GLUCOSE-6-PHOSPHATE-1-EPIMERASE FROM BAKER'S YEAST. A NEW ENZYME

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1. Introduction

Salas et al. [1] reported that phosphoglucose isomerase from yeast not only catalyses the isomerization of glucose-6-phosphate to fructose-6-phosphate but also the conversion of α-D-glucopyranose-6-phosphate to the aldehyde form of glucose-6-phosphate. Also Carlson et al. [2] could show that this enzyme accelerates the mutarotation of β-D-glucopyranose-6-sulphate. Investigating this intrinsic anomerase activity of PGI [3] in a quantitative way we discovered another enzyme in the yeast cell which, at the branch point of glucose metabolism, catalyses the equilibrium of the anomeric forms of glucose-6-phosphate. The separation and partial characterization of this glucose-6-phosphate-1-epimerase will be described in this paper.

2. Materials and methods

All chemicals of pro analys grade were purchased from E. Merck AG, Darmstadt; Lab-Trol was obtained from DADE Division, Miami, Florida. Sephadex G-25, G-100 and Blue Dextran were bought from Pharmacia, Uppsala, glucose-6-phosphate, ATP, NADP, hexokinase, glucose-6-phosphate dehydrogenase, glucose oxidase, peroxidase and cytochrome *c* from Boehringer Mannheim GmbH, and α-D-glucose, *o*-dianisidine, ribonuclease, carboxypeptidase A, ovalbumin, bovine serum albumin, trypsin and α-chymotrypsin from Serva, Heidelberg. Protein was determined by the biuret method [4] using Lab-Trol as a standard. With minor modification methods for enzyme activity determinations were taken from [5] and metabolites were assayed according to [6]. Glucose-6-phosphate-1-epimerase activity was determined at 25° in standard buffer 50 mM imidazole/HCl, 50 mM KCl and 8 mM $MgSO_4$, pH 7.6 in the test systems described below with the following conditions: System 1: 1.5 mM α-D-glucopyranose, 2 mM ATP, 2 mM $NADP^+$, 0.1 U/ml HK (v_{system}) and 30 U/ml G6PDH; System 2: 30–50 μM G6P equilibrated, 2 mM $NADP^+$, 30–80 U/ml G6PDH. The enzymes were freed from ammonium sulphate by elution through columns of Sephadex G-25 [7].

For stopped flow experiments the equipment developed by Hess et al. [8] was used with the monochromator set at 340 nm. The flow system of the apparatus has a total dead time of 2 msec, and a flow velocity of 5 m/sec, d = 1 cm. The molecular weight was estimated by gel filtration [7, 9]. A Sephadex G-100 column (93 × 2.6 cm) was equilibrated with standard buffer + 3 mM NaN_3 and loaded with 10 mg protein/ml. The elution velocity was 40 ml/hr giving fractions of 6.6 ml. The void volume V_0 was determined using Blue Dextran. For the following reference compounds the elution volume V_e was estimated: cytochrome *c* (M.W. 13,000), ribonuclease (M.W. 13,600), trypsin (M.W. 24,000), carboxypeptidase A (M.W. 34,300), ovalbumin (M.W. 45,000),

Abbreviations:

α-G6P	: α-D-glucopyranose-6-phosphate;
β-G6P	: β-D-glucopyranose-6-phosphate;
γ-G6P	: aldehyde form of D-glucose-6-phosphate;
6PGδL	: 6-phosphogluconic acid δ-lactone;
G6P-1-E	: glucose-6-phosphate-1-epimerase;
PGI	: glucose-6-phosphate isomerase (E.C. 5.3.1.9);
G6PDH	: glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49).
HK	: hexokinase (E.C. 2.7.1.1).

bovin serum albumin (M.W. 67,000) and enolase (M.W. 82,000). The molecular weight of glucose-6-phosphate-1-epimerase was obtained from the regression line of a plot V_e/V_0 versus log molecular weight.

