Partial Purification and Characterization of Glucose-6-phosphate Isomerase from *Dictyostelium discoideum*

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(Received 4 August 1980; revised 19 February 1981)

Glucose-6-phosphate isomerase was partially purified from cells of *Dictyostelium discoideum* in the culmination stage of development. The enzyme had a pH optimum of about 8.5 in Tris/HCl buffer. Activity was not inhibited by *p*-chloromercuribenzoate or iodoacetate. The enzyme exhibited Michaelis–Menten kinetics and the K_m values for glucose 6-phosphate and fructose 6-phosphate were 2 mM and 0.1 mM, respectively. Erythrose 4-phosphate was a strong competitive inhibitor of the enzyme with a K_i value of 3.8 μ M, whereas 6-phosphogluconate was less effective with a K_i of 0.1 mM.

INTRODUCTION

A recent report from this laboratory indicated that *Dictyostelium discoideum* contains all the enzymes necessary for both oxidative and non-oxidative pentose phosphate metabolism during development (Thomas, 1979). One of these enzymes, glucose-6-phosphate isomerase (D-glucose-6-phosphate ketol-isomerase; EC 5.3.1.9), may play a role in regulating the activity of these two branches of metabolism. The isomerase is responsible for catalysing the interconversion of glucose 6-phosphate and fructose 6-phosphate. During development of *D. discoideum* the concentrations of these two sugar phosphates increase approximately threefold between the aggregation and culmination stages, whereas isomerase activity remains relatively constant (Thomas, 1979; Cleland & Coe, 1968). Several groups of workers (Salas *et al.*, 1965; Zalitis & Oliver, 1967; Grazi *et al.*, 1960; Venkataraman & Racker, 1961) have demonstrated that the isomerases isolated from plant and animal sources are sensitive to inhibition by specific pentose phosphate intermediates. Based on these inhibitor studies, some authors (Grazi *et al.*, 1960; Parr, 1956; Stadtman, 1966) have suggested that glucose-6-phosphate isomerase may be an important regulatory enzyme involved in the control of carbohydrate metabolism. Although hexose metabolism has been extensively studied during development in *D. discoideum*, little information is available concerning the properties of the isomerase in this organism.

This paper reports the partial purification and kinetic characterization of glucose-6-phosphate isomerase from *D. discoideum*.

METHODS

Chemicals. Bovine serum albumin, NAD, NADP, streptomycin sulphate, resorcinol, and glucose-6-phosphate dehydrogenase (EC 1.1.1.49; yeast enzyme) were obtained from Sigma. All other chemicals used were reagent grade.

Growth conditions. *Dictyostelium discoideum* strain NC-4 (ATCC 24697) was grown with *Escherichia coli* on nutrient agar sheets as previously described (Marshall *et al.*, 1970). Amoebae were harvested, washed free of bacteria, and spread on 2% (w/v) agar sheets (38 × 25 cm) containing 10 mM-phosphate buffer pH 6.5 and 1 mM-EDTA. The agar sheets were incubated at 15 or 22 °C.

Enzyme assay. Enzyme activity in the direction of glucose 6-phosphate formation was measured using the coupled enzyme system of Noltmann (1966). The standard reaction mixture contained (total volume 1.0 ml): 100 μmol Tricine/KOH buffer pH 7.6, 2 μmol MgCl_2 , 1 μmol NADP^+ , 5 μmol fructose 6-phosphate, 2 units glucose-6-phosphate dehydrogenase and enzyme. Enzyme activity was followed by monitoring the increase in absorbance at 340 nm, at about 23 °C.

Glucose-6-phosphate isomerase activity was also measured in the direction of fructose 6-phosphate formation using the colorimetric technique of Roe & Papadopoulos (1954). The reaction mixture contained (total volume 1.0 ml): 100 μmol Tricine/KOH buffer pH 7.6, 5 μmol glucose 6-phosphate and enzyme. The reaction tubes were incubated at 25 °C and the reaction was terminated after 10 min by the addition of Roe's reagent [3 ml conc. HCl plus 1.0 ml 1% (w/v) resorcinol in 95% (v/v) ethanol]. The colour was developed by heating the mixture at 80 °C for 10 min. After rapid cooling to room temperature the absorbance of the solution was measured at 530 nm.

One unit of activity is defined as the amount of enzyme catalysing the formation of 1 μmol product min^{-1} under the assay conditions. Specific activity is expressed as units $(\text{mg protein})^{-1}$. Protein was determined by Lowry's method, using bovine serum albumin as the standard.

