ENZYME-CATALYZED ANOMERIZATION OF D-GLUCOSE-6-PHOSPHATE

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1. Introduction

Recently we reported the discovery of the enzyme glucose-6-phosphate 1-epimerase in baker's yeast [1]. This enzyme catalyzes the equilibration of the anomeric forms of D-glucose-6-phosphate at the branch point of D-glucose metabolism. In addition following the experimental results of Salas et al. [2] and Carlson et al. [3], it was shown, that glucosephosphate isomerase catalyzes not only the isomerization of α- and β-D-glucopyranose-6-phosphate to α- and β-D-fructofuranose-6-phosphate [4, 5] but also the anomerization of α- to β-D-glucopyranose-6-phosphate [4, 5] and the anomerization of α- to β-D-fructofuranose-6-phosphate [5].

From these observations arose the questions whether glucose-6-phosphate 1-epimerase is present in other organisms as well, and whether glucosephosphate isomerase from other organisms will also catalyze the anomerization of D-glucose-6-phosphate. In this paper the results of an investigation of the occurrence of enzyme catalyzed anomerization of D-glucose-phosphate in *E. coli*, *Rhodotorula gracilis*, potato tubers, rat muscle, rat liver and rat kidney are described.

In *E. coli* and *Rhodotorula gracilis* glucose-6-phosphate 1-epimerases with molecular weights of about 30 000 were detected, and in potato tubers two glucose-6-phosphate 1-epimerases with molecular weights of about 30 000 and 45 000 respectively were discovered. In rat muscle, rat liver and rat kidney the occurrence of glucose-6-phosphate 1-epimerase could not be demonstrated. Glucosephosphate isomerases from all six biological sources catalyze the anomerization of D-glucose-6-phosphate.

2. Materials and methods

All chemicals of p.a. grade were purchased from E. Merck AG, Darmstadt; Sephadex G-75 was bought from Pharmacia, Uppsala, D-glucose-6-phosphate, ATP, $NADP^+$, hexokinase, glucose-6-phosphate dehydrogenase, glucose oxidase, peroxidase and cytochrome *c* from Boehringer, Mannheim GmbH, and polyethyleneglycol 20 000, α-D-glucose, *o*-dianisidine, trypsin and ovalbumin from Serva, Heidelberg.

E. coli (ATCC 9637) was grown aerobically in Sakaguchi medium (0.25% $(NH_4)_2HPO_4$, 0.15 % KH_2PO_4, 0.5% NaCl, 0.01% $MgSO_4 \cdot 7H_2O$, 0.3% sodium glutaminate, 0.3% D-glucose) at 30°C.

Rhodotorula gracilis 5 Fres–Harrison was grown aerobically in a mineral salts medium (0.12% $(NH_4)_2SO_4$, 0.07% $MgSO_4 \cdot 7H_2O$, 0.1% KH_2PO_4, 0.05% NaCl, 0.053% $CaCl_2 \cdot 2H_2O$, 0.00083% $FeCl_3$) containing 2% glycerol and 0.25% Difco yeast extract, at 30°C.

Cells of *E. coli* and *Rhodotorula gracilis* were harvested at the end of the logarithmic phase of growth and were washed twice with distilled water.

Cell-free extracts of *E. coli* and *Rhodotorula gracilis* were prepared in a glass beads homogenizer (Braun, Melsungen) [6]. In a 100 ml glass bottle 34 ml of a suspension of *E. coli* (17 g of cells wet weight plus 17 ml of 0.3 M $(NH_4)_2SO_4$) together with 30 g of glass beads (Ø 0.18 mm) or 40 ml of a suspension of *Rhodotorula* (20 g of cells wet weight plus 20 ml of 0.3 M $(NH_4)_2SO_4$) together with 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 *Rhodotorula*

supernatant was used directly for Sephadex G-75 chromatography. The *E. coli* supernatant was dialyzed against 3 M $(NH_4)_2SO_4$. After centrifugation for 10 min at 30 000 *g* the precipitate was dissolved in 30 ml H_2O.

