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α-Phosphoglucomutase from Escherichia coli ATCC 25922 – pilot studies

α-Fosfoglukomutaza z Escherichia coli ATCC 25922 – badania pilotażowe

INTRODUCTION

α-Phosphoglucomutase (alpha-D-glucose-1,6-phosphomutase; PGM; EC 5.4.2.2, formerly EC 2.7.5.1) is an enzyme that catalyzes reversible isomerization of α-D-glucose-1-phosphate (Cori ester, α-D-glucose-1P) to α-D-glucose-6-phosphate (Robison-Embden ester, α-D-glucose-6P). In α-PGM catalysis, the active enzyme is phosphorylated at conserved serine residue in the catalytic site and binds bivalent metal ion, generally one Mg(II). Initially, phosphate group is transferred from the phosphoserine of enzyme to substrate α-D-glucose-1P. It creates the biphosphorylated intermediate (α-D-glucose-1,6-bisphosphate, α-D-glucose-1,6P_2). The intermediate must become reoriented and a phosphate group must be transferred back to the protein, regenerating the active form of enzyme. Finally, the product of PGM reaction α-D-glucose-6P is released [4, 12, 18].

α-PGM is a widely distributed and evolutionary conserved enzyme, found in organisms ranging from Escherichia coli to humans [16]. The formation of bacterial biofilm is connected with activity of α-phosphoglucomutase. For example, it is postulated the presence of pcgA (yhxB), an important gene encoding α-PGM in Bacillus subtilis strains N38, OG-N-1 and OG-G-1 isolated from patients with persistent periapical periodontitis. These strains are responsible for production a large amount of exopolysaccharides, which are compounds of extracellular polymeric substances (EPS) of bacterial biofilm [21]. PGM deficiency is associated with a reduced virulence of various pathogen bacteria e.g. Streptococcus pneumoniae [9] and Streptococcus iniae [5].

E. coli α-PGM mutants are partially blocked in metabolism of glucose-1P when grown on galactose or maltose. These strains grow poorly as pink colonies on MacConkey galactose plates. They stain blue with iodine when grown in the addition galactose [1, 14].

The PGM from E. coli has been purified and characterized from various strains: ATCC 26 [11], ATCC 35218 [8, 20] and few clinical isolates [20].

The aim of the present work was to describe the isolation and some important biochemical, physical and kinetic properties of α-phosphoglucomutase from reference strain ATCC 25922 of E. coli.

EXPERIMENTAL PROCEDURES

Bacterial strain. The reference strain E. coli ATCC 25922 was a kind contribution from the Department of Microbiology, Medical University in Lublin. For liquid culture a synthetic medium, enrichment broth (Biomed, Poland) supplemented with 1% maltose was used. 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. Liquid cultures were grown at 37°C and aerated with constant shaking.

Reagents and materials. All reagents were of analytical grade and unless stated otherwise, supplied by Sigma Chemical Co., Fluka Chemie or ICN Biomedicals inc. Blue Dextran 2000 and Sephadex G-200 were obtained from Pharmacia Fine Chemicals AB, Sweden. Bio-Rad protein reagent was a product of Bio-Rad Laboratories. Mark12™ wide–range protein standards were purchased from Novex, USA.

Assay of PGM activity. The catalytic activity of phoshoglucomutase was measured by the glucose-6-phosphate formation using dehydrogenase glucose-6P (EC 1.1.1.49) as auxiliary enzyme (coupled assay). The standard reaction mixture was composed according to Szynal [20]. In our experiments, 0.6 mL solution contained: 33.0 mM TRIS/HCl buffer pH 8.0, 0.8 mM α-D-glucose-1P, 0.1 mM α-D-glucose-1,$6P_2$, 5 mM $MgCl_2$, 0.3 mM $NADP^+$ and 1 U of dehydrogenase glucose-6P. The reaction mixture was incubated at room temperature (RT) for 1 min and the assays were started by the addition of the PGM preparations. The reaction was followed by 3-5 min. The activity was measured at 340 nm using spectrophotometer Genesys™6 (Thermo Electron Co., USA). One unit (U) of phosphoglucomutase was defined as the amount of enzyme catalyzing 1 μmol of $NADP^+$ reduction per min under conditions. Control assays in which assay mixtures lacked $NADP^+$, α-D-glucose-1P, or crude extract were also carried out.

