soman, G. and Philip, G., unpublished). The purified sample was found to move as a single band on polyacrylamide-gel electrophoresis. The specific activity of the purified enzyme was found to be 40 (μM phosphate liberated per min per mg protein at 30 °C when assayed with 0.016 M glucose 1-phos phate, 1 mM AMP and 1% glycogen in 15 mM cysteine—20 mM sodium β-glycerophosphate, pH 6.8). Preliminary investigations revealed that the enzyme contained about 2 moles of pyridoxal phosphate per mole of protein assuming a molecular weight of 185 000.

The reciprocal plot for glucose 1-phosphate (glucose-1-*P*)was linear with the fish enzyme as with the rabbit enzyme. However, in contrast to the rabbit enzyme, the plot for AMP in the range of AMP concentrations $4 \cdot 10^{-6}$—10^{-3} M was linear with fish phosphorylase *b*. The K_m values for the fish enzyme for glucose-1-*P* and AMP were comparable with those for the rabbit and lobster enzymes (see below).

One of the important predictions of the model of Monod, Wyman and Changeux [5] for allosteric enzymes is that cooperativity of sites for substrate and activator will increase in the presence of inhibitors because of a shift in the $R \rightleftharpoons T$ equilibrium to the *T* state. This has been demonstrated with rabbit muscle phosphorylase *b* using glucose, ATP and glucose 6-phosphate (glucose-6-*P*) as allosteric inhibitors [6,7]. With the fish enzyme reported here we have employed this method to analyse its allosteric property.

Fig.1 shows the influence of glucose, glucose-6-*P* and ATP on the

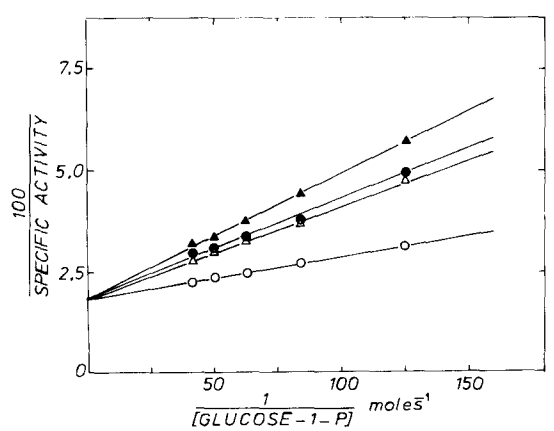

Fig.1. Reciprocal plot for glucose-1-*P* in the presence of glucose, glucose-6-*P* and ATP for α-glucan phosphorylase *b* from *Indocibium guttattam*.Phosphorylase *b* was assayed in the direction of glycogen synthesis with varying concentrations of glucose-1-*P* in the absence and presence of glucose, glucose-6-*P* or ATP at 30 °C in 15 mM cysteine—20 mM sodium β-glycerophosphate buffer, pH 6.8 and initial rates measured. Concentrations of AMP and glycogen in assay mixtures were 1 mM and 1%, respectively. Specific activity is expressed as μM phosphate liberated per min per mg protein. ○——○, no inhibitor; ●——●, with 10 mM glucose-6-*P*; △——△, with 10 mM ATP and ▲——▲, with 10 mM glucose.

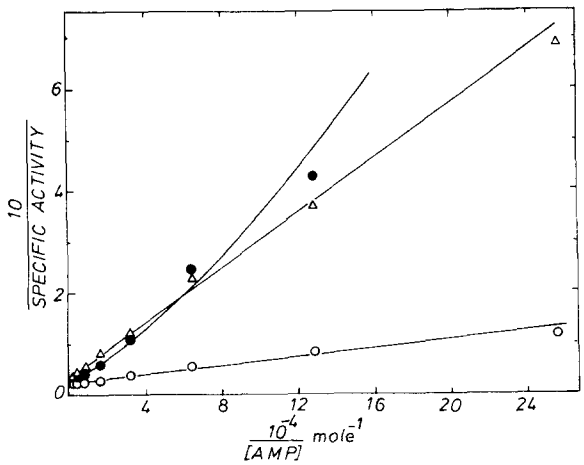

Fig.2. Reciprocal plot for AMP in the presence of glucose and glucose-6-*P* for α-glucan phosphorylase *b* from *Indocibium guttattam*. Phosphorylase *b* was assayed with varying concentrations of AMP in the presence of glucose and glucose-6-*P* at 30 °C and initial rates measured. The concentration of glucose-1-*P* was 16 mM. Other details were as in Fig.1. ○——○, no inhibitor; ●——●, with 2 mM glucose-6-*P* and △——△, with 10 mM glucose.