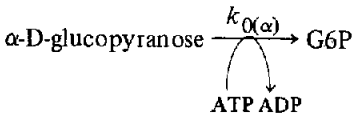
Electrofocusing experiments were carried out using an LKB 8101 column (110 ml) with a 2% ampholine pH 5–7 gradient stabilised by sucrose (4° and 600 V).

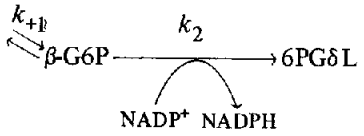
3. Results and discussion

3.1. *Test systems*

Because of the specificity of glucose-6-phosphate dehydrogenase for β-G6P [1, 10–13] the spontaneous and enzyme catalysed formation of β-G6P from α-G6P can be tested in the following systems with G6PDH as indicator-enzyme [1, 3, 10, 11, 14]:

System 1





If the substrates α-D-glucopyranose, ATP and $NADP^+$ are saturating their respective enzymes and the following conditions hold:

$$V_{max(G6PDH)} \gg v_{system} = k_{0(\alpha)} = V_{max(HK)} \text{ and}$$

$$k_2 = \frac{V_{max(G6PDH)}}{K_{m(\beta\text{-G6P})}} \gg k_{+1} \text{ then, } [\beta\text{-G6P}] \to 0, \text{ and the}$$

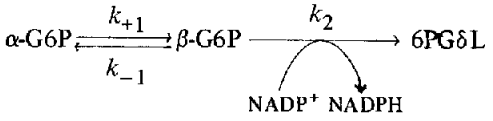
system can be treated as being irreversible. The hexokinase reaction follows zero order, the anomerisation reaction as well as the reaction of G6PDH are first order. Under steady state conditions the velocity of the irreversible reaction sequence is given by:

$$v_{system} = k_{0(\alpha)} = k_{+1}[\alpha\text{-G6P}_{ss}] = k_2[\beta\text{-G6P}_{ss}] =$$

$$= \frac{d\,NADPH}{dt} = \text{constant.}$$

The system is started by addition of freshly dissolved α-D-glucopyranose. As soon as the steady state is reached, after reaction time of 1 min [14], the reactions are quenched by addition of 0.2 ml 4 N $HClO_4$ (per 1 ml volume) and the steady state concentration of G6P can be assayed after neutralisation with 4 N KOH. k_{+1} can be computed from v_{system} and $[G6P_{ss}] \approx [\alpha\text{-G6P}_{ss}]$.

System 2



The reactions are started by addition of equilibrated G6P and recorded until the reaction is complete. For $k_2 = V_{max(G6PDH)}/K_{m(\beta\text{-G6P})} \gg k_{+1}$ during the fast initial reaction $[\beta\text{-G6P}] \to 0$; the following slow reaction of first order represents the formation of β-G6P from α-G6P. The reaction velocity constant k_{+1} can be obtained from first order plots.

In both test systems the same reaction velocity constant of first order k_{+1} is obtained. After subtraction of the value of the velocity constant for the spontaneous reaction ($k_{+1(s)}$) the first order reaction velocity constant for the G6P-1-epimerase catalysed reaction $k_{+1(G6P\text{-}1\text{-}E)} = k_{+1} - k_{+1(s)}$ can be computed. This constant (activity constant) per mg protein is defined as specific activity constant in $min^{-1} \times mg^{-1}$. In both test-systems neither V_{max} nor K_m for G6P-1-epimerase can be determined. In the presence of PGI (in cell extracts) the formation of β-G6P from α-G6P can only be investigated in system 1. Then, the reaction velocity constant for the G6P-1-epimerase catalysed reaction is obtained from $k_{+1(G6P\text{-}1\text{-}E)} = k_{+1} - k_{+1(s)} - k_{+1(PGI)}$.

3.2. *Separation*

From 30 g of fresh baker's yeast in 70 ml suspension of 0.2 M $(NH_4)_2SO_4$ cell extracts were prepared in a Braun disintegrator [15]. In a 100 ml glass bottle 35 ml suspension plus 30 g of glass beads (Ø 0.5 mm) were shaken for 2 min at a frequency of 4000/min whilst cooling with liquid CO_2. After centrifugation for 30 min at 30,000 *g* the supernatant was used for $(NH_4)_2SO_4$ fractionation (pH 5.8, 2–4°, initial protein concentration 40 mg/ml) [4, 16].