Potato tuber extracts were prepared in a blendor (Star Mix, Electrostar). Two hundred grams of new potato tubers (washed and cut into small pieces) plus 200 ml standard buffer (see below) were homogenized for 2 min at 4°C. The homogenate was filtered through a cotton cloth and the filtrate was centrifuged for 30 min at 27 000 *g*. The supernatant (300 ml) was dialyzed against 1 litre of 20% polyethyleneglycol in order to concentrate the solution about 20-fold.

Fresh rat muscle (27.5 g) plus 55 ml of standard buffer, or 3 g of fresh rat kidney plus 9 ml of standard buffer were homogenized using an Ultra-Turrax blade homogenizer (Janke and Kunkel, Staufen), and 18.5 g of fresh rat liver plus 18.5 ml of standard buffer were homogenized in a Potter-Elvehjem homogenizer (Braun, Melsungen). After centrifugation for 30 min at 30 000 *g* the supernatants were used for Sephadex G-75 chromatography. Glucose-6-phosphate 1-epimerase was separated from glucosephosphate isomerase by chromatography on a Sephadex G-75 column (80 × 5.2 cm), which was equilibrated with standard buffer (50 mM imidazole-HCl, 50 mM KCl, 8 mM $MgSO_4$, 2 mM EDTA, 5 mM mercaptoethanol, 3 mM NaN_3, pH 7.6). To 10 ml of each extract 20 mg of cytochrome *c* (as a marker protein) were added and the mixture was applied to the Sephadex G-75 column. Chromatography was carried out at 4°C; elution velocity was 100 ml/hr; fractions of 17 ml volume were collected. In order to estimate the molecular weights [7, 8] of the glucose-6-phosphate 1-epimerase the elution volume was determined for the following reference proteins: cytochrome *c* (mol. wt 13 000), trypsin (mol. wt. 24 000) and ovalbumin (mol. wt. 45 000).

The determination of substrate concentrations and enzyme activities was performed at 25°C in standard buffer pH 7.6 (see above) using an Eppendorf photometer conncected to a recorder, wavelength 366 nm. With minor modifications substrates were assayed according to [9], and methods for enzyme activity determinations were taken from [10]. The enzymes were freed from ammonium sulphate by dialysis.

The activity constant of glucose-6-phosphate 1-epimerase was determined in test system 2 described in [1] in the presence of 50 μM D-glucose-6-phosphate, 2 mM $NADP^+$ and 20 U/ml glucose-6-phosphate dehydrogenase.

The anomerase activity of glucosephosphate isomerase was determined as described in [4] at 2 mM α-D-glucose, 2 mM ATP, 2 mM $NADP^+$, 0.1 U/ml hexokinase and 20 U/ml glucose-6-phosphate dehydrogenase. The anomerase activity of glucosephosphate isomerase from potato tubers (in the fractions of Sephadex G-75 chromatography) could only be determined after inactivation of apyrase [11] by heat treatment (30 min at 53°C).

3. Results

Glucose-6-phosphate 1-epimerase was found to be present in extracts of *E. coli, Rhodotorula gracilis* and potato tubers. In the Sephadex G-75 chromatography, shown in the figure, the enzyme was eluted in fractions 53–59 in all three cases, and with potato tuber extracts an earlier, larger peak was found in fractions 47–53. By comparison with the elution volumes of the reference proteins it was calculated that the molecular weights of the enzymes extracted from *E. coli* and *Rhodotorula gracilis* were about 30 000 and the molecular weights of the two enzyme fractions from potato tubers were about 30 000 and 45 000. No elution peaks containing glucose-6-phosphate 1-epimerase were observed with extracts obtained from rat muscle, rat liver or rat kidney.

Since it was shown that glucosephosphate isomerase from yeast [2–5] and from rabbit muscle [12] exhibit anomerase activity towards D-glucose-6-phosphate, it was investigated whether glucosephosphate isomerases from other biological sources also catalyze the anomerization of D-glucose-6-phosphate. In these experiments glucosephosphate isomerases, eluted in fractions 34–42 of the Sephadex G-75 chromatography, were analyzed. It was found, that glucose phosphate isomerases from all six sources were capable of catalyzine the anomerization of D-glucose-6-phosphate.