Purification of glucose-6-phosphate isomerase. Enzyme was purified from cells in the culmination stage of development. Vegetative amoebae were harvested, washed free of bacteria and replated on non-nutrient agar (Marshall *et al.*, 1970). Cells were allowed to differentiate at 22 °C until they reached the culmination stage of development (18 to 20 h). The cells were washed off the agar with cold 50 mM-Tris/HCl buffer pH 7.5. The cell suspension was stored frozen at -20 °C. After thawing at room temperature, suspensions were passed through a Yeda press at 17.5 MPa. The supernatant fraction (crude extract) after centrifugation at 27 000 g for 20 min at 4 °C contained all of the isomerase activity. The crude extract was treated with streptomycin sulphate to a final concentration of 5% (w/v) and stirred at 4 °C for 1 h, then centrifuged at 27 000 g for 20 min. This procedure removed 95% of the nucleic acids. The fraction of the supernatant precipitating between 70 and 80% $(\text{NH}_4)_2\text{SO}_4$ saturation was collected, dissolved in 50 mM-Tris/HCl buffer pH 7.5 and dialysed at 5 °C overnight against 100 vol. of the same buffer. A slurry of alumina C γ gel was added to the dialysed $(\text{NH}_4)_2\text{SO}_4$ fraction until all of the isomerase was absorbed. The gel containing the absorbed enzyme was collected after centrifugation for 5 min at 3000 g . After washing the gel with 50 mM-Tris/HCl buffer pH 7.5, the isomerase was eluted by two successive extractions with 5 vol. of 0.5 M- $(\text{NH}_4)_2\text{SO}_4$ in 50 mM-Tris/HCl buffer pH 7.5. The enzyme was precipitated by addition of $(\text{NH}_4)_2\text{SO}_4$ to 80% saturation. After centrifugation at 27 000 g for 20 min the enzyme was dissolved in 2.0 ml 50 mM-Tris/HCl buffer pH 7.5 and dialysed overnight against 100 vol. of the same buffer. The partially purified isomerase preparation was stored at 5 °C. Table 1 gives a summary of the purification procedure.

RESULTS

The purpose of this investigation was to obtain an isomerase preparation essentially free of interfering enzyme activities in order to determine the relevant kinetic constants. The partially purified isomerase fraction from step 4 (Table 1) was tested for several enzymes of hexose and pentose phosphate metabolism and found to contain low levels of glucose-6-phosphate dehydrogenase activity (0.25% of the isomerase activity). No transketolase, transaldolase, phosphofructokinase or 6-phosphogluconate dehydrogenase activities were detected. The preparation did contain measurable levels of ribose-5-phosphate isomerase (11%) and ribulose-5-phosphate epimerase (6%) activity.

The purified isomerase from *D. discoideum* was stable to overnight dialysis (5 °C) against 50 mM-Tris/HCl buffer pH 7.5 and was also relatively stable during storage at 5 °C (6% loss in activity in 7 d). Incubation of the enzyme preparation at 37 °C in 100 mM-Tris/HCl buffers at pH values below 7.0 resulted in a rapid loss of activity. Maximal stability was obtained at approximately pH 7.2 under these conditions.

The activity of the purified enzyme was determined over the pH range 7.2 to 9.0 using two buffer systems: Tris/HCl and Tricine/NaOH. The isomerase had a broad pH activity range with a maximum at pH 8.5 in Tris/HCl buffer. In Tricine/NaOH buffer the enzyme activity was slightly lower.

The purified isomerase showed Michaelis-Menten kinetics when assayed at five concentrations of either substrate (0.5, 1, 2, 5 and 10 mM for glucose 6-phosphate; 0.05, 0.1, 0.2, 0.5 and 1 mM for fructose 6-phosphate). The apparent Michaelis constants (K_m) for glucose 6-phosphate and fructose 6-phosphate were approximately 2 mM and 0.1 mM, respectively. The isomerase from *D. discoideum* appears to exhibit a high degree of substrate

Table 1. *Partial purification of glucose-6-phosphate isomerase from D. discoideum*

Fraction	Volume (ml)	Protein (mg ml ⁻¹)	Total activity (units)	Specific activity [units (mg protein) ⁻¹]	Purification factor
1. Crude extract	50	19.2	181	0.188	—
2. Streptomycin sulphate supernatant	47	8.9	126	0.302	1.6
3. Ammonium sulphate 70–80% pellet	11	4.6	82.9	1.64	8.7
4. Alumina Cγ gel concentrate	2.7	1.1	42.2	14.2	75.5

Table 2. *Inhibition of glucose-6-phosphate isomerase from D. discoideum*

Inhibitor	Concn (mM)	Percentage inhibition*
Ribose 5-phosphate	1.0	12
Fructose 1,6-bisphosphate	1.0	4
Xylulose 5-phosphate	0.5	1
Fructose 1-phosphate	1.0	10
Ribulose 5-phosphate	0.5	48
Sedoheptulose 7-phosphate	1.0	10
Erythrose 4-phosphate	0.02	58
Glucose 1-phosphate	1.0	0
6-Phosphogluconate	0.2	52
ATP	1.0	1
UDPGlucose	0.1	0
Phosphate	1.0	0
p-Chloromercuribenzoate	0.1	2
Iodoacetate	0.1	0

* The glucose-6-phosphate isomerase assay was performed in both directions; the percentage inhibition was similar in both directions for each inhibitor.

specificity as other sugar phosphates such as ribose 5-phosphate, ribulose 5-phosphate and xylulose 5-phosphate were inactive as substrates. Substrate activity was measured using the pentose phosphate assay of Cooper *et al.* (1958).

