Biochimica et Biophysica Acta, 302 (1973) 129–134

BBA 66820

TWO FORMS OF UDPGLUCOSE-4-EPIMERASE FROM MAMMALIAN LIVER

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(Received August 22nd, 1972)

SUMMARY

UDPglucose-4-epimerase (EC 5.1.3.2) is shown to exist in two distinct forms in goat and beef liver. The two forms can be distinguished easily by their interaction with sugar phosphates and with substrate. The minor form comprising about 20% of the total activity, is activated by both Gal-1-*P* and Gal-6-*P* and is partially inhibited by Glc-1-*P*. None of these sugars have any effect on the major form except Gal-6-*P*, which inhibits its activity partially only in the case of goat liver. The major form, however, undergoes inhibition by substrate at higher concentrations. The two forms behave similarly in their requirement for NAD for activity and in their inhibition by UMP. The induced epimerase in yeast fails to show the existence of these two forms.

INTRODUCTION

UDPglucose-4-epimerase (EC 5.1.3.2) is an obligatory enzyme for galactose metabolism in all cells studied so far and has been extensively purified from the yeast *Saccharomyces fragilis*[1] and *Escherichia coli*[2]. The enzyme was also purified from calf liver[3]. The enzyme occupies a special position for the metabolism of galactose, since unlike the galactokinase (EC 2.7.1.6) and the Gal-1-*P* uridyltransferase (EC 2.7.7.10) it is needed both for the synthesis and for the degradation of the galactose moiety[4]. In spite of the obvious importance of this enzyme, very little is known about the modulation in the enzyme activity in the presence of various metabolites closely related to this pathway. During the course of our study with epimerase from goat liver[5] we have obtained two distinct forms of this enzyme which behave significantly differently with regard to substrate and some sugar phosphates. Beef liver epimerase was also shown to have these two distinct forms.

Methods and Materials

All the reagents were from Sigma Co. Some UDPglucose dehydrogenase (EC 1.1.1.22) was obtained from Sigma as a gift. The coupled assay of epimerase was

performed according to the method of Darrow and Rodstrom[1]. For the two-step assay the method of Maxwell *et al.*[6] was followed. One unit of the epimerase was defined as the amount of enzyme needed to epimerize 1 mole of UDPGal per min.

RESULTS

Separation of the two forms on calcium phosphate gel

All the operations were carried out between 0 and 6 °C. 12 g of frozen goat liver were homogenized in a Waring blender and then centrifuged at 12 000 × *g* for 30 min. 2% (w/v) protamine sulphate solution was added to the crude supernatant till the volume of added protamine sulphate was 15% of the total volume. After standing for 15 min, the solution was centrifuged and the supernatant was subjected to fractionation by $(NH_4)_2SO_4$. The enzyme which precipitated between 35 and 55% of $(NH_4)_2SO_4$ was redissolved in 5 ml of 0.02 M potassium phosphate buffer (pH 7.4) containing 0.002 M EDTA and 0.001 M mercaptoethanol. The $(NH_4)_2SO_4$ fraction was dialyzed for 5 h against the same buffer and was then treated with calcium phosphate gel prepared according to the method of Keilin and Hartree[7]. To 5 ml of the enzyme solution (approx. 45 mg/ml), 2.5 ml of the centrifuged gel (26 mg/ml) were then added. The mixture was stirred for 5 min and then centrifuged off. To the gel supernatant, 5 ml of fresh centrifuged gel were added. The portion of the epimerase which then became absorbed in the gel precipitate could be completely extracted from it with 5 ml of 0.12 M potassium phosphate buffer (pH 7.4). The epimerase that came out of the gel at this stage is designated epimerase B. The supernatant of the second gel treatment was retreated for the third time with 2.5 ml of the centrifuged gel and the precipitated gel was discarded. The supernatant after this final gel treatment retained the major fraction of the epimerase. The epimerase obtained in this fraction is termed epimerase A. This fraction contained about 75–80% of the total epimerase activity. Following the same fractionation procedure, the beef liver epimerase could also be separated in these two forms and the relative ratios were also of the same order. The specific activities of forms A and B from goat liver at this stage were 0.16 and 0.03, respectively.