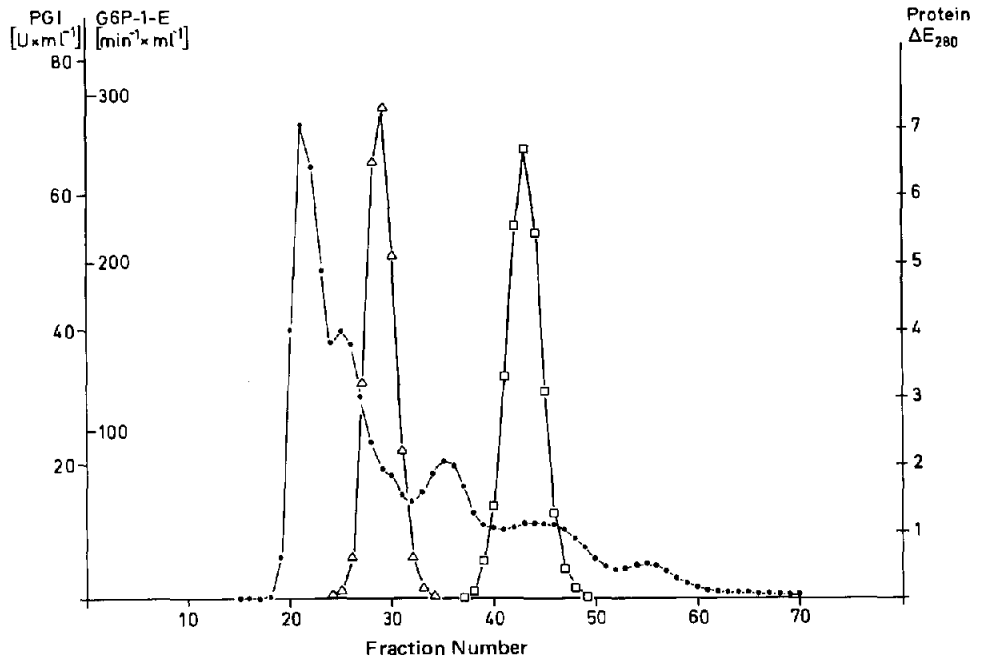


Fig. 1. Elution profile of Sephadex G-100 chromatography. Elution velocity 40 ml/hr; fractions of 6.6 ml were collected. (△—△—△): PGI activity; (□—□—□): G6P-1-epimerase activity constant; (●—●—●): protein ΔE_{280}.

The fraction 1.9–2.3 M $(NH_4)_2SO_4$ was dissolved in standard buffer + 3 mM NaN_3 and placed on the Sephadex G-100 column described above. The elution profile in fig. 1 demonstrates the separation of a G6P-1-epimerase of low molecular weight from PGI. It should be mentioned that PGI still maintains its intrinsic anomerase activity [1–3]. The fractions 40–46 were concentrated by pressure filtration (Amicon, PM 10, 3.5 atmospheres) and dialysed against a saturated solution of $(NH_4)_2SO_4$, giving 10 mg of a crude preparation of G6P-1-epimerase with a specific activity constant of 500 $min^{-1} \times mg^{-1}$ and a 30-fold purification with a recovery of 50%.

3.3. *Partial characterization*

The G6P-1-epimerase activity was completely destroyed by heat treatment (10 min at 80°) as well as by incubation with trypsin and α-chymotrypsin (10 min at 25°, pH 7.6). By gel filtration on Sephadex G-100 the molecular weight of the G6P-1-epimerase was estimated to be 35,000, and in electrofocusing experiments an isoelectric point of pH 5.8 was determined.

Table 1

Calculation of the first order velocity constants (activity constants) $k_{+1(G6P\text{-}1E)}$ of the enzyme catalyzed conversion of α-G6P to β-G6P.

