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α-GLYCEROLPHOSPHATE DEHYDROGENASE: A REGULATORY ENZYME*

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SUMMARY

1. Glycerol-3-phosphate dehydrogenase catalyses the reduction of dihydroxyacetone phosphate together with the hydrolysis of *p*-nitrphenol acetate.

2. Cooperative heterotropic effects from the substrates for the two distinct reactions have been demonstrated. These effects are specific for dihydroxyacetone phosphate and glycerol phosphate, although, phosphate alone is also capable of eliciting them.

3. The experimental evidence presented here points to two separate sites for the esterolytic and dehydrogenase activity of glycerol-3-phosphate dehydrogenase.

INTRODUCTION

Combined kinetic and chemical modification studies[1] strongly suggest that in the active center of glycerol-3-phosphate dehydrognease (glycerol-3-phosphate:NAD^+ oxidoreductase, EC 1.1.1.8), there are two highly reactive histidine residues, at least one of which is essential for enzymatic activity. Furthermore, the "activating" effect of *N*-ethylmaleimide and other N-substituted maleimides[2] and the esterolytic activity of glycerol-3-phosphate dehydrogenase[3], seems to be associated with the basic form of a histidine residue relatively exposed on the surface of the enzyme molecule.

The question then arises if both dehydrogenase and esterase activities involve the same active site, as is the case for glyceraldehyde-3-phosphate dehydrogenase[4], or if they pertain to separate sites on the enzyme.

The present communication describes the effect of the substrates for the dehydrogenase activity, dihydroxyacetone phosphate and glycerol-3-phosphate, on the kinetics of the esterolytic reaction.

MATERIALS AND METHODS

Glycerol-3-phosphate dehydrogenase was isolated from rabbit muscle according

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to a published procedure[4]. Alternatively, a commercial preparation obtained from Boehringer-Mannheim was also used. All preparations were extensively dialyzed against distilled water, containing 1 mM EDTA, pH 7.0, before use.

Dihydroxyacetone phosphate was obtained from Boehringer-Mannheim as the dimethylketal, dicyclohexylammonium salt. DL-Glycerol-3-phosphate and *p*-nitrophenyl acetate were obtained from Sigma Chemical Co., St. Louis. Glycerol was purchased from Merck-Darmstadt and β-glycerol phosphate was a preparation from Calbiochem, California. All other reagents were of the best purity commercially available.

The kinetics of hydrolysis of *p*-nitrophenyl acetate was measured by monitoring the formation of *p*-nitrophenoxide ion at 400 nm (ref. 5). Calculation of total product concentration (phenol + phenolate) was done by use of $\varepsilon_{400\ nm} = 1.84 \cdot 10^4$ for *p*-nitrophenolate and a pK_a value of 7.13 for *p*-nitrophenol[6]. Protein concentration was measured by the method of WADDELL[7] (see also ref. 8).

RESULTS AND DISCUSSION

Incubation of glycerol-3-phosphate dehydrogenase with *p*-nitrophenyl acetate, a substrate for the esterolytic reaction, produces, as was observed for *N*-ethylmaleimide[2], activation of the dehydrogenase reaction. However, the following differences can be observed: *N*-ethylmaleimide is a better activator than *p*-nitrophenyl acetate; *i.e.*, a 100% activation is obtained with a molar ratio of *N*-ethylmaleimide to enzyme of $1 \cdot 10^4$:1, whereas a molar ratio of *p*-nitrophenyl acetate to enzyme of $34 \cdot 10^4$:1 produces only a 75% activation. Activation elicited by *N*-ethylmaleimide requires an incubation time of only a few seconds at 0°, with *p*-nitrophenyl acetate, incubation periods of several minutes (5–10) at room temperature (23°–25°) are needed in order to elicit the effect. Finally, a recently prepared enzyme seems to be a requirement for the activation mediated by *p*-nitrophenyl acetate. These differences

TABLE I

EFFECT OF THE SUBSTRATES FOR THE DEHYDROGENASE REACTION ON THE ESTEROLYTIC ACTIVITY OF GLYCEROL-3-PHOSPHATE DEHYDROGENASE

Incubation conditions: enzyme, 10 μM; *p*-nitrophenyl acetate, 10 mM; Tris–HCl buffer, 50 mM, pH 7.13, 25°.

