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Phosphoglucose isomerase from Escherichia coli ATCC 25922 – pilot studies

Izomeraza fosfoglukozowa z *Escherichia coli* ATCC 25922 – badania pilotowe

INTRODUCTION

Phosphoglucose isomerase (D-glucose-6-phosphate aldose-ketose-isomerase; PGI; EC 5.3.1.9) is an enzyme that catalyzes reversible isomerization of glucose-6-phosphate (Robinson-Embden ester, glucose-6P) to D-fructose-6-phosphate ester (Neuberg ester, fructose-6P).

PGIs are represented by two evolutionarily distinct protein families: the PGI superfamily and the cupin superfamily. The PGI-superfamily comprises both the crenarcheal phosphogucose isomerase/ phosphomannose isomerase family (PGI/PMI family) and the PGI family, which are present in almost all bacteria including *Eschericha coli* (*E. coli*), eucarya and few euryarchaea. On the other hand, only few cupin type PGIs (cPGI) are known [8].

Phosphoglucose isomerase has sometimes been described as a "workhouse" enzyme of sugar metabolism [3]. PGI is a vital link between the Embden-Meyerhoff-Parnas, Entner-Duodoroff and pentose-phosphate pathways [14]. PGI is also involved in the gluconeogenesis. PGI has a number of other "moonlighting" functions [12].

The *E. coli* PGI mutant is able to grow glucose medium, although at reduced rate [6]. Disruption of phosphoglucose isomerase in this bacteria resulted in use the of the pentose phosphate pathway as the primary route of glucose. Furthermore, the PGI knockout *E. coli* demonstrated unexpected glyoxylate shunt activation. The Entner-Duodoroff pathway also contributed to a minor fraction of the glucose catabolism in this mutant strain [10]. In *E. coli* cells phosphoglucose isomerase is localized in two cellular compartments. Primarily the enzyme is located in the cytoplasm. About 6–10% of the PGI activity is localized in the periplasmic space. The cytoplasmic PGI participates in glucose catabolism [6]. The function of periplasmic PGI is the conversion of extracellular fructose-6P into glucose-6P, which is the only extracellular inducer for the hexose-phosphate transport system [7].

PGI is located at 91 min on the *E. coli* chromosome [1]. It does not seem to be a member of an operon or located close to other genes encoding glycolytic enzymes. The *E. coli* JM101 PGI is more

similar to the *Clarkia* (plant) sequence (87.6%) than the pig one (65.9%) [5]. The higher amino acids (aa) sequence similarity between plant plastid PGI isoenzyme and the *E. coli* PGI is consistent with the hypothesis that the nuclear gene encoding the former enzyme derived from the prokaryotic symbiont that evolved into the chloroplast [21].

The biofilm formation is recognized as an important virulence factor in many bacterial strains (e.g. *Staphylococcus aureus, Staphylococcus epidermidis* and *E. coli*). Bacterial biofilms are now known to play an important role in a range of chronic infections. Being crucial for the organism's survival, PGIs metabolizing sugars may be treated as potential targets for therapeutic control of bacterial infections.

This report describes preliminary results of the isolation, partial purification and characterization of catalytic and physicochemical properties of the phosphoglucose isomerase from *Escherichia coli* ATCC 25922. However, further research is needed to confirm and develop already performed research.

EXPERIMENTAL PROCEDURES

Bacterial strain. The reference strain *E. coli* ATCC 25922 was a kind gift from the Department of Microbiology, Medical University of Lublin. For liquid culture was used a synthetic medium, enrichment broth (Biomed, Poland) supplemented with 1% glucose. Inocula (~ 1.50 x 10^8 CFU/ml, 0.5 McF standard scales) were prepared with fresh cultures of bacteria which were streaked on CM0337 Mueller-Hinton Agar (Oxoid, England) petri plates with sterile 0.9% NaCl. Cultures were grown at 37°C and aerated with constant shaking. In our preliminary experiments the growth of *E. coli* on different carbon sources (glucose, maltose and glycerol) at final concentration 1% was measured. After inoculation the growth of bacteria were observed in nephelometer Phoenix Spec (Becton-Dickinson, USA).

Reagents and materials. All reagents were analytical quality grade and unless stated otherwise, supplied by Sigma Chemical Co., Fluka Chemie or ICN Biomedicals INC. Blue Dextran 2000 and Sephadex G-200 were purchased from Pharmacia Fine Chemicals AB, Sweden. Bio-Rad protein reagent was obtained from Bio-Rad Laboratories. DEAE-cellulose (DE-52 cellulose) was purchased from Whatman Ltd, England. N-Brom-succinimid (NBS) was obtained from Schuchardt, München, Germany. Diaflo YM-30 (cutoff, 30 kDa) ultrafiltration membranes were purchased from Amicon, Inc., USA.