Preparation of crude extract of PGM. E. coli ATCC 25922 was grown in 1 L Erlenmayer flasks containing 0.25 L medium for 21 hours at 37°C. The bacteria cells were collected by centrifugation. Next, E. coli cells were washed three times in chilled 50 mM sodium-phosphate buffer, pH 7.4 and resuspended in the same chilled buffer. The suspensions of cells (2.0 mL) were frozen at -18°C until used. For the lysis of bacteria cells, a modification of the composition of lysis buffer described by Szynal was used [20]. The suspension of cells (4 mL) was resuspended in 20 mL phosphate buffer saline (PBS), pH 7.6 containing 1.5 mM D,L-dithiothreitol (DTT), 0.8 mM EDTA, 0.05 mM phenylmethylsulfonyl fluoride (PMSF) and the addition of lysozyme to a final concentration 0.3 mg/mL. The lysis of bacteria was performed in the incubator shakers (Innova™42, New Brunswick Scientific, USA; 50 cpm at 37°C for 2 h). The treated cells of E. coli ATCC 25922 were ruptured by sonication with apparatus UPH 100 (24 kHz, Hielscher Ultrasonics, GmbH, Germany) using 3 cycles of 5 min pulses followed by a 2 min rest on ice water bath. The lysate was centrifuged at 3314 x g for 45 min at 4°C (centrifuge type 3-16 K, rotor 12159, Sigma, USA). The supernatant, designated as "crude extract" was collected and then dialysed overnight against 10 mM sodium-potassium phosphate buffer, pH 7.4 with 2.5 mM mercapthoethanol (ME).

Other methods. Protein concentration was measured with Bio-Rad protein reagent using bovine serum albumin as standard [3] or spectrophotometrically at 280 nm.

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 [6] 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 mM per tube) in 7% acetic acid. The solution for active PGM stainings was always freshly prepared and comprised of sources almost 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. The staining was stopped by rinsing the gels in deionized water. Purple band showed the presence of activity PGM.

The PGM activity was tested over pH range 7.1–9.7. We tested the following buffers: imidazole/HCl, pH 7.1; sodium-potassium phosphate buffer, pH 7.4; 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)aminomethan (TRIS)/HCl, pH 7.65, 7.8; 8.0 and glycine/NaOH, pH 9.7.

To examine the effect of metal cations (3.4 mM), of thiols (2 mM) and of compounds making mercaptide linkage and alkylating, the assay was preceded by the enzyme dialysis against 25 mM (N-[2-hydroxy-ethyl]piperazine-N'-[2-ethanesulfonic acid]) (HEPES) buffer, pH 7.6 at 4°C.

The effect of α-D-glucose-1,6P_2 on PGM activity was investigated over a concentration range of 0–1 mM.

α-D-Xylose-1P, α-D-glucuronic-1P, α-D-galactosamine-1P, α-D-N-acetylgalactosamine-1P and α-D-glucose-1P (0.8 mM) were tested as possible substrates. Kinetic parameters of PGM were determined by measuring the initial velocity of the PGM reaction at varying α-D-glucose-1P concentrations (0 to 0.5 mM) keeping α-D-glucose-1,6P_2 constant (0.1 mM).

The effect of potential inhibitors (chemical modifying cysteine, the chelators and the anions) of PGM was tested directly after the addition 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 phoshoglucomutase was estimated by gel filtration on Sephadex G-200 (20 x 2.6 cm). To determine the void volume (V_o), the sample of Blue dextran was used. Molecular weight standards included: aldolase fructose-1,6P_2 from rabbit muscle (158 kDa), BSA (66 kDa), lysozyme from chicken egg white (14.4 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 PGM V_e to the V_o was compared with corresponding ratios obtained for the standard proteins in order to determine the molecular weight by linear regression. Protein standards were assayed by measuring the absorbance at 280 nm. The elution of phosphoglucomutase from Sephadex G-200 column was monitored by the catalytic activity assessment.

The molecular mass of PGM subunit was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in 12% resolving gel. It was performed on apparatus Minipol-2 (Kucharczyk, Poland) followed by staining with Coomassie brillant blue R–250 according to conventional procedure [13]. The standards for SDS-PAGE were phosphorylase b from rabbit muscle (97.4 kDa), BSA (66.3 kDa), glutamic dehydrogenase from bovine liver (55.4 kDa), lactate

dehydrogenase from porcine muscle (36.5 kDa), carbonic anhydrase from bovine erythrocyte (31 kDa), trypsin inhibitor from soybean (21.5 kDa) and lysozyme from chicken egg white (14.4 kDa). The calibration curve was prepared by plotting the logarithms of molecular weights of protein standards vs the distances of all using standards from the line start.

