GALACTOSE-6-PHOSPHATE DEHYDROGENASE: A NEW ENZYME FROM MAMMALIAN LIVER

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1. Introduction

During the course of our study with the UDP glucose-4-epimerase from goat-liver [1], we have detected a novel NAD-dependent dehydrogenase from the same source which specifically utilizes galactose-6-phosphate as its substrate. The enzyme has also been detected in rat-liver. The presence of Gal-6-P was demonstrated by Inouye et al. [2] in erythrocytes of patients suffering from galactosemia. Further, Posternak and Resselet had shown that Gal-1-P could be converted to Gal-6-P in the presence of phosphoglucomutase [3]. It is likely therefore that some Gal-6-P is formed under normal metabolic conditions though its fate either in that case or under pathological conditions remains unknown.

2. Methods and materials

All the reagents including Gal-6-P were obtained from Sigma Co. The enzyme can be assayed spectrophotometrically conveniently after some purification. The assay mixture contained in a total volume of 1 ml, 100 μmoles of Tris buffer pH 8.1 and 0.5 μmoles of NAD. The reaction was started by the addition of 1 μmole of Gal-6-P. The rate remained linear for more than 5 min and was measured by following the increase in absorbancy at 340 nm. One unit of the enzyme was defined as the amount of enzyme needed for the formation of 1 μmole of NADH per minute.

3. Results

3.1. *Purification and separation from glucose-6-phosphate dehydrogenase*

For purification of the enzyme, all the operations were carried out at 4°. 12 g of frozen goat-liver were homogenized in a Waring blender for 1 min in 36 ml of 0.02 M potassium phosphate buffer pH 7.4, containing 0.002 M EDTA and 0.002 M mercaptoethanol. The homogenate was centrifuged at 10,000 *g* for 30 min. To the crude supernatant, 2% protamine sulphate was slowly added with stirring till its final volume became 15% of the crude supernatant. The accumulated precipitate was removed by centrifugation after standing for 10 min. The protamine sulphate supernatant was now subjected to fractionation by ammonium sulphate. The enzyme which precipitated between 35–55% cut was redissolved in 5 ml of the same buffer and was dialyzed against the same buffer for 6 hr. Along with Gal-6-P dehydrogenase activity, this portion contained considerable amount of glucose-6-P dehydrogenase activity. These two activities could, however, be completely separated during purification by gel treatment. To 5 ml (approx. 45 mg/ml of protein) of this enzyme fraction, 5 ml of centrifuged calcium phosphate gel (18 mg/ml), prepared according to the method of Keilin and Hartree [4] was slowly added with stirring. After 10 min the gel was centrifuged off and to the supernatant another 5 ml of gel was added. This second portion of the gel was also discarded. G-6-P dehy-

Table 1
Purification of Gal-6-P dehydrogenase from goat-liver.

Step	Total activity (units)	Total protein (mg)	Yield (%)	Specific activity (units/mg protein $\times 10^2$)
1 Crude			–	–
2 Protamine sulphate	1.08	510	100	0.21
3 Ammonium sulphate	1.01	230	94	0.44
4 Calcium phosphate gel	0.89	26	83	3.4

drogenase was partially absorbed during the first gel treatment and was now completely absorbed in the second gel fraction. The enzyme could be extracted from this portion of the gel with 2 ml of 0.2 M K-PO_4 buffer pH 7.4. For the further purification of the second gel treatment, 2 ml of gel was added for the third time. The enzyme which came out in the precipitate with the gel was eluted from it by extraction with 5 ml of 0.1 M K-PO_4 buffer pH 7.4 containing 0.001 M mercaptoethanol and 0.002 M EDTA. The enzyme is about 16-fold purified over the protamine sulphate supernatant fraction (table 1) and is completely free from G-6-P dehydrogenase, glucose dehydrogenase and phosphoglucomutase.

3.2. *Substrate specificity*

The enzyme shows a remarkable specificity with regard to the substrate (table 2). None of the various sugar phosphates and free sugars could be even partially utilized as substrates. Total lack of reaction in presence of galactose distinguishes this enzyme from the NAD dependent galactose dehydrogenase obtained earlier from rat liver by Segal's group [5, 6]. NAD however can be partially replaced by NADP and other NAD analogues.

3.3. *Properties of the enzyme*

The enzyme follows typical Michaelis-Menten kinetics both with regard to Gal-6-P and NAD. The K_m for Gal-6-P and NAD were determined to be 6×10^{-4} M and 1.2×10^{-4} M, respectively. The enzyme was found to have an optimum pH at 8.1.

Table 2
Specificity of the reaction.

Set A	NADH formed (nmoles)
Complete	17.2
G-6-P	–
Gal-1-P	–
F-6-P	–
Glucose	–
Galactose	–
Man-6-P	–
Mannose	–
Set B	**NADH formed (nmoles)**
Complete	21.2
NADP	2.5
3-Acetyl pyridine DPN	3.4
3-Acetyl pyridine deamino DPN	3.2

The complete reaction mixture contains in 1 ml, 100 μmoles of Tris buffer pH 8.1, 120 μg of purified enzyme, 1 μmole of NAD and the reaction is started with 1 μmole of Gal-6-P. The reaction is followed at 340 nm for 6 min. In Set A, Gal-6-P is replaced by other compounds as indicated. In Set B, NAD is replaced.

4. Discussion

Kirkman [7] had previously shown that Gal-6-P could act as a weak substrate for glucose-6-phosphate dehydrogenase when the enzyme was isolated from human erythrocytes. The product for this *in vitro* reaction was 6-phosphogalactonic acid and the reaction was completely dependent on NADP. Our enzyme on the other hand is absolutely specific for Gal-6-P and utilizes NADH for oxidation. Further, preliminary studies showed that the aldehydic group remained unaffected during the enzymatic oxidation and thus a product other than 6-phosphogalactonic acid was formed. Existence of an alternate route has been suggested by various workers. In fact, Segal and Cuatrecasas [6] has demonstrated the existence of an alternate route for galactose metabolism in rat liver where galactose is first oxidized to galactonic acid. The wide variation in severity for children lacking in transferase also indicates possibilities for existence of alternate paths for the metabolism of galactose. Even the presence of UDP-galactose pyrophosphorylase [9] at low levels is not adequate enough to explain this varia-

tion in severity of the disease. Whether Gal-6-phosphate dehydrogenase along with phosphoglucomutase and other undiscovered enzymes leads to an alternate path in mammalian liver remains to be established.

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