Assay of PGI activity. The catalytic activity of phosphoglucose isomerase was evaluated by a coupled assay or direct assay for fructose-6P. In the coupled assay, the activity of PGI was estimated by the glucose-6P formation using dehydrogenase glucose-6P (EC 1.1.1.49) as auxiliary enzyme. The standard reaction mixture in 1 mL solution contained: 50 mM TRIS/HCl buffer, pH 7.65, 1 mM D-fructose-6P, 5 mM $MgCl_2$, 0.5 mM $NADP^+$ and 1 U of dehydrogenase glucose-6P. The reaction mixture was incubated at room temperature (RT) for 3 min and the reaction was initiated by the addition of the PGI preparations. The reaction was followed by 3 min. The activity was measured at 340 nm using spectrophotometer Genesys^{TM}6 (Thermo Electron Corporation, USA) by monitoring the NADPH formation from $NADP^+$. One unit (U) of PGI activity was defined as the amount of enzyme catalyzing 1 μmol of $NADP^+$ reduction per min under above conditions.

Phosphoglucose isomerase activity was also measured by the decreased amount of fructose-6P using colorimetric technique of Lever [13]. To our knowledge, we were the first to use p-hydroxybenzoic acid hydrazide (PAHBAH) as a reagent for the direct determination of PGI activity. The standard direct assay mixture in 0.5 mL contained: 10 mM TRIS/HCl buffer, pH 7.65, 0.15 mM D-fructose-6P, 1 mM $MgCl_2$ and various amounts of PGI. After various times of incubation at 37°C, 1.5 mL of *ex tempore* mixing liquids of 5% PAHBAH in 0.5 M HCl and 0.5 M NaOH (1: 4 v/v) were added. Next, the samples were heated at 100°C for 10 min. After cooling on ice water bath, the absorbance was measured at 410 nm using spectrophotometer Genesys™6. One unit (U) of PGI activity was defined as the amount required for the decrease of 1 μmol fructose-6P per min under above conditions. The specific activity in both methods was defined as units of enzyme activity per mg of protein. The choice of the assay to determine activity of the characterized enzyme was frequently caused by the kind of the performed experiment.

Partial purification of PGI activity. *E. coli* ATCC 25922 was grown in 1 L Ehrlemayer flasks containing 0.25 L medium for 24 hours. The bacteria cells were collected by centrifugation. Next, *E. coli* cells were washed twice in chilled 50 mM TRIS/HCl buffer, pH 7.65 and resuspended in the same chilled buffer. The suspensions of cells (2.0 mL) were frozen at -18°C until used. All purification steps were done at 4°C unless otherwise specified.

Step 1: preparation of crude extract. The suspension of cells (4 mL) was resuspended in 20 mL 50 mM TRIS/HCl buffer, pH 7.65 containing 2 mM D,L-dithiothreitol (DTT) and 5 mM $MgCl_2$. The cells were lysed by the addition of lysozyme to a final concentration 0.4 mg/mL and the viscosity of the solution was reduced by the addition of DNase I to an activity of 1 U/mL. The suspension was shaken in water bath (amplitude 6, 50 cpm Elpan, Poland,) at 37°C for 1 h. The treated cells were ruptured by sonication with apparatus Unipan UD 20 (IPPT, 180 W, 22 kHz, TECHPAN, Poland), using 3 cycles of 3 min pulses followed by a 2 min rest on ice water bath. The lysate was centrifuged at 3314 x g for 30 min at 4°C (centrifuge type 3-16K, rotor 12159, Sigma, USA) and the supernatant was collected.

Step 2: ammonium sulfate fractionation. The liquid, which was designated "crude extract", was placed in an ice bath on a magnetic stirrer and solid $(NH_4)_2SO_4$ was added, slowly to 45% saturation and the precipitate appeared was removed by a conventional centrifugation. The supernatant liquid was then brought to 70% saturation by the slow and continual addition of solid $(NH_4)_2SO_4$. The precipitate was collected by centrifugation (type 3-16K, rotor 12159, 1753 x g) dissolved in a minimal volume of 25 mM TRIS/HCl buffer pH 7.65 with 2 mM DTT (Buffer A) and dialyzed overnight against the same buffer (AS-fraction).

Step 3: DEAE-cellulose chromatography. The AS-fraction was applied to a column of DEAE cellulose (2.6 x 10 cm) that had been equilibrated with 0.2 M TRIS/HCl buffer pH 7.65 and Buffer A. The column was washed thoroughly with the same Buffer A (250 mL) and the wash, which did not contain activity of PGI, was discarded. PGI was eluted from the column with a 0.05–0.5 M linear gradient of NaCl in Buffer A (total volume of gradient 300 mL). Most active fractions were pooled and desalted immediately by ultrafiltration on the Amicon filtration apparatus (Millipore, Billarica, MA) using a YM-30 membrane and kept frozen in Buffer A.