The thermostability of PGM activity over a range of 25-65°C was tested in sealed vials, which were incubated at temperatures, as indicated. The vials were then cooled on ice water bath for 1 min, and the residual activity was tested at RT by using α-D-glucose-1P as substrate in the coupled enzyme assay.

RESULTS

ISOLATION OF THE E. COLI ATCC 25922 PGM

Electropherogram of native preparation PGM after Sephadex G-200 from E. coli ATCC 25922 growing on maltose revealed the presence of two bands (Fig. 1). α-PGM activity was detected as the band which migrated more slowly to anode. The presence of other one was possibly a result of proteolysis of preparation PGM or an artifact of other enzymatic activity.

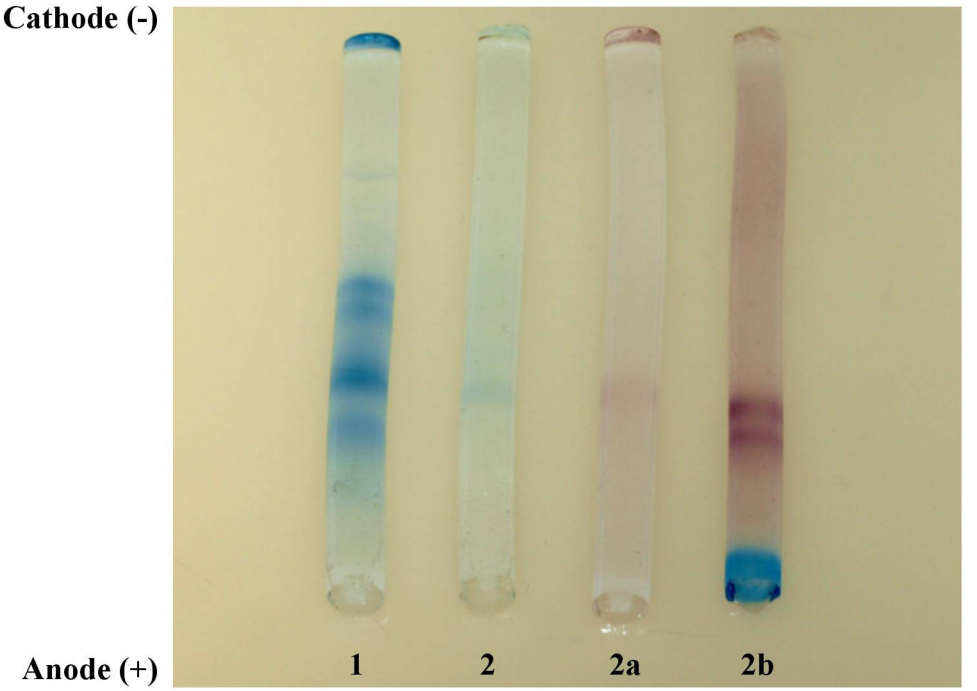

Figure 1. PAGE analysis of preparations of α-PGM from E. coli ATCC 25922.

Lanes: 1 – crude extract; 2 – after Sephadex G-200; 2a – after Sephadex G-200 (staining for 30 min at 37°C); 2b – after Sephadex G-200 (staining several h at RT); Lanes 1, 2 – staining with amido black for protein; Lanes: 2a, 2b – activity staining in situ

SOME PROPERTIES OF α-PGM FROM E. COLI ATCC 25922

The influence of buffers on enzyme activity. The experiment of activity evaluation using various types of buffers (37.5 mM), showed the highest PGM activity only in TRIS/HCl buffer, pH 8.0 (Fig. 2A). When we tested course of the enzyme reaction in this buffer but at different concentrations (6.6-132 mM) we chose 33 mM as optimal (Fig. 2B).

