FEBS Letters 581 (2007) 4423–4429

Occurrence of more than one important source of ADPglucose linked to glycogen biosynthesis in *Escherichia coli* and *Salmonella*

María T. Morán-Zorzano, Nora Alonso-Casajús, Francisco J. Muñoz, Alejandro M. Viale[1], Edurne Baroja-Fernández, Gustavo Eydallin, Javier Pozueta-Romero*

Instituto de Agrobiotecnología, (CSIC, UPNA, Gobierno de Navarra), Mutiloako etorbidea zenbaki gabe, 31192 Mutiloabeti, Nafarroa, Spain

Received 4 June 2007; revised 30 July 2007; accepted 6 August 2007

Available online 15 August 2007

Edited by Judit Ovádi

Abstract To explore the possible occurrence of sources, other than GlgC, of ADPglucose linked to bacterial glycogen biosynthesis we characterized *Escherichia coli* and *Salmonella* Δ*glgCAP* deletion mutants lacking the whole glycogen biosynthetic machinery. These mutants displayed the expected glycogen-less phenotype but accumulated ADPglucose. Importantly, Δ*glgCAP* cells expressing the glycogen synthase encoding *glgA* gene accumulated glycogen. Protein chromatographic separation of crude extracts of Δ*glgCAP* mutants and subsequent activity measurement analyses revealed that these cells possess various proteins catalyzing the conversion of glucose-1-phosphate into ADPglucose. Collectively these findings show that enterobacteria possess more than one important source of ADPglucose linked to glycogen biosynthesis.

Keywords: Carbohydrate metabolism; Enterobacteria; Glycogen synthase; UDPglucose

1. Introduction

Glycogen is a branched homopolysaccharide of α-1,4-linked glucose subunits with α-1,6-linked glucose at the branching points. Synthesized by both glycogen synthase (GlgA) and branching enzyme, glycogen accumulates in enterobacteria under conditions of limited growth in the presence of an excess of a carbon source [1].

Regulation of glycogen biosynthesis in *Escherichia coli* and *Salmonella* is highly interconnected with a wide variety of cellular processes [2] and involves a complex assemblage of factors that are adjusted to the physiological status of the cell. At the level of enzyme activity for instance, the glycogen biosynthetic process is subjected to the allosteric regulation of GlgC, a protein that catalyzes the enzymatic activity designated as ADPglucose (ADPG) pyrophosphorylase (AGPase) (EC 2.7.7.27) [1,3,4]. Moreover, depending on carbon source, phosphoglucomutase (Pgm) and adenosine diphosphate sugar pyrophosphatase have been shown to play an important role in glycogen biosynthesis [2,5,6]. At the level of gene expression, the process depends on the regulation of *glgBX*, *glgS* and *glgCAP* operons [1,7–10], the latter encoding the GlgC and GlgA anabolic enzymes as well as the catabolic glycogen phosphorylase [11].

Since the initial demonstration that ADPG serves as the precursor molecule for both plant starch and bacterial glycogen biosynthesis [12,13], it has been considered that AGPase is the sole enzyme activity catalyzing the production of ADPG [1,3]. However, a number of in vivo and in vitro experimental data indicate the presence in plants of enzyme activities other than AGPase that may catalyze the synthesis of ADPG [14–19]. To investigate the possible occurrence of various source(s) of ADPG linked to glycogen biosynthesis in bacteria we have expressed *glgA* in glycogen-less Δ*glgCAP* deletion mutants of *E. coli* and *S. enterica* lacking the whole glycogen biosynthetic machinery. The rationale behind our experimental approach was that, if ADPG is exclusively produced by GlgC, *glgA* expressing Δ*glgCAP* cells will display a glycogen-less phenotype. Conversely, if ADPG linked to glycogen biosynthesis results from both GlgC and other enzyme(s), *glgA* expressing Δ*glgCAP* cells will be able to accumulate glycogen.

Results presented in this communication show for the first time the occurrence of important source(s) of ADPG, other than GlgC, linked to glycogen biosynthesis in enterobacteria.

