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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

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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

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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

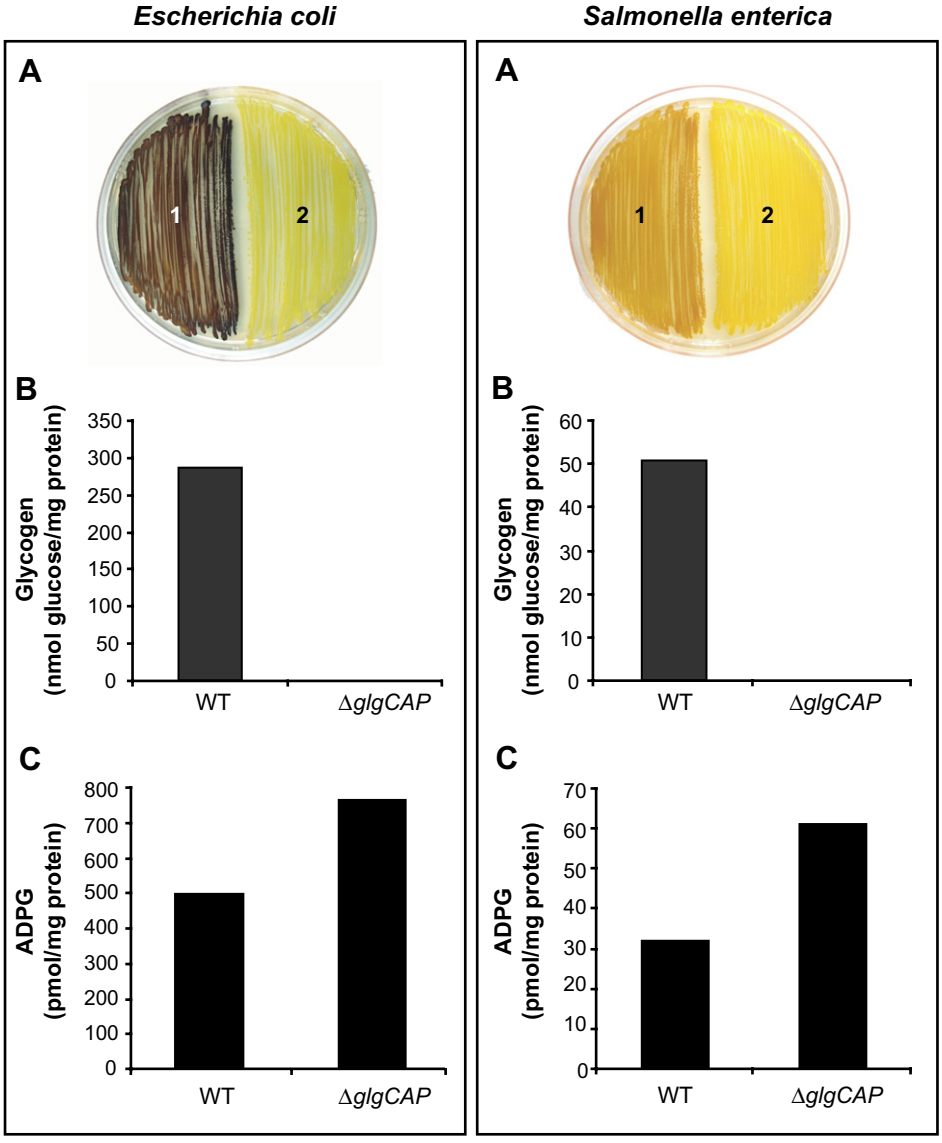

Fig. 1. The *glgCAP* operon highly controls glycogen production but not ADPG accumulation in both *E. coli* K-12 W3110 and *S. enterica* LT2. (A) Iodine staining of WT and Δ*glgCAP* deletion mutants (Sections 1 and 2, respectively) cultured in solid Kornberg medium supplemented with 50 mM glucose. (B) 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.

reaction was subjected to HPLC analyses essentially as described in [22].

One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μmol of product per min.

2.5. ADPG extraction and measurement

ADPG was extracted essentially as described in [22]. Freshly harvested cells (ca. 2.5 g fresh weight) were resuspended in 1 ml of 1 M $HClO_4$, left on ice for 1 h and centrifuged at $10000 \times g$ for 15 min. The supernatant thus obtained was neutralized with K_2CO_3, centrifuged at $10000 \times g$ and ADPG was measured by using either one of the following methods:

Assay A: by HPLC on a system obtained from P.E. Waters and Associates fitted with a Partisil-10-SAX column [22].