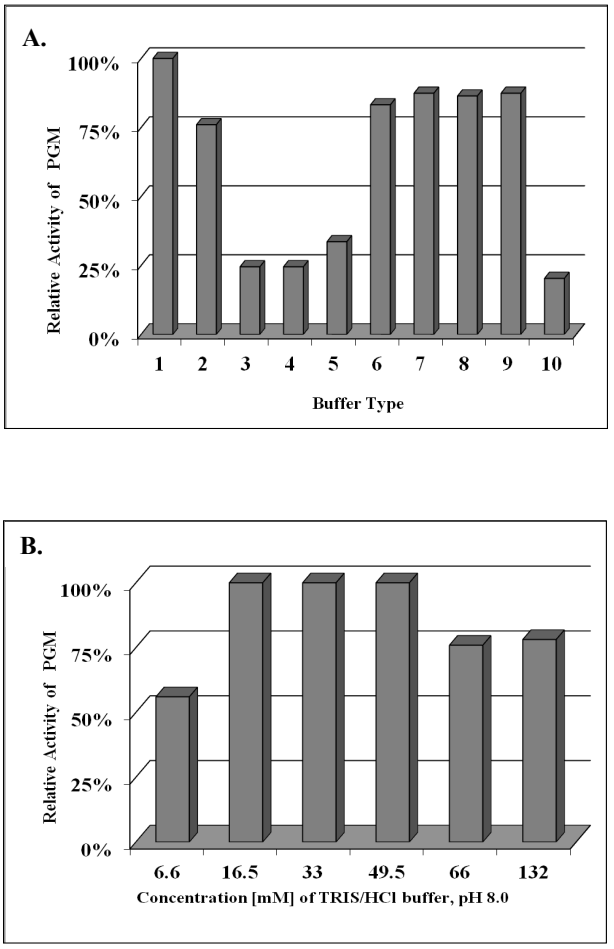

Figure 2 A. The influence of kind buffers (37.5 mM) on α-PGM activity from E. coli ATCC 25922.
1 – TRIS/HCl, pH 8.0; 2 – Imidazole/HCl, pH 7.1; 3 – Sodium-potassium phosphate, pH 7.4;
4 – TES, pH 7.6; 5 – PBS, pH 7.6; 6 – MOPS, pH 7.6; 7 – TRICINE, pH 7.6;
8 – TRIS/HCl, pH 7.65; 9 – TRIS/HCl, pH 7.8; 10 – glycine/NaOH, pH 9.7.
B. Influence of concentration of buffer TRIS/HCl, pH 8.0 on α-PGM activity from E. coli ATCC 25922.

The effect of metal ions on enzyme activity. Tested cations: Mn(II), Cu(II), Ca(II), Co(II) and Cd(II) at 3.4 mM concentration completely inhibited the activity of PGM. Other tested cation Ni(II) at this concentration significantly inhibited enzymatic activity, by 80%. Then we tested the influence of Mg(II) concentration (1-15 mM), we observed 5-6 folds increase

of enzymatic activity in comparison with control without cations. Based on our results, we decided to use 5 mM Mg(II) as chloride salts.

The effect of thiols on enzyme activity. Among sulfhydryl compounds tested, at 2 mM concentration, the activity was slightly stimulated by D,L-dithiotreitol, D,L-dithioerythritol, L-cysteine, reduced gluthathione and mercaptoethanol with 141%, 137%, 129%, 120% and 107% relative activity compared to the control without thiols, respectively. N-acetylocysteine and thioglycolate appeared to have no effect on the activity of characterized PGM at tested concentrations.

The influence of α-D-glucose-1, $6P_2$ on enzyme activity. This compound was not considered to be a substrate or coenzyme but activating cofactor. We observed 9-17 fold stimulation of the activity tested enzyme (Table 1). Interestingly, E. coli ATCC 25922 phosphoglucomutase obtained as described above was able to be responsible for transferring phosphoryl group between the C1(O) and C6(O) position of glucose phosphate in the absence of added glucose-1,$6P_2$. The lack of the requirement of enzyme for this activator may be the effect of the impurities α-D-glucose-1P (substrate). Hypothesis that isolated protein was in phosphorylated form seems to be improbable. Based the highest price of this compound and our results we decided to use 0.1 mM of α-D-glucose-1,$6P_2$ in reaction mixture. It was not replaced by α-D-fructose-1,$6P_2$ (ester of Harden–Young).