Other methods. Protein concentration was measured with Bio-Rad protein reagent using bovine serum albumin (BSA) as standard [2] or spectrophotometrically at 280 nm. In the pool of protein concentration (mg/mL) was calculated by formula: 1.55 x A_{280nm} – 0.76 x A_{260nm}. Native polyacrylamide gel electrophoresis (PAGE) was performed on 6% gel in tubes on apparatus 175 Tube Cell (Bio-Rad, Austria). PAGE was run according to the method described by Davis [4] in TRIS/HCl continuous buffer system at pH 8.3. Gels were stained with amido black 10B in 7% acetic acid to detect protein at RT for 30 min. The destaining was done by electrophoresis (10 mA per tube) in 7% acetic acid. The solution for active PGI stainings was always freshly prepared and comprised of sources almost the same as mixture for coupled assay activity but it included 0.4 mg nitroblue tetrazolium chloride (NBT) and 0.04 mg phenazine methosulfate (PMS) per 1 mL, respectively. The native gels were incubated in the staining solution immediately after electrophoresis in the darkness at 37°C for about 30 min. The staining was stopped by rinsing the gels in deionized water. Purple bands showed the presence of activity PGI.

The PGI activity was tested over pH range 7.4–8.0 by using coupled and direct activity assays. We tested the following buffers: glycyl-glycine, pH 7.4; (N-[2-Hydroxy-ethyl]piperazine-N'-[2-ethanesulfonic acid]) (HEPES), pH 7.6; morpholinopropane sulfonic acid (MOPS), pH 7.6; N-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid) (TES), pH 7.6; N-tris-[hydroxymethyl]-methyl glycine (TRICINE), pH 7.6; tris-(hydroxymethyl)amino-methan (TRIS)/HCl, pH 7.65; triethanolamine (TEA)/NaOH, pH 7.7; N-tris(hydroxy-methyl)methyl-3-aminopropane sulfonic acid (TAPS), pH 7.8 and TRIS/HCl, pH 8.0.

For the determination of the effect of metal cations (5 mM) or thiols (10 mM) on PGI activity, the enzyme was dialyzed against 25 mM HEPES buffer, pH 7.6. In these experiments the catalytic activity was determined by direct assay. \

Dihydroxyacetone phosphate (DHAP), D-fructose, D-fructose-1P, D-fructose-6P, D-fructose-1,6P_2 and tagatose-6P (2 mM) were tested as possible substrates with coupled activity assay. Kinetic parameters of PGI were determined with coupled assay by varying the concentration of D-fructose-6P (0.5–15 mM).

The typical PGI inhibitors such as D-erythrose-4P, D-fructose-1P, D-fructose-1,6P_2, D-mannose-6P and 6-phospho-D-gluconate (6-phosphogluconate) (2 mM) were tested as potential inhibitors of phosphoglucose isomerase from *E. coli* ATCC 25922. The mM concentration range of phosphates sugar is usually used in the study of various types PGIs [19, 20]. To examine the effect of other inhibitors (chemical modyfing aa and the chelators, Table 2) on PGI activity, the assay was preceded by the enzyme dialysis against 25 mM HEPES buffer, pH 7.6. The tested inhibitors concentrations are usually used in the biochemical research of the enzymes. In both experiments the D-fructose-6P concentration was 1 mM and the activity was determined by a coupled assay. Inhibitors were added directly to the assay mixture. No effect of the tested inhibitors on the auxiliary enzyme was observed at the given concentration.

The molecular weight of the native phosphoglucose isomerase (isoenzyme I) was estimated by gel filtration on Sephadex G-200 (2.6 x 22 cm). To determine the void volume (V_o), the sample of Blue Dextran 2000 was used. Molecular weight standards included: apoferritin (443 kDa), alcohol

dehydrogenase (150 kDa), BSA (66 kDa) and cytochrome C (13 kDa). The calibration curve was prepared by plotting V_e/V_o values versus the logarithms of molecular weights of protein standards. The ratio of PGI V_e to the V_o was compared with corresponding ratios obtained for the standard proteins in order to determine the molecular weight of PGI by linear regression. Protein standards were assayed by measuring the absorbance at 280 nm. The elution of PGI from Sephadex G-200 column was monitored by the enzymatic activity assessment.

RESULTS

ISOLATION AND PARTIAL PURIFICATION OF THE *E. COLI* ATCC 25922 PGI

In our preliminary experiments, we determined the influence of different carbon sources (glucose, maltose and glycerol) on reference strain *E. coli* ATCC 25922 growth. The growth of characterized cells is almost equivalent to 1% glucose and 1% maltose. Little growth was found for cells cultured on 1% glycerol (data not shown). We detected a difference in enzymatic activity of PGI, although the characterized cells growth on glucose and maltose was almost identical. We estimated a two-fold increase of PGI catalytic activity for *E. coli* ATCC 25922 growing on glucose (using fructose-6P as substrate) comparing with those growing on maltose (data not shown). PGI was purified from *E. coli* ATCC 25922 cell-free extract using conventional techniques (Table 1).