Assay B: by HPLC with pulsed amperometric detection on a DX-500 system (Dionex) fitted to a CarboPac PA10 column [15].

We checked the reliability of the two chromatographic methods of ADPG detection and measurement by adding known amounts of commercially available ADPG to cell extracts. In addition, we confirmed that samples digested with purified *E. coli* adenosine diphosphate sugar pyrophosphatase [5,6] do not possess ADPG (Supplementary Fig. 3).

We checked the effectiveness of the method of ADPG extraction by adding known amounts of commercially available ADPG to bacterial samples (final concentration in the homogenate being 10, 30 and 50 μM). Recovery in all cases exceeded 95%.

2.6. Chromatographic separation of AGPases

S. enterica LT2 cells grown in three liters of liquid M9 minimal medium supplemented with glucose were centrifuged at $10000 \times g$ for 15 min. The pelleted bacteria were then resuspended in 10 ml of extraction buffer (40 mM Tris/HCl, pH 7.5), disrupted by sonication and centrifuged at $30000 \times g$ for 30 min. The supernatant thus obtained was loaded onto a Q-sepharose column (Amersham Biosciences) equilibrated with 40 mM Tris/HCl (pH 8.0) and eluted with a linear gradient of 0–0.5 M NaCl in 40 mM Tris/HCl (pH 8.0). The eluted fractions (2 ml) were then subjected to analysis of AGPase activity as described above and to immunoblot analyses using GlgC-antisera.

2.7. Analytical procedures

Bacterial growth was followed spectrophotometrically by measuring the absorbance at 600 nm. Glycogen was determined using an amyloglucosidase/hexokinase/glucose-6P dehydrogenase-based test kit from Sigma. Protein content was measured by the Bradford method using a Bio-Rad prepared reagent. Iodine staining of colonies on solid Kornberg medium was performed as described in [23].

2.8. Electron microscopic (EM) analyses

We proceeded as described in [24]. Cells entering the stationary phase were pre-fixed with 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 2 h at 4 °C, rinsed in 0.25 M sucrose/0.1 M cacodylate buffer, and post-fixed in 1% OsO_4 in phosphate buffer (pH 7.3) at 4 °C. After two washes with Veronal sodium (pH 7.4), the cells were embedded in 2% molten Noble Agar. The gel was dehydrated and embedded in Epon-812, and ultrathin sections were placed on nickel grids, post-stained for 30 min with uranyl acetate and for 15 min with lead citrate, and observed under a Zeiss EM 10CR electron microscope.

3. Results and discussion

3.1. ΔglgCAP deletion mutants of E. coli and S. enterica are totally devoided of glycogen but accumulate ADPG

The *glgCAP* operons of *E. coli* W3110 and *S. enterica* LT2 were disrupted by insertional mutagenesis (Supplementary