Table 1. Effect of various concentration of α-D-glucose-1,$6P_2$ on E. coli ATCC 25922 α-phosphoglucomutase

Concentration of α-D-glucose-1,$6P_2$ [mM]	Activity [U/mL]	Relative activity [%]
0	0.0103	100
0.01	0.0941	914
0.05	0.1529	1484
0.1	0.1716	1666
0.25	0.1825	1772
0.5	0.1787	1735
1	0.1760	1709

Substrate specificities and kinetic properties. Several sugars with orthophosphate moiety at position 1 of carbon atom (accessible in our Department) were tested as possible substrates for PGM. The activities were detected using α-D-glucose-1P, α-D-galactosamine-1P and α-D-N-acetylgalactosamine-1P with 100%, 12% and 6% of relative activity, respectively. Other tested sugar phosphates such as α-D-xylose-1P and α-D-glucuronic-1P were inactive as substrates. The rate dependence on α-D-glucose-1P concentration followed the Michaelis-Menten kinetics. The K_m value for α-D-glucose-1P and the V_{max} value were calculated from Lineweaver-Burk plot. At RT, K_m of PGM was estimated to be 25 x 10^{-5} M/L for α-D-glucose-1P with the V_{max} of 0.0985 μM/min.

The effect of inhibitors on enzyme activity. The effect of various inhibitors on the PGM activity from E. coli ATCC 25922 is shown in Table 2. The tested

compounds can be divided into three groups. The first group consists of making mercaptide bonds (p-chloro mercuric benzoic sodium salt (pCMB) and $HgCl_2$) and alkylating compounds (N-ethylmaleimide (NEM) and iodoacetic acid). The second group is composed of the following chelators: 2,4'-bipyridyl and ethylenediaminetetracetic acid (EDTA). Finally, the third group comprises anions: fluoride and chloride.

Table 2. Effect of various inhibitors on E. coli ATCC 25922 α-phosphoglucomutase

Inhibitor	Concentration of the inhibitor [mM]	Inhibition [%]
Control	-	0
p-chloro mercuric benzoic sodium salt (pCMB)	0.01	45
	0.1	77.5
$HgCl_2$	0.01	100
	0.1	100
N-ethylmaleimide (NEM)	0.1	31
Iodoacetic acid	1	10
2,4'-bipyridyl	5	19.5
Ethylenediaminetetraacetic acid (EDTA)	5	42
Fluoride (F^-)	5	1
	50	13
Chloride (Cl^-)	500	11
	1000	42

According to data from Table 2 we stated the extremely sensitivity of E. coli ATCC 25922 α-PGM to pCMB and $HgCl_2$, which affected thiol groups of cysteine. However, enzymatic activity was not significantly inhibited by alkylation compounds. It suggests that in this case the enzyme is modified chemically at other Cys residue. Chelating agents (2,4'-bipyridyl and EDTA) did not cause of important destabilizing effect on enzyme activity. This result indicated that the enzyme is not metallprotein.

Molecular weight of enzyme. Based on the gel filtration chromatography which was conducted on Sephadex G-200, the active phosphoglucomutase from E. coli ATCC 25922 had a molecular weight about 65 kDa. Preparation of PGM from gel chromatography column contained several bands of different color intensities as visualized by SDS-PAGE. One of these strong bands appeared molecular mass of approximately 60 kDa.

The thermostability of enzyme. As judged on Fig. 3, the course of catalyzed reaction was reasonably below 50°C. The optimum temperature of PGM was found to be around 45°– 50°C.

The stability of α-PGM activity. Twice of freezing and thawing of E. coli ATCC 25922 α-phosphoglucomutase resulted in about 2-fold loss of catalytic activity.

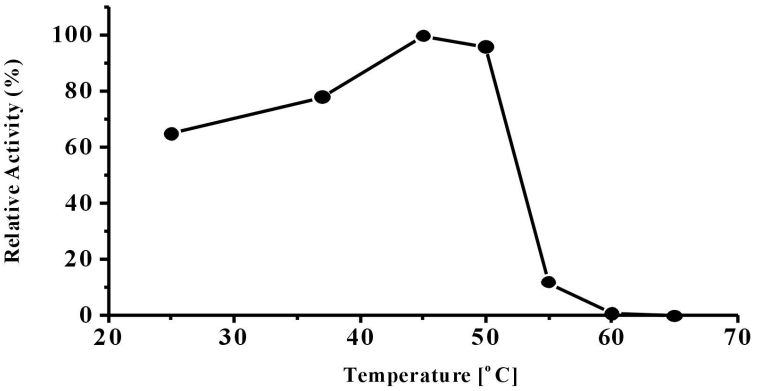

Figure 3. Thermostability of E. coli ATCC 25922 α-PGM

DISCUSSION

The comparison of some properties of α-PGM from E. coli ATCC 25922 from other E. coli strains like ATCC 26 and ATCC 35218 indicates similarities.