Table 1. Purification of PGI from *E. coli* ATCC 25922 grown on 1% glucose

Purification step	Total activity[X] [U]	Total protein [mg]	Specific activity [U/mg]	Purification [fold]	Yield [%]
Crude extract	6.80	15.300	0.444	-	100
AS fraction	4.18	6.907	0.605	1.4	61
DEAE-cellulose (peak I)	1.13	0.999	1.131	2.5	17
DEAE-cellulose (peak II)	0.55	1.610	0.342	0.8	8

[X] – Enzymatic activity of PGI was measured by the coupled assay

After ion-exchange chromatography on DEAE-cellulose and determination of AS fraction, two peaks of PGI activity, termed I and II according to their elution sequence, were detected (Fig. 1). They were pooled separately.

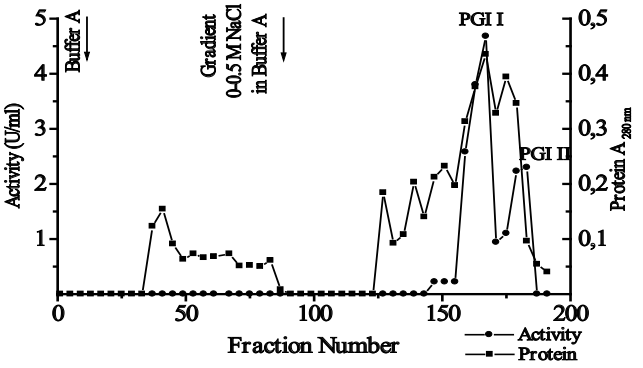

Fig. 1. Chromatography of AS fraction from *E. coli* ATCC 25922 on DEAE-cellulose column. Protein fractionation was recorded at 280 nm. The direct assay method was used to measure activity of PGI

PAGE of the PGI preparation obtained from chromatography on DEAE-celulose (peak I and peak II as one pool) showed 5 protein bands of different intensities (Fig. 2 A). Zymography of the preparation confirmed that *E. coli* ATCC 25922 has two PGI isoenzymes. The PGI isoenzymes exhibited different activities in the same method of activity detection *in situ* on the gel. A minor isoenzyme of PGI migrated more slowly to the anode than the major one and must therefore differ in size or charge (Fig. 2 B).

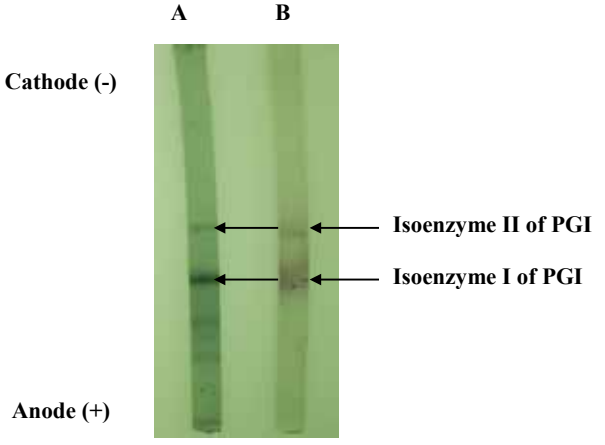

Fig. 2. PAGE analysis of pooled fractions of PGI from E. coli ATCC 25922 from DEAE-cellulose column. Lane A- staining with amido black for protein, Lane B- activity staining *in situ*

SOME PROPERTIES OF PGI (ISOENZYME I) FROM *E. COLI* ATCC 25922

The influence of buffers on enzyme activity. The experiment of activity evaluation using PAHBAH, showed enzyme activity only in TRIS/HCl buffer, pH 7.65 and glycyl-glycine buffer, pH 7.4 with 100% and 16% of relative activity, respectively. Fig. 3 ilustrates the dependence of PGI activity on buffers after determination by the coupled assay. When we determined the enzyme activity by this assay, the kind of buffer was less essential. However, the catalytic activity of PGI was higher in TRIS/HCl buffer, pH 7.65 and TRIS/HCl buffer, pH 8.0 with 100% and 98% relative activity, respectively.

The effect of metal ions on enzyme activity. The metal cations requirement for enzymatic activity was determined by the direct assay to avoid complications due to coupling enzyme. PGI from *E. coli* ATCC 25922 showed activity without addition of metal cations, but 5 mM Mg(II) stimulated activity. Then we tested the influence of Mg(II) concentration (1–15 mM) on the activity of the PGI and we observed a decrease of enzymatic activity at higher Mg(II) concentrations (data not shown). We suggest that unspecific inhibition of *E. coli* ATCC 25922 PGI resulted from the increased concentration of chloride anions. 5 mM concentration of Ca(II) and Co(II) significantly inhibited the activity of *E. coli* ATCC 25922 PGI, by 65% and 74% respectively. Other tested cations: Mn(II), Ni(II), Cd(II), Fe(II), Zn(II) and Fe(III) at this concentration completely inhibited (100% inhibition) the activity of phosphoglucose isomerase. Based on our results, we decided to use 5 mM Mg^{2+} during the direct assay of the PGI activity.