4. Discussion

The occurrence of glucose-6-phosphate 1-epimerase has been demonstrated in baker's yeast, *E. coli, Rhodo-*

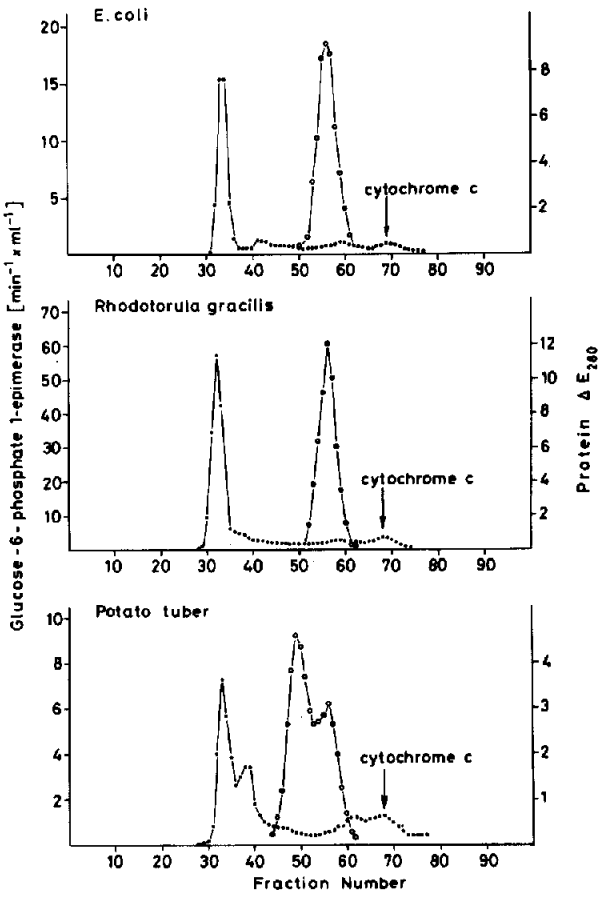

Fig. 1. Sephadex G-75 chromatography of extracts from *E. coli, Rhodotorula gracilis* and potato tubers. Fractions of 17 ml volume were collected, elution velocity 100 ml/hr. Activity constant of glucose-6-phosphate 1-epimerase (o–o–o) and protein ΔE_{280} (•–•–•) were determined in the fractions. Cytochrome *c* was added to the extracts as a marker protein.

torula gracilis and potato tubers. Glucose-6-phosphate 1-epimerases differ from aldose 1-epimerases (mutarotases) in exhibiting little or no activity towards D-glucose. Using a test system comprising glucose oxidase, peroxidase and *o*-dianisidine [13, 14] the activity constant of glucose-6-phosphate 1-epimerase from baker's yeast towards D-glucose was determined to be 70 000 times smaller than towards D-glucose-6-phosphate [15]. On the other hand it was shown that aldose 1-epimerase from beef kidney [13, 16] and from *E. coli** [17] do not catalyze the anomerization of D-glucose-6-phosphate. In the Sephadex G-75 chromatography, fractions containing glucose-6-phosphate 1-epimerase were analyzed for aldose 1-epimerase activity after dialysis against buffer containing no EDTA and mercaptoethanol. In *Rhodotorula* no aldose 1-epimerase activity could be detected whereas with *E. coli* and potato tubers peak fractions of glucose-6-phosphate 1-epimerase showed a little aldose 1-epimerase activity (0.5–5% of glucose-6-phosphate 1-epimerase activity). In *E. coli* an aldose 1-epimerase has already been described [18, 19].

As shown in fig. 1, in the Sephadex G-75 chromatography of potato tuber extract we obtained two species of glucose-6-phosphate 1-epimerase which differ in molecular weight. Since rechromatography on Sephadex G-75 of the species with higher molecular weight did not result in the formation of measureable amounts of the species with lower molecular weight, an association–dissociation equilibrium between the two species can be excluded. Furthermore, the possibility exists that the species with low molecular weight is the product of proteolytic digestion of the species with higher molecular weight. However, in three preparations of extracts of potato tubers, which were stored for different time periods before Sephadex G-75 chromatography the ratios of the activity constants of the two species were identical. Thus, the conclusion of the presence of two types of the enzyme in potato tubers seems to be justified.

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