An apparent molecular weight of α-phosphoglucomutase from E. coli ATCC 26 [11] and E. coli ATCC 25922 was almost the same: 62–65 kDa and 65 kDa, respectively. However, the molecular weight of α-PGM from E. coli ATCC 35218 was high and its value was about 80 kDa [8].

E. coli ATCC 25922 α-phosphoglucose mutase appears, like E. coli ATCC 26 [11], E. coli ATCC 35218 [8], recombinant Acetobacter xylinum (A. xylinum) [12] and rabbit muscle α-PGMs, to consists one polypeptide chain [4].

The α-PGMs, which exist in reference strains like E. coli ATCC 25922 and E. coli ATCC 35218 had the greatest activities at pH 8.0. It is in contrast to optimal value of pH 9.0 for E. coli ATCC 26 α-phosphoglucose mutase [11]. The difference was perhaps due to different methods of determination of enzymatic activity. For the first strains, the coupling assay was used but for the last one- Bartlett's colorimetric phosphate estimation.

Interestingly, the activity of characterized α-PGM did not depend on Mg(II) cation. However we observed positive response (stimulation of activity) to magnesium chloride addition. E. coli ATCC 25922 α-phosphoglucose mutase was not activated by e.g. Cu(II) cations. α-PGM from E. coli ATCC 35218 had a high level of activity in the presence of 5 mM concentration of cations: especially Mg(II), Mn(II), Fe(II) and Cu(II) [8].

We did not observe the significant activation by sulfhydryl agent cysteine. It was not agreed with results for α-phosphoglucose mutases from E. coli ATCC 26 [11] and from E. coli ATCC 35218 [8].

We did not determine the value of K_m for α-D-glucose-1,6P_2, but we chose 0.1 mM concentration of this compound as optimal. For example, K_m for α-D-glucose-1,6P_2 isolated from the recombinant A. xylinum α-phosphoglucomutase was 0.2 μM [12].

α-phosphoglucomutase from E. coli ATCC 25922 exhibited a high specificity for α-D-glucose-1P alike α-PGMs from other sources (e.g. E. coli ATCC 26 [11] and recombinant

A. xylinum [12]). The respective K_m for range size (10^{-5} M/L) for this substrate was similar to that reported for E. coli ATCC 26 [11]. The studies performed by Dworniczak et al. indicated the wide substrate specificities and the smaller affinity of PGM from E. coli ATCC 35218 to α-D-glucose-1P [8]. Currently, we are not able to verify the presence of β-phosphoglucomutase (beta-D-glucose 1,6-phosphomutase, beta-PGM, EC 5.4.2.6) in cells of reference strain E. coli ATCC 25922. The BRENDA data enzymes showed the sequence of nucleotides for beta-PGM from E. coli O157:H7 EC869 (219 aa, molecular weight about 23.6 kDa). The presence of β-specific PGM activity has been described for Euglena gracilis [2], Lactobacillus (L.) brevis [15] and L. lactis susp. lactis [19]. Lactococci β-PGM activity is induced by maltose in the growth medium but is repressed by presence of glucose [17]. We did not have access to D-glucose-1P and D-glucose-1,6P_2 in forms of β-anomers.

We suggest the presence of Cys in the E. coli ATCC 25922 α-phosphoglucose mutase active site. First, the activity of this protein was significantly inhibited by organic and non- preparation of Hg(II) with the advantage of the last. Second, cysteine is absent in the structure of dehydrogenase glucose-6P from Leuconostoc mesenteroides [10], which was auxiliary enzyme in the activity assay of α-PGM.

Many chelating agents of metal cations can change the activity α-PGMs. EDTA used at low concentration (range mM) inhibited probably both the enzyme characterized in this study and that one isolated from E. coli ATCC 35218 [8] by removal of Mg(II) cations from reaction mixtures. We used buffer with compound TRIS as optimal for enzyme activity assay. In accordance with our results, the α-phosphoglucomutase from E. coli ATCC 25922 does not contain an essential metal in catalytic site.