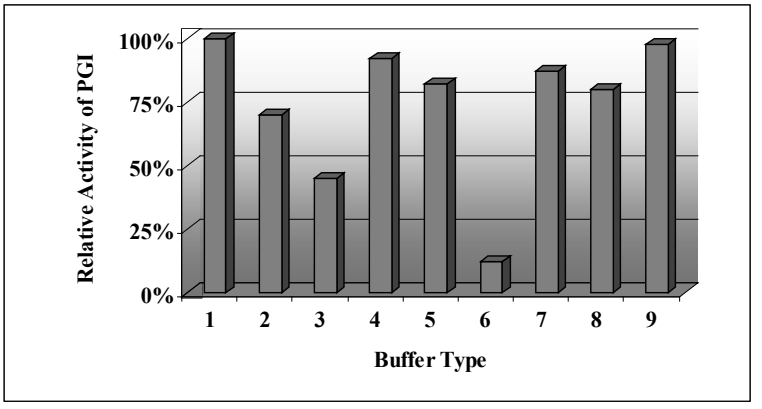

Fig. 3. Influence of PGI activity from *E. coli* ATCC 25922 on buffers.
The concentration of buffers was 50 mM. 1 – TRIS/HCl, pH 7.65; 2 – Glycyl-glycine, pH 7.4; 3 – HEPES, pH 7.6; 4 – MOPS, pH 7.6; 5 – TES, pH 7.6; 6 – TRICINE, pH 7.6; 7 – TEA/NaOH, pH 7.6; 8 – TAPS, pH 7.8; 9 – TRIS/HCl, pH 8.0

The effect of thiols on enzyme activity. D,L-dithioerythritol and D,L-dithiothreitol at constant concentrations (10 mM) activated *E. coli* ATCC 25922 PGI with 165%

and 131% relative activity compared to the control without thiols, respectively. Glutathione (reduced), D,L-cysteine and mercaptoethanol appeared to be inhibitors (data not shown). However, only mercaptoethanol completely inhibited (100%) the activity of characterized PGI at tested concentrations.

Substrate specificities and kinetic properties. Several phosphoketoses were tested as possible substrates for PGI. The activities were detected using D-fructose-6P and D-fructose-1,6P_2 with 100% and 5.5% of relative activity, respectively. The PGI activity detected using D-fructose-1,6P_2 as substrate probably resulted from impurity the agent by D-fructose-6P. The phosphoglucose isomerase from *E. coli* ATCC 25922 appeared to exhibit a high degree of D-fructose-6P substrate specificity. Other tested sugar phosphates such as DHAP, D-fructose-1P and tagatose-6P were inactive as substrates. This PGI was unable to isomerize non-phosphorylated sugar like D-fructose. The characterized PGI displayed Michaelis-Menten kinetics. The K_m value for fructose-6P and the V_{max} value were calculated from Lineweaver-Burk plot. At RT, K_m of PGI was estimated to be 1 mM for fructose-6P with the V_{max} of 0.177 μM/min (data not shown).

The effect of inhibitors on enzyme activity. The effect of various inhibitors on the PGI activity from *E. coli* ATCC 25922 was shown in Table 2. The tested compounds can be divided into three groups. The first group consists of phosphorylated compounds as D-erythrose-4P, D-fructose-1P, D-fructose-1,6P_2, D-mannose-6P and 6-phosphogluconate. The second group is composed of reagents for the aa modification such as p-chloro mercuric benzoic sodium salt (pCMB), N-ethylmaleimide (NEM), NBS, succinic anhydride and N-acetyloimidazole. Finally, the third group comprises the following chelators: EDTA and 1,10-phenanthroline. From the first group, phosphorylated agents, only two caused significant inhibition. The residual activities of 56% and 39% were monitored in the presence of D-mannose-6P and 6-phosphogluconate (at 2 mM), respectively. Especially, interesting was the lack of inhibition of PGI from *E. coli* ATCC 25922 by D-erythrose-4P. The inhibitors: pCMB (making mercaptide linkage) and NEM (alkylating compound) inhibited the enzyme activity indicated the participation of –SH group of cysteine (Cys) in enzymic catalysis. NBS at 10 mM concentration, which is a specific reagent for tryptophan (Trp) modification in proteins, did inhibit a half activity of PGI. Probably, the active site of phosphoglucose isomerase contains lysine (Lys) and tyrosine (Tyr), because this prokaryotic enzyme was inhibited in addition of succinic anhydride and N-acetyloimidazole, respectively. The fact that the tested chelators (EDTA and 1,10-phenanthroline at mM concentration) different in their structures did not significantly affected the PGI activity from *E. coli* ATCC 25922 indicated that the enzyme is not a metallprotein.