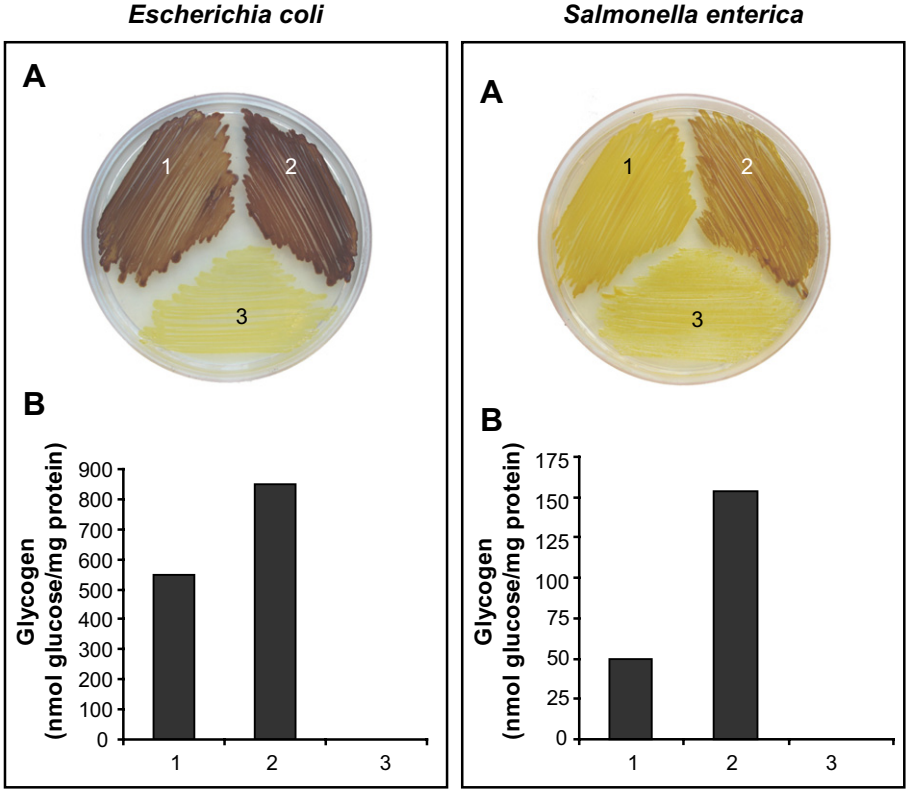

Fig. 2. Occurrence of source(s) of ADPG linked to glycogen biosynthesis different to GlgC in both *E. coli* and *S. enterica*. (A) Iodine staining of cells cultured in solid Kornberg medium supplemented with 50 mM glucose. (B) Glycogen content in cells cultured in liquid M9 minimal medium supplemented with 50 mM glucose. Cells were harvested at the end of the exponential growth phase. (1) WT cells transformed with pACYCDuet; (2) Δ*glgCAP* cells transformed with pACYCDuet-glgA; (3) Δ*glgCAP* cells transformed with pACYCDuet. GlgA activities of these cells are shown in Supplementary Table 3.

Fig. 1A). As illustrated in Supplementary Fig. 1B, the resulting Δ*glgCAP* cells totally lacked *glgCAP* transcripts.

Iodine staining analyses on solid cultures supplemented with 50 mM glucose (Fig. 1A) revealed that both *E. coli* and *S. enterica* Δ*glgCAP* cells displayed the expected negative (yellow) stain, whereas their corresponding wild type (WT) cells gave a brown stain phenotype that is due to glycogen accumulation. Subsequent glycogen measurement analyses on liquid cultures showed that, in conformity with the results of Fig. 1A, Δ*glgCAP* cells are totally devoided of glycogen (Fig. 1B). Importantly however, abolition of glycogen biosynthesis in these bacteria was not accompanied by a reduction of the intracellular ADPG levels (Fig. 1C). Collectively these results (a) show that *glgCAP* is essential for glycogen production in both *S. enterica* and *E. coli* and (b) indicate that these enterobacterial species possess source(s) of ADPG different to GlgC.

3.2. ADPG accumulating in ΔglgCAP deletion mutants of *E. coli* and *S. enterica* is linked to glycogen biosynthesis

We explored whether ADPG accumulating in Δ*glgCAP* deletion mutants can be linked to glycogen biosynthesis. Towards this end we compared the glycogen contents between WT and Δ*glgCAP* cells transformed with either pACYCDuet or pACYCDuet-glgA. As shown in Supplementary Table 3, GlgA activities in Δ*glgCAP* cells transformed with pACYCDuet-glgA were threefold higher than those occurring in the corresponding WT cells.

Iodine stain analyses on solid cultures of both *E. coli* and *S. enterica* cells showed that Δ*glgCAP* cells transformed with pACYCDuet displayed a negative glycogen stain as compared with WT cells (Fig. 2A). Remarkably however, Δ*glgCAP* cells transformed with pACYCDuet-glgA displayed a dark brown iodine stain phenotype, strongly indicating that *glgA*-expressing Δ*glgCAP* cells accumulate high levels of glycogen. We then measured the content of this polysaccharide in liquid cultured cells. As illustrated in Fig. 2B, WT cells, but not Δ*glgCAP* cells transformed with pACYCDuet, accumulated glycogen. Most importantly, and confirming the results shown in Fig. 2A, *glgA*-expressing Δ*glgCAP* cells accumulated high levels of glycogen (Fig. 2B).

EM analyses further substantiated that *glgA*-expressing Δ*glgCAP* cells, but not Δ*glgCAP* cells, accumulate glycogen granules (Fig. 3). Resembling the topographic distribution pattern of glycogen granules occurring in different bacterial species [24–26], glycogen granules deposited in both the periphery and poles of *glgA*-expressing Δ*glgCAP* cells.

The overall results thus strongly indicate that both *E. coli* and *S. enterica* possess important source(s), other than GlgC, of ADPG linked to glycogen biosynthesis.