The partially purified *D. discoideum* isomerase was tested for inhibition by a number of sugar phosphates (Table 2). Inhibition of the isomerase was similar in the forward and backward reactions. Of those phosphorylated compounds tested only three caused significant inhibition. Erythrose 4-phosphate was a competitive inhibitor in the presence of either substrate. An apparent K_i value of 3.8 μ M was obtained using enzyme assays in the direction of glucose 6-phosphate formation at three concentrations of fructose 6-phosphate (1.0, 0.5 and 0.2 mM) and erythrose 4-phosphate concentrations from 0 to 0.1 mM. In control experiments it was demonstrated that erythrose 4-phosphate did not affect the activity of the coupling enzyme, glucose-6-phosphate dehydrogenase. 6-Phosphogluconate and ribulose 5-phosphate were also inhibitors of the *D. discoideum* isomerase; however, somewhat higher concentrations were needed to obtain the same degree of inhibition (Table 2). Only 6-phosphogluconate inhibition was studied in detail, and it was found to be a competitive inhibitor of both substrates, with an apparent K_i of 0.1 mM. Enzyme assays in the direction of glucose 6-phosphate formation were performed with 0.5 and 0.2 mM-fructose 6-phosphate. The concentrations of 6-phosphogluconate were from 0 to 2.0 mM. When the isomerase was assayed at a lower pH (7.0), about 20% less inhibition was observed for the same concentration of 6-phosphogluconate. The *D. discoideum* enzyme does not appear to be sensitive to inhibition by sedoheptulose 7-phosphate and in this respect is different from two mammalian isomerases (Salas *et al.*, 1965; Venkataraman & Racker, 1961).

The enzyme was not inactivated when assayed in the presence of the thiol reagents, *p*-chloromercuribenzoate and iodoacetate (Table 2).

DISCUSSION

Glucose-6-phosphate isomerase has previously been characterized in a wide variety of organisms, including mammals (Salas *et al.*, 1965; Venkataraman & Racker, 1961; Noltmann, 1966), plants (Takeda *et al.*, 1967), insects (Saunders *et al.*, 1969) and micro-organisms (Klungsoyr & Endersen, 1964; Matsushima & Simpson, 1965). In general, the properties of the *D. discoideum* enzyme appear to be similar to those noted for isomerases isolated from other sources. The enzyme has a broad pH range with optimum activity at pH 8.5. The enzyme exhibited Michaelis-Menten kinetics with kinetic constants comparable to those of other isomerases. The enzyme was competitively inhibited by erythrose 4-phosphate and 6-phosphogluconate. However, several other phosphorylated intermediates (fructose 1-phosphate, fructose 1,6-bisphosphate, ribose 5-phosphate, xylulose 5-phosphate, sedoheptulose 7-phosphate, ATP and phosphate) which were found to inhibit yeast (Salas *et al.*, 1965) and rabbit muscle (Zalitis & Oliver, 1967) isomerases had little effect on the activity of the *D. discoideum* enzyme (Table 2).

The intracellular concentration of 6-phosphogluconate reaches a maximum of about 0.018 mM based on packed cell volume during the culmination stage of development in *D. discoideum* (Thomas, 1979). This is some fivefold below the measured K_i (0.1 mM) of the *D. discoideum* enzyme for this intermediate. If compartmentalization is not a factor, then 6-phosphogluconate may have a minimal effect in vivo on glucose-6-phosphate isomerase activity. However, the enzyme became more sensitive to 6-phosphogluconate when the pH of the assay was lowered to 7.0. The pH effect has been described previously and suggested as a possible control mechanism for modulation of isomerase activity (Geewater *et al.*, 1965). Although direct measurement of intracellular pH has yet to be done in *D. discoideum*, it is interesting to note that the developmental medium for this organism is buffered in the acid range (pH 6.5).

The inhibition of glucose-6-phosphate isomerase by erythrose 4-phosphate and the high affinity of the enzyme for this intermediate suggests that it may function in a regulatory manner in carbohydrate metabolism. Under conditions where the metabolism of fructose 6-phosphate by the transketolase-transaldolase catalysed reactions (EC 2.2.1.1; sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate glycolaldehydetransferase - EC 2.2.1.2; sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate dihydroxyacetone-transferase) might lead to an accumulation of erythrose 4-phosphate, further formation would be prevented by inhibition of the isomerase step. On the other hand, it would appear that this reaction is close to equilibrium in vivo, based on the similarity of the mass action ratios (0.32 to 0.34) to the equilibrium constant (0.36) throughout differentiation (Thomas, 1979). However, in the absence of a dynamic model of this metabolic network, it is difficult to assess the extent to which the equilibrium would be displaced if regulation by erythrose 4-phosphate did occur.

This investigation was supported by U.S. Public Health Service grant no. AG00649 to D.T. and National Institutes of Health on Aging grants no. AG00433 and AG00260 to B. E. Wright.

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