Table 2. Effect of various inhibitors on *E. coli* ATCC 25922 phosphoglucose isomerase

Inhibitor	Concentration of the inhibitor [mM]	Inhibition [%]
K	-	0
Erythrose-4P	2	0
Fructose-1P	2	5
Fructose-1,6P_2	2	20
Mannose-6P	2	44
6-phosphogluconate	2	61
pCMB	1	8
	5	45
NEM	5	73
	10	100
NBS	5	29
	10	50
Succinic anhydride	5	45
	10	92
N-acetyloimidazole	5	36
	10	38
EDTA	5	26
	10	33
1,10-phenanthroline	5	2
	10	8

M o l e c u l a r w e i g h t . Based on the gel filtration on Sephadex G-200, the active PGI I from *E. coli* ATCC 25922 had a molecular weight about 120 kDa.

DISCUSSION

In this pilot study we described the procedure of partial purification and characterized the kinetic and physicochemical properties of phosphoglucose isomerase involved in the reversible isomerization of glucose-6P to fructose-6P in *E. coli* ATCC 25922. In this research, we performed the evidence that *E. coli* ATCC 25922 cells contain two cytosolic isoenzymes of PGI. This result was

in agreement with preliminary reports of Schreyer and Böck [16] and Szynal [18] for PGIs which were isolated from *E. coli* K-10 and *E. coli* ATCC 35218 strains, respectively.

The comparison of some properties of PGI isoenzyme from *E. coli* ATCC 25922 with isoenzyme I from other *E. coli* strains like K-10 and ATCC 35218 indicates similarities. An apparent molecular weight of PGI isoenzyme I from *E. coli* K-10 [16] and *E. coli* ATCC 25922 determined by gel chromatography on Sephadex G-200 was the same 120 kDa. Although the molecular weight of PGI isoenzyme I from *E. coli* ATCC 35218 was low and its value was about 100 kDa [18]. Isoenzymes I from *E. coli* K-10 [16] and ATCC 35218 [18] strains have a homodimeric structure. We did not perform SDS-PAGE for the determination of subunit structure of PGI isoenzyme I from *E. coli* ATCC 25922, because our preparation of the enzyme was partially purified. Nevertheless, the characterized enzyme is probably homodimeric, because almost all PGIs have been previously described as homodimers of about 100–130 kDa [15].

The classical PGI from different sources were active over a broad pH range 7.5–10.0 [20]. Isoenzyme I from *E. coli* K-10 exhibited the maximum activity at pH 8.0 [16]. We determined routinely the activity of PGI from *E. coli ATCC* 25922 at pH 7.65 of TRIS/HCl buffer, but we observed a high activity at pH 8.0 of the same buffer.

The activity of PGI isoenzyme I did not depend on metal cofactors, like PGIs from other sources [14]. However, after determination of enzyme activity by direct assay, we observed that 5 mM concentration of Mg^{2+} slightly stimulated the activity of phosphoglucose isomerase isoenzyme I from *E. coli* ATCC 25922.

In our studies, a direct assay indicates the high activity of the PGI isoenzyme I from *E. coli* ATCC 25922 in the presence of D,L-dithioerythritol and D,L-dithiothreitol (10 mM). These thiols were interfered in many analytical procedures. In the coomassie protein assay, D,L-dithioerythritol is obligatory used at a lower concentration than D,L-dithiothreitol. Despite this fact, we routinely used 2 mM D,L-dithiothreitol as the protective agent of Cys –SH groups of PGI isoenzyme I from *E. coli* ATCC 25922. The present of this aa in the characterized enzyme active site is highly probable. There are two pieces of evidence for that. Firstly, the catalytic activity of PGI was significantly inhibited by non- or organic preparation of Hg^{2+} and alkylating compound NEM. Secondly, Cys are absent in the dehydrogenase glucose-6P from *Leuconostoc mesenteroides* [11], which was a coupling enzyme in the activity assay of PGI.

Phosphoglucose isomerase from *E. coli* ATCC 25922 exhibited a high specificity for D-fructose-6P substrate alike PGIs from other sources. The respective K_m value for this substrate was about 5-fold higher for the PGI from *E. coli* ATCC 25922 compared with those preliminary reported for the other *E. coli* PGIs [16, 18]. This difference is difficult to interpret. However, we evaluated the kinetic parameter by using partial purificated of enzyme (isoenzyme).