Separation on DEAE-cellulose column

The two forms of the epimerase could also be separated on a DEAE-cellulose column after the $(NH_4)_2SO_4$ step. A 15 cm × 1.4 cm column was packed with medium-grained DEAE-cellulose which was equilibrated with 0.02 M potassium phosphate buffer (pH 7.4). 0.75 ml of the enzyme solution containing 32 mg of protein and 0.5 unit of total epimerase was absorbed in the column. The column was eluted successively with 48 ml each of 0.02, 0.05, 0.08 and 0.12 M concentrations of the same buffer. Fractions having a volume of 3 ml were collected using a flow rate of 0.6 ml/min. The major fraction of the enzyme, epimerase A was eluted between Tubes 40 to 44, during elution with 0.08 M buffer. Epimerase B was eluted between Tubes 55 to 59 during elution with 0.12 M buffer (Fig. 1). About 70% of the total activity was recovered from the column.

Epimerase A and B, when separated on the gel and remixed in equal volumes, could again be separated on a similar DEAE column which was now equilibrated against 0.075 M potassium phosphate buffer (pH 7.4). In this case, epimerase A

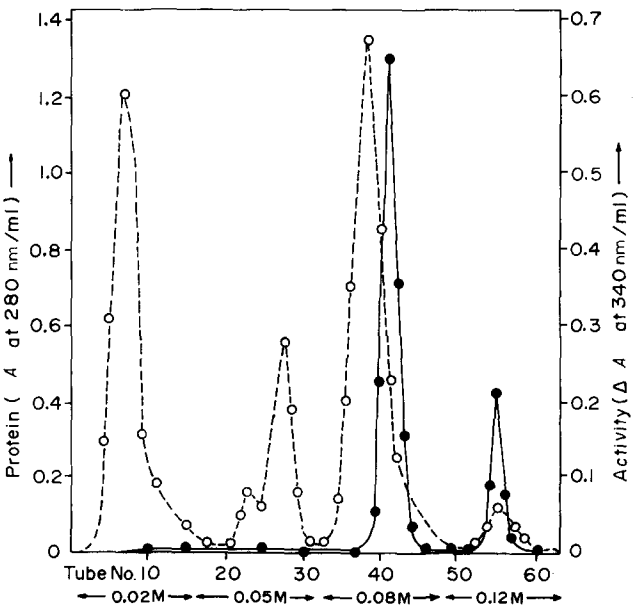

Fig. 1. Chromatographic separation of the two forms of epimerase from goat liver. ○---○ indicates the elution pattern of proteins and ●—● indicates the elution pattern of epimerase activity.

