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Escherichia coli ORF *ybhE* is *pgl* gene encoding 6-phosphogluconolactonase (EC 3.1.1.31) that has no homology with known 6PGLs from other organisms

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Abstract

The pentose-phosphate pathway (PPP) is an important part of central metabolism in many organisms. A *pgl*$^-$ mutation that decreases the efficiency of the second stage of PPP has been described and mapped at approx. 17.2 min of the *Escherichia coli* chromosome more than 30 years ago. Although it has recently been shown that deletion of ORF *ybhE* leads to earlier detected Pgl$^-$ phenotype for *E. coli* mutant strain, 6-phosphogluconolactonase from this organism has not been characterized. In the present, independent investigation we show that the Pgl$^-$ phenotype of Δ*ybhE* MG1655 could be complemented by insertion of the well-characterized *pgl* gene from *Pseudomonas putida* whose protein product has no visible homology with *E. coli* YbhE. Moreover, a final confirmation that *ybhE* actually encodes 6PGL in *E. coli* was obtained through overexpression of the cloned gene, purification of the protein product, followed by direct determination of its enzymatic activity in vitro.

Keywords: 6-Phosphogluconolactonase; Pgl; 6PGL

1. Introduction

The pentose phosphate pathway (PPP) is an important part of the central metabolism in many organisms. The oxidative branch of PPP provides reducing power, and phosphorylated carbohydrates obtained in the non-oxidative branch of PPP, are precursors for nucleotide biosynthesis, aromatic amino acids and vitamins. The oxidative branch of PPP includes three consecutive reactions shown in Fig. 1. The first and the third reactions are catalyzed by the well-known enzymes – glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and gluconate-6-phosphate dehydrogenase (6PGDH, EC 1.1.1.44) encoded in *Escherichia coli E. coli* by *zwf* and *gnd* genes, respectively.

As could be seen from the Fig. 1, δ-6-phosphogluconolactone (δ-6-P-G-L), one of the products of the reaction catalyzed by G6PDH could convert to γ-6-phosphogluconolactone (γ-6-P-G-L). But only δ-6-P-G-L could be spontaneously hydrolyzed into 6-phosphogluconate, and namely this reaction is catalyzed by known 6-phosphogluconolactonases (6PGL, EC 3.1.1.31) [1]. It has been shown, that the rate of δ-6-P-G-L izomerization into γ-6-P-G-L is higher than that for spontaneous conversion of δ-6-P-G-L into 6-P-G-A. So, the absence of 6PGL leads to accumulation of

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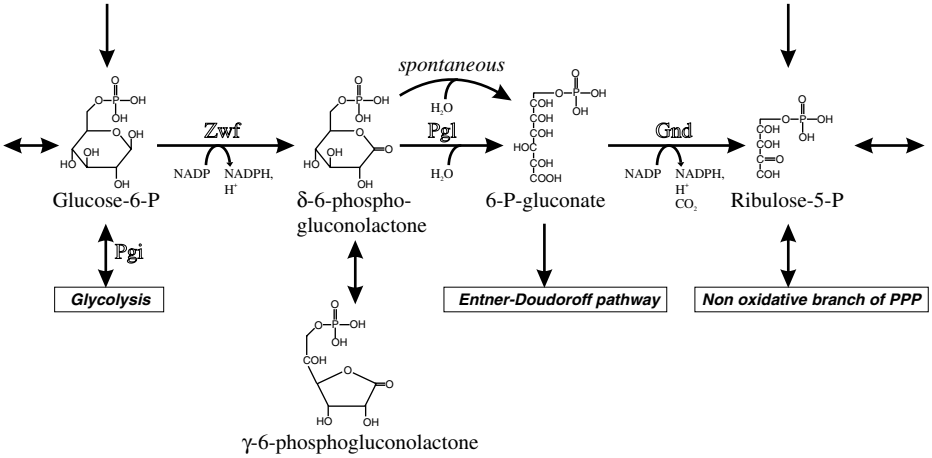

Fig. 1. Metabolic conversions in oxidative branch of pentose-phosphate pathway.

γ-6-P-G-L, and thus to reduction of the PPP efficiency. Also, high electrophilic reactivity of the phosphogluconolactones (6-P-G-Ls) reveals another possible role of 6PGL in protecting the cell from toxic compounds [1].

E. coli pgl gene hypothetically encoding 6PGL, has been mapped between *attλ* and *chlD* (later *modC*) more than 30 years ago [2]. It has been shown that *pgl* mutation only slightly decreases the growth rate of wild-type *E. coli* on glucose, but leads to dramatic growth retardation of pgi^- mutant strain [3]. The maltose-blue phenotype has been demonstrated for pgl^--strains: these mutants grown on maltose turn blue after iodine treatment [2]. And at last, it has been shown that the specific 6PGL activity measured by qualitative assay is significantly lower in mutant strain comparing with wild-type strain [2].

When the first variant of this article has been prepared for publication, the report by Thomason et al. [4] about identification of *ybhE* gene as *pgl* in *E. coli* appeared. Their proofs really points that *ybhE* gene is concerned with exhibition of 6PGL activity in *E. coli*, but the fact that *ybhE* is the structural gene encodes 6-phosphogluconolactonase has not been established in their paper. On the other hand, the experimental detection of 6PGL has been described for several pro- and eukaryotic organisms: *Trypanosoma brucei* [5], *Plasmodium falciparum* [6], *Pseudomonas aeruginosa* [7], bull [8], bass [9], human [10]. Surprisingly, no homologue of 6PGLs could be found in *E. coli* [7].

Present study could be considered as the further development of investigation of *E. coli pgl* gene initiated in [4]. We additionally found that Pgl^- phenotype of constructed Δ*ybhE* MG1655 *E. coli* strain could be complemented by expression of the well-characterized *pgl* gene from *Pseudomonas putida* whose protein product has no visible homology with *E. coli* YbhE. Finally the confirmation that *ybhE* actually encodes 6PGL in *E. coli* has been obtained due to the cloned gene overexpression, purification of protein product followed by direct determination of its enzymatic activity in vitro.

2. Materials and methods

2.1. Bacterial strains, plasmids and oligonucleotides

The bacterial strains and plasmids are listed in Table 1. Oligonucleotides are listed in Table 2.

All chromosome modifications were obtained by one-step replacement method developed by Datsenko and Wanner [11], using *E. coli* BW25113 as the parent strain. DNA fragments carrying the selective markers were obtained from pACYC184 for Cm^R and pUC4Kan for Km^R by PCR with overhanging primers. These fragments carried 36 nt homologous extensions for targeting recombination events. Replacements were verified by PCR with checking primers.

Double mutants were constructed using antibiotic dependent P1-duction followed by PCR verification.

2.2. Maltose blue test

The modified method previously described by Kupor and Fraenkel [2] was exploited for detection of maltose-blue phenotype. An overnight culture grown on rich media was diluted 50 times and grown up to $OD_{540} \sim 1.0$. Then, cells were precipitated and resuspended in M9 minimal media with 0.8% maltose. After 90 min of cultivation, iodine solution containing 0.01 M I_2 and 0.03 M KI was added in proportion of 50 μl per 1 ml of the culture. The obtained color of the mixture was visually scored as "blue" or "not blue".