D-Erythrose-4P affected the PGI isoenzyme from *E. coli* ATCC 25922 different from other PGIs. This compound is a strong inhibitor for PGI from *Dictyostelium discoideum* [19] and for PGI/PGM from *Aeropyrum pernix* [9] with K_i values of 3.8 μM and 35±4 μM, respectively. D-erythrose-4P could hardly inhibit cupin PGI from *Pyrococcus furiosus* (K_i value of 3.9 mM) [8]. Meanwhile, this intermediate of pentose-phosphate pathway did not show (at 2 mM concentration) the inhibitory effect on PGI from *E. coli* ATCC 25922. The activity of phosphoglucose isomerase was not significantly

inhibited by other classical PGIs inhibitors such as D-fructose-1P and D-fructose-1,6P_2. It was only inhibited by D-mannose-6P and 6-phosphogluconate (Table 2).

The discovery of present aa like Cys, Trp, Lys and Tyr in PGI from *E. coli* ATCC 25922 is consistent with the results reported previously for PGI from *E. coli* ATCC 35218 [18]. The occurence of Lys in the PGIs active sites from other sources was postulated by Noltmann [15].

Many binding agents (chelators) of metal ions like EDTA, 1,10-phenantroline, 2,2'-bipyridyl, azide and TRIS are known. Metalloenzymes, e.g. ribitol dehydrogenase (Zn^{2+} as ion metal biding in the active site) from *Mycobacterium smegmatis* are sensitive towards these compounds [17]. PGI isoenzyme I from *E. coli* ATCC 25922 was more sensitive to EDTA than 1,10-phenanthroline at the same concentration (Table 2). Probably, this phenomenon results from a higher ability of EDTA to chelate Mg^{2+} cations existing in the assay mixture as compared with 1,10-phenanthroline. Other chelator – TRIS, did not inhibit the activity of the studied isoenzyme. In accordance with our data, the PGI isoenzyme I from *E. coli* ATCC 25922 does not have essential metal ion in the active site.

It is necessary to carry out more studies concerning isoenzymes of PGI from *E. coli* ATCC 25922. The most important is to answer the question about the physiological role of the existence of two phosphoglucose isomerase forms.

CONCLUSIONS

The existence of two different forms of PGIs (isoenzymes) in the reference strain of *E. coli* ATCC 25922 was reported. A similarity between the properties of the major phosphoglucose isomerase isoenzyme *E. coli* ATCC 25922 and isoenzymes isolated from other *E. coli* strains like K10 and ATCC 35218 was discovered. Our preliminary results indicated that PGI from reference *E. coli* ATCC 25922 strain may be included to PGI superfamily.

REFERENCES

1. Bachman B.J.: Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev., 47, 180, 1983.
2. Bradford M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72, 248, 1976.
3. Davies C. et al.: The structure of human phosphoglucose isomerase complexed with a transition-state analoque. Acta Crystallogr. Sect. D Biol. Crystallogr., 59, 1111, 2003.
4. Davis B.: Disc electrophoresis. II Method and application to human serum proteins. Ann N.Y. Acad. Sci., 121, 404, 1964.
5. Froman B.E. et al.: Isolation and characterization of the phosphoglucose isomerase gene from *Escherichia coli*. Mol. Gen. Genet., 217, 126, 1989.
6. Fraenkel D.G., Levisohn S.R.: Glucose and gluconate metabolism in an *Escherichia coli* mutant lacking phosphoglucose isomerase. J. Bacteriol., 93, 1571, 1967.
7. Friedberg I.: Localization of phosphoglucose isomerase in *Escherichia coli* and its relation to the induction of the hexose phosphate transport system. J. Bacteriol., 112, 1201, 1972.

8. Hansen T. et al.: Cupin-type phosphoglucose isomeraes (Cupin-PGIs) constitute a novel metal-dependent PGI family representing a convergent line of PGI evolution. J. Bacteriol., 187, 1621, 2005.
9. Hansen T. et al.: Bifunctional phosphoglucose/phosphomannose isomerase from the Archaea *Aeropyrum pernix* and *Thermoplasma acidophilum* constitute a novel enzyme family within the phosphoglucose isomerase superfamily. J. Biol. Chem., 279, 2262, 2004.
10. Hua Q. et al.: Response of the central metabolism in *Escherichia coli* phosphoglucose isomerase and glucose-6-phosphate dehydrogenase knockouts. J. Bacteriol., 185, 7053, 2003.
11. Ishaque A. et al.: On the absence of cysteine in glucose 6-phosphate dehydrogenase from *Leuconostoc mesenteroides*. Biochem. Biophys. Res. Commun., 59, 894, 1974.
12. Jeffery C.J.: Moonlighting proteins. Trends Biochem. Sci., 24, 8, 1999.
13. Lever M.: A new reaction for colorimetric determination of carbohydrates. Anal. Biochem., 47, 273, 1972.
14. Mathur D., Garg L.C.: Functional phosphoglucose isomerase from *Mycobacterium tuberculosis* H37Rv: rapid purification with high yield and purity. Protein Expr. Purif., 52, 373, 2007.
15. Noltmann E.: Aldose-ketose isomerases. [In] Boyer P., editor. The Enzymes, 6, 3rd edn., 271, Academic Press, New York 1972.
16. Schreyer R., Böck A.: Phosphoglucose isomerase from *Escherichia coli* K10: purification, properties and formation under aerobic and anaerobic condition. Arch. Microbiol., 127, 289, 1980.
17. Swatko M., Szumiło T.: Isolation and partial characterization of a novel ribitol dehydrogenase from mycobacteria. Annales UMCS, Sect. DDD, XII/XIII, 169, 1999/2000.
18. Szynal K.: Purification and evaluation of biological role of phosphoglucomutase (PGM) and phosphoglucoisomerase (PGI) from bacteria cells of *Escherichia coli*. Master's thesis. Medical University of Lublin, 2008.
19. Thomas A.D.: Partial purification and characterization of glucose-6-phosphate isomerase from *Dictyostelium discoideum*. J. Gen. Microbiol., 24, 40, 1981.
20. Verhees C. et al.: The phosphoglucose isomerase from hyperthermophilic archaeon *Pyrococcus furiosus* is a unique glycolytic enzyme that belongs to the cupin superfamily. J. Biol. Chem., 276, 40926, 2001.
21. Weeden N.F.: Genetic and biochemical implications of the endosymbiotic origin of the chloroplast. J. Mol. Evol., 17, 133, 1981.