2. Materials and methods

2.1. Bacterial strains, plasmids and culture media

Bacterial strains and plasmids used in this work and their characteristics are summarized in Supplementary Table 1. *E. coli* W3110 cells and *S. enterica* serovar Typhimurium LT2 cells were used for production of *glgCAP* and *otsBA* deletion mutants (Δ*glgCAP* and Δ*otsBA*, respectively), as well as for *glgA*-expression experiments. DNA manipulations were conducted by following the procedures indicated by Ausubel et al. [20]. All plasmid constructs (see below) were propagated in *E. coli* XL1 Blue grown in LB medium (1% tryptone, 1% NaCl, 0.5% yeast extract) with the appropriate selection. For biochemical analyses, cells were grown in either Kornberg (1.1% K_2HPO_4, 0.85% KH_2PO_4, 0.6% yeast extract from Duchefa, Haarlem, The Netherlands) or M9 minimal (4 mM NaCl, 9 mM NH_4Cl, 0.1 mM $CaCl_2$, 2 mM $MgSO_4$, 48 mM Na_2HPO_4 and 22 mM KH_2PO_4) liquid media supplemented with 50 mM glucose and the appropriate selection antibiotic. In every case, the bacteria were grown with rapid gyratory shaking at 37 °C

*Corresponding author. Fax: +34 948232191.
E-mail address: javier.pozueta@unavarra.es (J. Pozueta-Romero).

[1]Present address: Instituto de Biología Molecular y Celular de Rosario (CONICET), Dpto. de Microbiología, Fac. de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina.

Abbreviations: ADPG, ADPglucose; AGPase, ADPG pyrophosphorylase; EM, electron microscopy; G1P, glucose-1-phosphate; GlgA, glycogen synthase; Pgm, phosphoglucomutase; U, unit of enzyme activity; UDPG, UDPglucose; WT, wild type

0014-5793/$32.00
doi:10.1016/j.febslet.2007.08.017

after inoculation with 1 volume of an overnight culture for 50 volumes of fresh medium. Solid Kornberg and M9 minimal media were prepared by addition of 1.5% bacteriological agar to liquid media.

2.2. glgCAP and otsBA disruptions

glgCAP and *otsBA* disruptions were performed essentially as described in [21]. A selectable antibiotic resistance gene was generated by PCR from a freshly isolated colony of *E. coli* MC4100 containing either a chromosomically located kanamycin resistance cassette or an apramycin resistance cassette, using 80 nucleotide-long primer pairs that included 60 nucleotides homology extensions for the targeted locus and 20 nucleotides priming sequences for the resistance genes (Supplementary Table 2). Deletion mutants were confirmed by both PCR and RT-PCR (Supplementary Fig. 1).

2.3. glgA-expression

The steps to produce *glgA*-expressing cells (Supplementary Table 3) are illustrated in Supplementary Fig. 2.

2.4. AGPase assays

Cells entering the stationary phase were harvested by centrifugation at 10000 × *g* during 15 min, rinsed with abundant M9 minimal medium, resuspended in 40 mM Tris/HCl, pH 7.5, sonicated and assayed for enzymatic activity. GlgA was assayed as described in [11]. AGPase activity was assayed in the ADPG-pyrophosphorolytic direction. Determination of glucose-1-phosphate (G1P) and ATP was performed in two steps. In step one, the reaction mixture contained 40 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 2 mM PPi, 2 mM ADPG and 2 mM fructose 1,6 bisphosphate. A control reaction lacking ADPG was run in parallel for all samples. After 60 min of incubation at 37 °C, the reaction was stopped by boiling in a dry bath for 2 min. G1P was determined spectrophotometically in a 300-μl mixture containing 50 mM Hepes (pH 8.0), 1 mM EDTA, 2 mM $MgCl_2$, 15 mM KCl, 0.4 mM NAD^+, 1 unit (U) each of Pgm and glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*, and 30 μl of the step-one reaction. After 20 min of incubation at 37 °C, the NADH production was monitored at 340 nm by using a Multiskan EX spectrophotometer (Labsystems, Chicago). For ATP measurement, 100 μl of the step-one