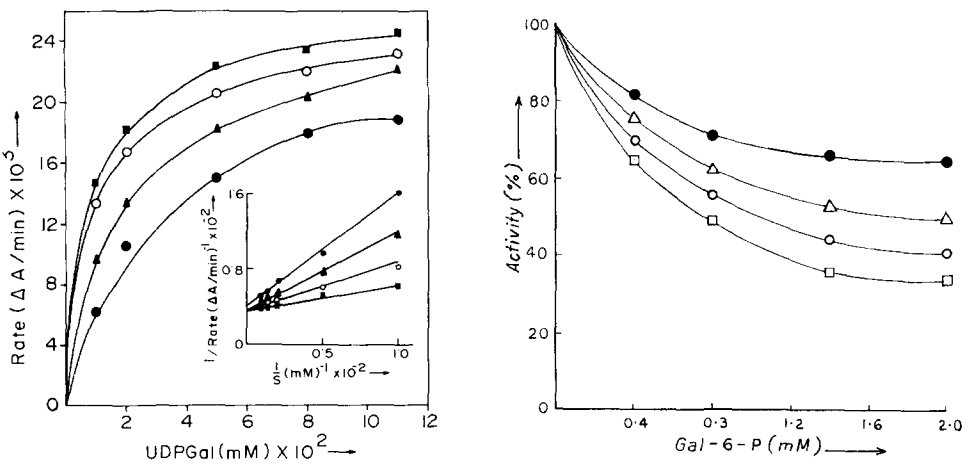

Fig. 2. Activation of epimerase B from goat liver with Gal-6-*P*. The lowest curve indicates the rate in absence of Gal-6-*P*. ▲—▲, ○—○ and ■—■ indicate the rates obtained with $2 \cdot 10^{-1}$ mM, $5 \cdot 10^{-1}$ mM and 1 mM concentrations of Gal-6-*P*. Similar activation was observed with epimerase B from beef liver. The insert in the figure was obtained by plotting the data by the Lineweaver–Burk method.

Fig. 3. Inactivation of epimerase A from goat liver with Gal-6-*P*. The extent of inhibition of the epimerase activity in presence of varying concentrations of Gal-6-*P* is indicated when the concentration of UDPGal was also varied. ●—●, △—△, ○—○ and □—□ show the per cent activity retained in presence of $2 \cdot 10^{-1}$ mM, $5 \cdot 10^{-1}$ mM, 1 mM and 2 mM concentrations of UDPGal, respectively.

passed through the column unabsorbed and the minor fraction was eluted with 0.12 M buffer.

Interaction with Gal-6-P

Galactose 6-phosphate showed totally different effects on the two forms of the epimerase from goat liver. Form B was quite strongly activated by Gal-6-*P*, whereas under similar conditions the other form was partially inhibited. Both the K_m and V changed during activation. At saturating concentrations of the substrate about 30% activation was observed but at lower concentrations of the substrate the effect was more pronounced (Fig. 2). However, the major fraction of the epimerase was inhibited about 60% at 2 mM concentration of Gal-6-*P* (Fig. 3). Further increase in Gal-6-*P* concentration did not result in any substantial increase in inhibition. Epimerase B from beef liver was similarly activated. Gal-6-*P*, however, had no inhibitory effect on the other form from beef liver.

Interaction with Gal-1-P

Epimerase B from both goat and beef liver was activated by Gal-1-*P*. The nature of the activation was very similar to that of Gal-6-*P* (Fig. 4). Gal-1-*P*, however, did not have any effect on the other form of the enzyme.

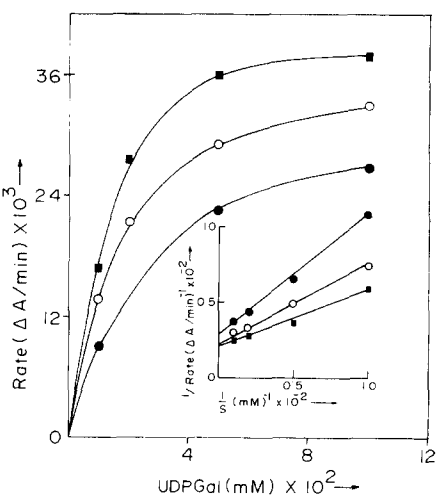

Fig. 4. Activation of epimerase B from goat liver with Gal-1-*P*. The lowermost curve indicates the rate in the absence of Gal-1-*P*. ○—○ and ■—■ indicate the rates with $5 \cdot 10^{-2}$ mM and $1 \cdot 10^{-1}$ mM concentrations of Gal-1-*P*, respectively. Similar activation was observed for epimerase B from beef liver in presence of Gal-1-*P*. The insert in the figure was obtained by plotting the data by the Lineweaver–Burk method.

Interactions with other sugar phosphates

Glucose 1-phosphate was found to have partial inhibitory effect on the minor form. Glc-1-*P* at a concentration of 2.0 mM inhibited the enzyme about 30%. Glc-6-*P*, Fruc-6-*P*, Fruc-1-*P*, had no effect on either of these two forms.

Inhibition with substrate

We had already noted that the yeast epimerase was strongly inhibited at high substrate concentrations[5]. In the case of the liver enzyme, however, only the major form, *i.e.* form A, was found to be inhibited by UDPGal at a concentration of $2 \cdot 10^{-1}$ mM (Fig. 5). The minor form showed no inhibition by substrate even at a much higher concentration. The two forms, however, had very similar K_m values.