kinetics with respect to glucose-1-*P* for fish phosphorylase *b*. The reciprocal plots are linear even at 10 mM concentrations of the inhibitors. Rabbit enzyme under these conditions gives nonlinear plots [6,7]. Reciprocal plots for AMP in the presence of inhibitors are given in Fig.2. The plot in the presence of 2 mM glucose-6-*P* is nonlinear and the Hill coefficient has been found to be 1.1 (n = 1.6 for rabbit enzyme under these conditions). In the presence of glucose the plot is still linear, in contrast to the rabbit enzyme. The present study thus emphasises that the allosteric control of phosphorylase in all animal species need not conform to a general pattern. AMP kinetics in the presence of ATP presented some problems probably due to contamination with AMP and hence could not be included here. The Hill coefficients for fish and rabbit enzymes are compared in Table I. It may be noted that even with 10 mM glucose-6-*P*, the n value for AMP has increased only to 1.3 indicating relatively decreased sensitivity of the AMP sites in fish phosphorylase *b*.

In striking contrast to the rabbit enzyme, with fish phosphorylase glucose, glucose-6-*P* and ATP behave as true competitive inhibitors of glucose-1-*P* (Fig.1), suggesting that in this case the binding of substrate and effectors involves same site. The kinetics for glucose-1-*P* was done at saturating level of AMP. The competitive kinetics exhibited by ATP here (Fig.1) is not surprising in view of the possibility of different modes of binding by substrate and effectors on same site as demonstrated in the case of rabbit phosphorylase *b* [8]. Moreover, it has been shown with rabbit enzyme that different molecules having a phosphate group can interact at a subsite on the enzyme resulting in partial or fully competitive inhibition of glucose-1-*P* by these molecules [9].

TABLE I

HILL COEFFICIENTS FOR PHOSPHORYLASE *b* FROM *INDOCIBIUM GUTTATTAM* AND RABBIT MUSCLE

Hill Coefficients were obtained from the slope of Hill plots. Experimental conditions were as in Figs. 1 and 2. The *n* values given for the fish enzyme are averages from three separate determinations and those for the rabbit enzyme are from refs. 6 and 8.

	Fish enzyme	Rabbit enzyme
n values for glucose-1-*P* sites		
Without inhibitors	0.97	0.93
With glucose (10 mM)	0.98	1.8
With glucose-6-*P* (10 mM)	0.95	1.73
With ATP (10 mM)	1.0	1.8
n values for AMP sites		
Without inhibitor	1.05	1.4
With glucose (10 mM)	0.96	—
With glucose-6-*P* (2 mM)	1.1	1.6
With glucose-6-*P* (10 mM)	1.33	—

The kinetics for glucose-1-*P* and AMP were analysed at different levels of each of them. Increasing concentration of AMP was found to decrease the K_m for glucose-1-*P* and vice versa showing that the fish enzyme possesses heterotropic cooperativity between these ligands. Thus in this respect fish phosphorylase *b* has qualitatively similar properties as the rabbit enzyme.

With rabbit phosphorylase *b* it has been shown that chemical modifications would yield partially desensitized derivatives in which homotropic cooperativity of some kind or all are abolished while heterotropic interactions are retained [7—9]. In some of these derivatives the inhibitors compete for the substrate or activator site. Thus it appears that the fish enzyme reported here represents the properties of an altered form of rabbit phosphorylase *b*.

We have pointed out the necessity for detailed study with hybrid phosphorylase in understanding the relationship between monomer—monomer interaction and allosteric property. The fish and rabbit enzymes with distinctly different allosteric properties seem to meet the requirement for such a study. Apart from this and its significance from a biological point of view the fish enzyme is important also for the study of the relationship between conformational aspects and allosteric properties of partially desensitized rabbit phosphorylase.

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