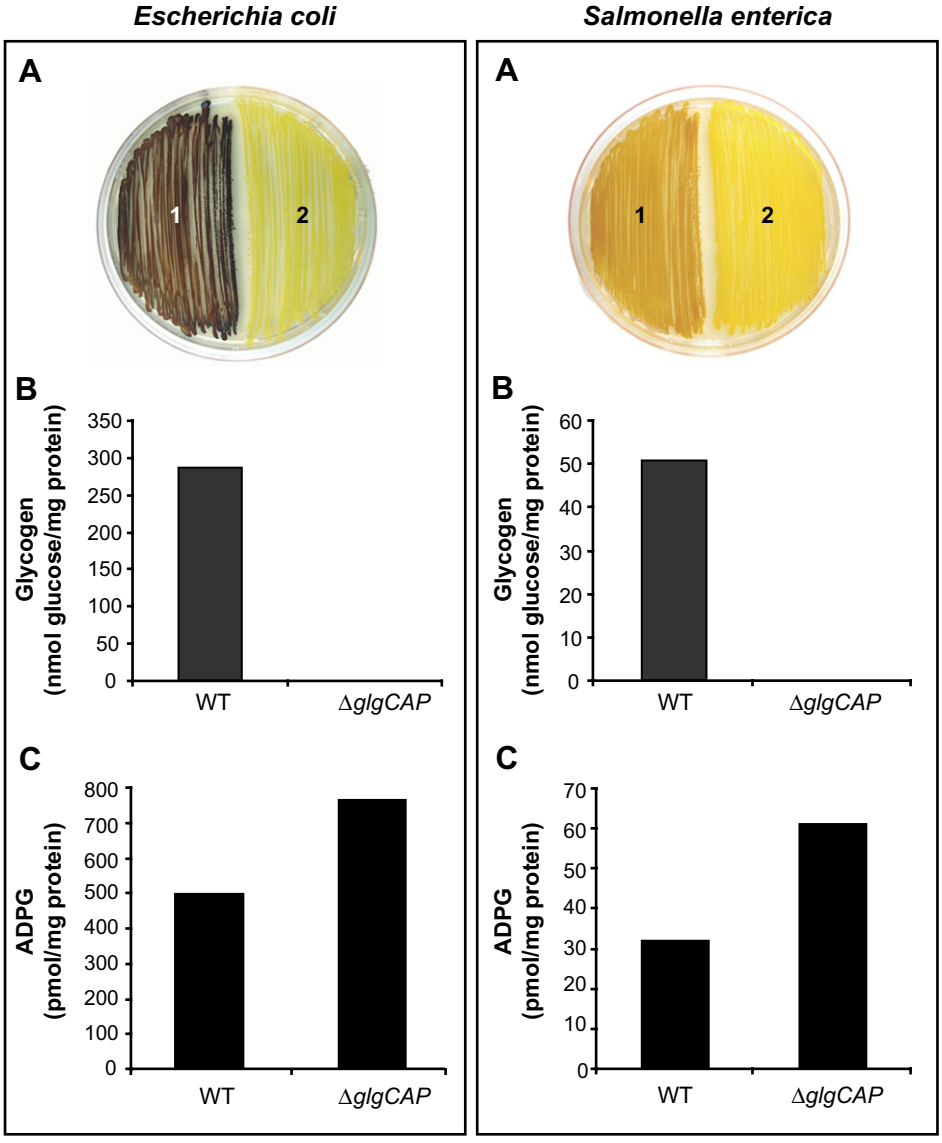

Fig. 1. The *glgCAP* operon highly controls glycogen production but not ADPG accumulation in both *E. coli* K-12 W3110 and *S. enterica* LT2. (A) Iodine staining of WT and Δ*glgCAP* deletion mutants (Sections 1 and 2, respectively) cultured in solid Kornberg medium supplemented with 50 mM glucose. (B) Glycogen and (C) ADPG contents in WT and Δ*glgCAP* cells. In B and C, cells were cultured in M9 minimal medium supplemented with 50 mM glucose and harvested at the end of the exponential growth phase.