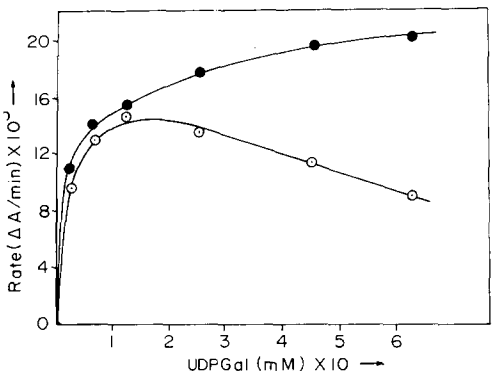

Fig. 5. Inhibition of Epimerase A from goat liver with substrate. ○—○ and ●—● indicate the variation in rate for epimerase A and epimerase B, respectively, with increasing concentrations of UDPGal. Similar inhibition by UDPGal was observed also for beef liver epimerase A.

TABLE I

In each tube, the specified enzyme was incubated in a medium containing 100 μmoles Tris buffer (pH 8.1), 0.1 μmole of UDPgalactose and an amount of enzyme that gave a rate of 0.03–0.04 absorbance unit per min in a total volume of 1 ml. Amount of NAD present in each tube is indicated in the table. After 8 min incubation, UDPglucose formed was assayed with UDPglucose dehydrogenase.

Enzyme	*$(NAD^+) \times 10^4$ (M)*	*UDPG formed (nmoles)*
Epimerase A	—	—
	0.2	14.3
	1.0	32.1
	5.0	32.6
Epimerase B	—	—
	0.2	12.3
	1.0	26.1
	5.0	29.3

Requirement of NAD and inhibition by UMP

Maxwell[3] had shown that, unlike the yeast epimerase, the calf liver enzyme has an absolute requirement for NAD added from the outside. Using the two-step assay, we found that both forms of the liver epimerase were completely inactive in the absence of NAD (Table I). Further, UMP which is a strong competitive inhibitor for yeast epimerase[5] also inhibited both the forms equally strongly and competitively.

DISCUSSION

In spite of our extensive knowledge about the control of galactose metabolism at the genetic level in the case of bacteria, little is known so far about the control of this pathway at the enzymatic level. Existence of the two forms of the epimerase in mammalian liver and their varying interactions with sugar phosphates and substrate may be significant in this respect. Apart from Gal-1-*P*, Gal-6-*P* has also been shown to accumulate during galactosaemic conditions[8]. Gal-6-*P* is also known to be formed from Gal-1-*P* by phosphoglucomutase[9,10]. Further, we have recently detected an enzyme in mammalian liver that specifically oxidizes Gal-6-*P* in presence of NAD[11]. Activation of the minor form by Gal-1-*P* and Gal-6-*P* may therefore be physiologically significant during galactosaemia, as increased synthesis of UDPGal from glucose could help to maintain the supply of galactose moiety for synthetic purposes. Gal-1-*P* was previously shown to inhibit phosphoglucomutase (EC 2.7.5.1)[12] and UDPG pyrophosphorylase (EC 2.7.7.9)[13]. However, its activating role was not known. In contrast to the sugar phosphates, the two forms behaved very similarly in their interactions with UMP and NAD (Table I). Requirement for NAD was absolute in both cases. Bhaduri *et al.*[14] and Darrow and Rodstrom[1] had shown previously that the yeast epimerase underwent molecular transitions in the presence of these metabolites. Whether such interactions take place in the case of these two forms of epimerase remains to be seen. In an earlier report, Cuatrecasas and Segal[15] had indicated the existence of the two forms of galactokinase in rat liver from developmental studies. However, the two forms were not physically separated. The induced epimerase in the case of the yeast *S. fragilis* fails to show the existence of these two forms. It is not unlikely that during induction only one of the forms of the epimerase is synthesized.

ACKNOWLEDGEMENT

The above work was financed by grants from Bhava Atomic Research Centre and Council of Scientific and Industrial Research (India).

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