SUMMARY

Phosphoglucose isomerase (EC 5.3.1.9; PGI) is an enzyme participating in the metabolism of the sugar and carrying the reversible isomerization of glucose-6-phosphate and D-fructose-6-phosphate. It plays a key role in the process of the extracellular polysaccharides (EPS) synthesis *in vivo* – creating the bacterial biofilm. The complete understanding of PGI catalysis and inhibition mechanisms may results in a discovery of new PGI inhibitors – antibacterial drugs.

In this pilot study we described the procedure of partial purification of *E. coli* ATCC 25922 PGI from cytosolic fraction. The activity of the enzymes was measured by the conversion of D-fructose-6P into D-glucose-6P. Two forms (isoenzymes I and II) of this enzyme were separated. Some properties of the dominant form (isoenzyme I) of PGI were studied. The K_m constant for D-fructose-6P was

1 mM. The enzyme had optimum activity in TRIS/HCl buffer, pH 7.65. Phosphoglucose isomerase had molecular weight 120 kDa. Our results indicated that amino acids – cysteine, tryptophan, tyrosine and lysine residues were to be presented in the active site of PGI from *E. coli* ATCC 25922. The lack of the activity inhibition of the characterized enzyme by chelators as EDTA, 1,10-phenantroline and TRIS indicated that the PGI is not a metallprotein. A large similarity between the properties of phosphoglucose isomerases isolated from *E. coli* ATCC 25922 and other *E. coli* strains such as K10 and ATCC 35218 was discovered.

Keywords: phosphoglucose isomerase; isoenzymes, PGI inhibitors, *Escherichia coli* ATCC 25922, biofilm

STRESZCZENIE

Fosfoglukoizomeraza (EC 5.3.9.1; PGI) jest enzymem uczestniczącym w metabolizmie cukrów, katalizującym odwracalną izomeryzację D-glukozo-6-fosforanu i fruktozo-6-fosforanu. Odgrywa kluczową rolę w procesie syntezy *in vivo* zewnątrzkomórkowych polisacharydów (EPS) tworzących bakteryjny biofilm. Pełne zrozumienie mechanizmów katalizy i inhibicji PGI może skutkować odkryciem nowych inhibitorów PGI – leków przeciwbakteryjnych. W pracy opisano procedurę częściowego oczyszczania PGI z frakcji cytozolowej *E. coli* ATCC 25922. Aktywność enzymu mierzono w kierunku od D-fruktozo-6P do D-glukozo-6P. Rozdzielono dwie formy (izoenzymy I i II) tego enzymu. Badano wybrane właściwości formy dominującej (izoenzymu I) PGI. Stała Michaelisa dla D-fruktozo-6P wynosiła 1 mM. Enzym miał optymalną aktywność w buforze TRIS/HCl, pH 7.65. Ciężar cząsteczkowy fosfoglukoizomerazy wynosił 120 kDa. Nasze wyniki wskazują na obecność aminokwasów: cysteiny, tryptofanu, tyrozyny i lizyny w centrum aktywnym PGI z *E. coli* ATCC 25922. Brak hamowania aktywności charakteryzowanego enzymu przez chelatory, takie jak: EDTA, 1,10-fenantrolinę i TRIS, dowodzi, że PGI nie jest metaloproteiną. Zaobserwowano duże podobieństwo właściwości pomiędzy izomerazami fosfoglukozowymi izolowanymi z *E. coli* ATCC 25922 i z innych szczepów *E. coli*, takich jak: K10 i ATCC 35218.

Słowa kluczowe: izomeraza fosfoglukozowa, izoenzymy, inhibitory PGI, *Escherichia coli* ATCC 25922, biofilm