	G6P-1-E [mg × ml^{-1}]	k_{+1} [min^{-1}]	$k_{+1(G6P\text{-}1E)} = k_{+1} - k_{+1(s)}$ [min^{-1}]
1)	0	4.2	0
2)	0.015	11.4	7.2
3)	0.030	19.8	15.6
4)	0.045	25.8	21.6
5)	0.060	32.0	27.8
6)	0.075	40.2	36.0

Velocity constants k_{+1} were evaluated from the slope of the regression lines of fig. 2. The velocity constant obtained from experiment 1 in the absence of G6P-1-epimerase corresponds to the velocity constant of the spontaneous reaction $k_{+1(s)}$.

The formation of β-G6P from α-G6P at varying G6P-1-epimerase concentrations was investigated with a stopped-flow technique using test system 2. The reaction courses computed as first order plots are demonstrated in fig. 2. The activity constants $k_{+1(G6P\text{-}1E)}$ were calculated using the reaction velocity

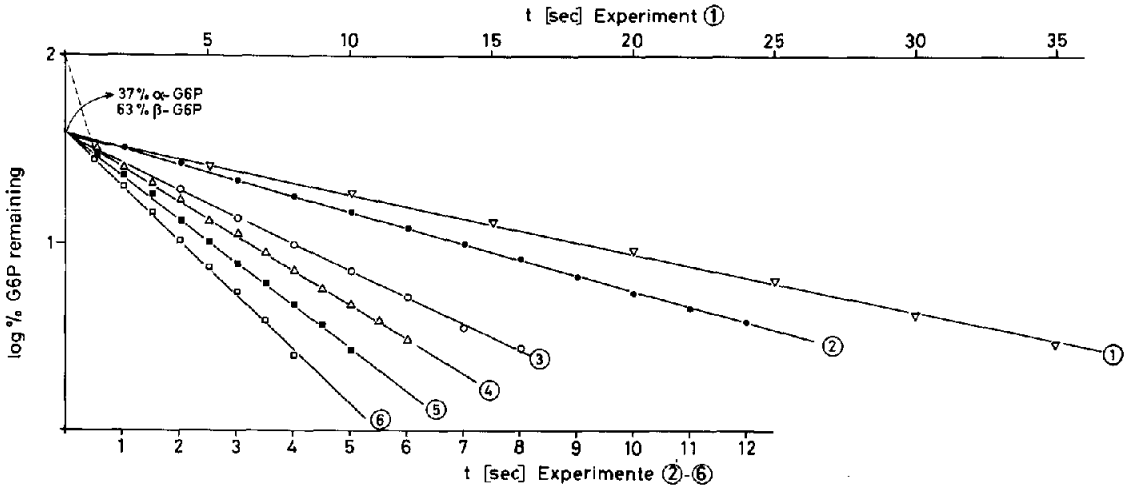


Fig. 2. Stopped flow experiments. Determination of the first order velocity constant k_{+1} for the conversion of α-G6P into β-G6P at various concentrations of G6P-1-epimerase, given in the table. Reactions were initiated by mixing equal volumes of a) 160 U/ml G6PDH + G6P-1-E + 2 mM NADP and b) 60 μM G6P equilibrated + 2 mM NADP. The rapid initial reaction represents the dehydrogenation of β-G6P present in the equilibrium mixture (broken line), the following slower reaction corresponds to the conversion of α-G6P to β-G6P. Velocity constants k_{+1} were evaluated from the slope of the regression lines (see table 1). From the intersection of the regression lines with the ordinate the equilibrium composition of G6P was calculated as 37% α-D-glucopyranose-6-phosphate and 63% β-D-glucopyranose-6-phosphate.

constants of fig. 2 as summarized in the table (2nd column). A good proportionality between the G6P-1-E concentration and the activity constant of the enzyme is found. Up to now we only know of glucose-6-phosphate as a substrate of G6P-1-epimerase. In contrast to aldose-1-epimerases (mutarotases) [17–21] G6P-1-epimerase does not catalyze the anomerization of D-glucose. This could be verified in a test system composed of glucose oxidase, peroxidase and *o*-dianisidine [6, 10] analogous to the test system 2 described above.

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