Anions have been shown to inhibit PGMs by both binding to enzyme and reducing concentration of free Mg(II) [12]. Buffer anions affects are seen at pH around 7.6 where a TRIS/HCl result in 3-fold higher enzyme activity then PBS is used as an assay buffer. The reaction of characterized enzyme was inhibited by sulfate of Mg(II) as compared to control with magnesium chloride (result not shown). E. coli ATCC 26 α-phosphoglucose mutase was negatively affected by sulfate [11]. Interestingly, the protein reported in this study was not inhibited by fluoride. These anions, which probably reduced pool of free Mg(II), are inhibitors of α-PGM from E. coli ATCC 35218 [8].

CONCLUSION

We agree with other scientists, that we need more knowledge of the activity α-phosphoglucomutase as enzyme involved in biosynthesis of bacterial exopolysaccharides and carbon flows between glycolysis (Embden-Mayerhof-Parnas, EMP pathway) and production of bacterial EPS [7].

The next step in our research on characterized enzyme would be its purification to homogeneity with the highest catalytic activity. It will be difficult, but crucial for testing the anti-biofilm agents targeting α-phosphoglucomutase as potential antibacterial drugs. In this context, from a biochemical point of view, molecular modelling studies of drug design would be very fascinating. However, we must remember, that new inhibitors must be effective to bacterial PGMs, but not humans.

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ABSTRACT

The α-phosphoglucomutase (α-PGM, EC 5.4.2.2) is an enzyme participating in the metabolism of the sugar and carrying the reversible isomerization of α-D-glucose-1-phosphate to α-D-glucose-6-phosphate.

Some properties of α-PGM from *E. coli* ATCC 25922 were studied. The K_m constant for α-D-glucose-1-phosphate was 25 x 10^{-5} M/L. The enzyme had optimum activity in TRIS/HCl buffer, pH 8.0. Its activity was stimulated by addition of α-D-glucose-1,6-bisphosphate, Mg(II) cations and several thiols. The α-phosphoglucomutase had molecular weight 65 kDa and consisted of one polypeptide chain. Our results indicated the significant sensitivity of enzymatic activity by non- and organic mercuric compounds. The lack of the activity inhibition of characterized enzyme by chelators as 2,4'-bipyridyl, EDTA and TRIS indicated that the α-PGM is not a metallprotein. The similarities and differences between properties of α-phosphoglucomutases isolated from *E. coli* ATCC 25922 and other *E. coli* strains such as ATCC 26 and ATCC 35218 were discovered.

Our pilot studies have an important implication in better understanding of virulence of enteric bacteria (*E. coli*).

Keywords: α-phosphoglucomutase; *Escherichia coli* ATCC 25922, biofilm

STRESZCZENIE

α-fosfoglukomutaza (α-PGM, EC 5.4.2.2) jest enzymem uczestniczącym w metabolizmie cukrów, katalizującym odwracalną reakcję izomeryzacji α-D-glukozo-1P do α-D-glukozo-6P. Badano wybrane właściwości α-PGM z E. coli ATCC 25922. Stała Michaelisa dla α-D-glukozo-1P wynosiła 25 x 10^{-5} M/L. Enzym miał optymalną aktywność w buforze TRIS/HCl, pH 8.0. Jego aktywność była stymulowana w obecności α-D-glukozo-1,6-bisfosforanu, kationów Mg(II) i kilku tioli. Ciężar cząsteczkowy α-fosfoglukomutazy wynosił 65 kDa, a enzym był zbudowany z jednego łańcucha polipeptydowego. Wykazano znaczącą wrażliwość aktywności enzymatycznej na nie i organiczne preparaty rtęciowe. Brak hamowania aktywności charakteryzowanego enzymu przez takie chelatory jak: 2,4'-bipirydyl, EDTA i TRIS dowodzi, że α-PGM nie jest metaloproteiną. Zaobserwowano podobieństwa i różnice pomiędzy α-fosfoglukomutazami izolowanymi z *E. coli* ATCC 25922 i z innych szczepów *E. coli* takich jak: ATCC 26 i ATCC 35218.

Przeprowadzone wstępne badania mają istotne implikacje dla lepszego zrozumienia wirulencji enterobakterii (*E. coli*).

Słowa kluczowe: α-fosfoglukomutaza; *Escherichia coli* ATCC 25922, biofilm