Additions	*nmoles of p-nitrophenol/10 min*
None	11.0
NAD^+ (1.4 mM)	11.2
NADH (1.4 mM)	11.3
Glycerol-3-phosphate	
0.32 mM	16.4
0.65 mM	21.2
2.60 mM	26.0
Dihydroxyacetone phosphate	
0.32 mM	18.0
0.62 mM	26.0

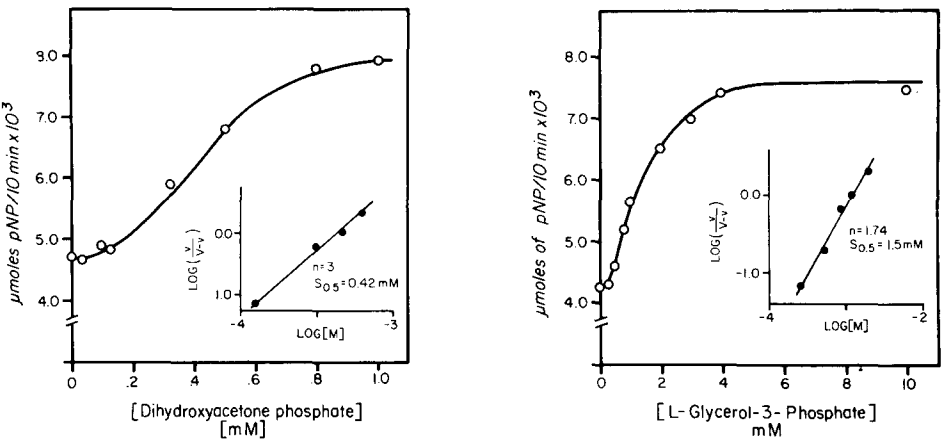

Fig. 1. Plot of reaction rate *versus* activator concentration for the effect of the substrates for the dehydrogenase reaction on the esterolytic activity of glycerol-3-phosphate dehydrogenase. Same conditions as in Table I but *p*-nitrophenyl acetate, 1 mM; and veronal buffer 20 mM. Insert: Hill plot of the data. pNP, *p*-nitrophenol.

may be due either to the inherently different reactivities of the compounds or to better fitting of *N*-ethylmaleimide to the appropriate site on the enzyme.

The converse of *p*-nitrophenyl acetate activation for the dehydrogenase reaction is also observed. The substrates for the dehydrogenase reaction, dihydroxyacetone phosphate and glycerol-3-phosphate, but not the coenyzme NAD^+ or NADH activate the esterolytic reaction. Dihydroxyacetone phosphate is a better activator than glycerol-3-phosphate (Table I).

A plot of esterolytic activity *versus* activator concentration, at a fixed concentration of *p*-nitrophenol acetate, is shown in Fig. 1. The sigmoidal shape of the resulting curves is apparent for both activators, dihydroxyacetone phosphate and glycerol-3-phosphate. From Hill plots of the data, shown in the same figure, *n* values of 1.8 and 3 for glycerol-3-phosphate and dihydroxyacetone phosphate, respectively, are

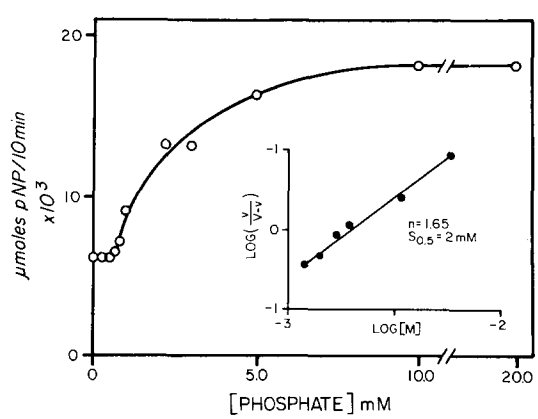

Fig. 2. Plot of reaction rate *versus* phosphate concentration for the esterolytic activity of glycerol-3-phosphate dehydrogenase. Same conditions as in Fig. 1.

obtained. This suggests the existence of positive heterotropic cooperativity[9]. It is interesting to note that the value for the half saturating concentration for those effectors, 0.42 mM for dihydroxyacetone phosphate and 1.5 mM for glycerol-3-phosphate are near the respective values of K_m for the dehydrogenase reaction[10].

Phosphate alone is also capable of producing activation of the esterolytic reaction. Fig. 2 shows a plot of reaction rate *versus* phosphate concentration. Here again, the curve has a sigmoid shape and a value of 1.65 for the Hill coefficient is obtained.

Neither glycerol nor β-glycerolphosphate produce activation, suggesting that this effect is specific for the normal enzyme substrates and that, most probably, the phosphate group is specifically involved in the interaction.

The fact that the substrates for the dehydrogenase and esterase activities do not directly compete with one another for binding sites indicates that these sites are at least partially distinct and, in conjunction with results of studies of *p*-chloromercuribenzoate inhibition and photo-inactivation of the enzyme[3], strongly suggests that they may be completely distinct.

Since the physiological reaction of glycerol-3-phosphate dehydrogenase is the reduction of dihydroxyacetone phosphate to glycerol-3-phosphate[13], it seems reasonable to associate the dehydrogenase activity of glycerol-3-phosphate dehydrogenase with the catalytic site of the enzyme whereas the esterolytic activity may be related to a regulatory site. Moreover, in our view, the esterolytic activity probably has no physiological significance but simply reflects the presence of a reactive and exposed histidine residue at or near a regulatory site.

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