3.3. Enterobacteria possess more than one protein entity endowed with AGPase activity

E. coli mutants lacking Pgm activity display a glycogen-less phenotype when cultured in the presence of glucose [2] suggesting that (a) G1P is the common substrate for both GlgC and the additional ADPG source(s) and (b) enzymes(s), other than GlgC, must occur in enterobacteria that catalyze the conversion of G1P to ADPG. To check the latter possibility, crude extracts of both WT and Δ*glgCAP S. enterica* LT2 cells were subjected to chromatographic separation on a Q-sepharose column, and AGPase activity was measured in the eluted fractions. Importantly, as illustrated in Fig. 4, AGPase activity was detected in the eluted fractions of Δ*glgCAP* cells, the AGPase activity profile of WT cells being similar to that of Δ*glgCAP* cells except that an additional peak of activity appeared in fractions of WT cells eluted at positions 48–66. These frac-

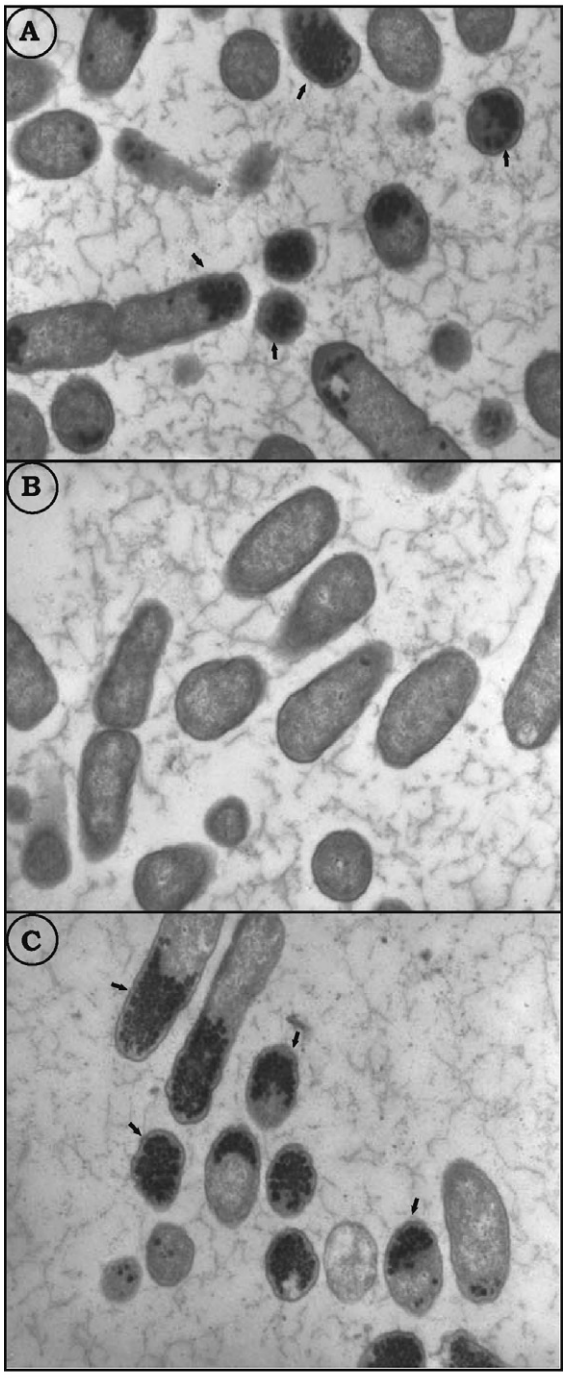

Fig. 3. EM analysis of glycogen granules in *E. coli* WT cells (A) and Δ*glgCAP* cells transformed with either pACYCDuet or pACYCDuet-glgA (B and C, respectively). Cells were cultured in liquid M9 minimal medium supplemented with 50 mM glucose and harvested at the end of the exponential growth phase. Arrows indicate the position of glycogen granules.

tions possess a ca. 48 kDa protein which is specifically recognized by GlgC specific antisera (Fig. 4C). The overall data thus indicate that enterobacteria possess more than one protein entity catalyzing AGPase activity.

3.4. Additional remarks

Results presented in this work show for the first time the occurrence of more than one important source of ADPG linked to glycogen biosynthesis in both *E. coli* and *S. enterica*. Since glycogen may play relevant roles in the survival of bacteria to sporadic periods of famine [1], and because glycogen metabolism is highly interconnected with multiple and important cellular processes [2], it is tempting to speculate that redundancy of ADPG sources was selected during bacterial evolution to guarantee the production of glycogen.

We are currently investigating the additional mechanism(s) of ADPG production in enterobacteria. Qu et al. [27] have found a novel trehalose glucosyltransferase activity in the ar-

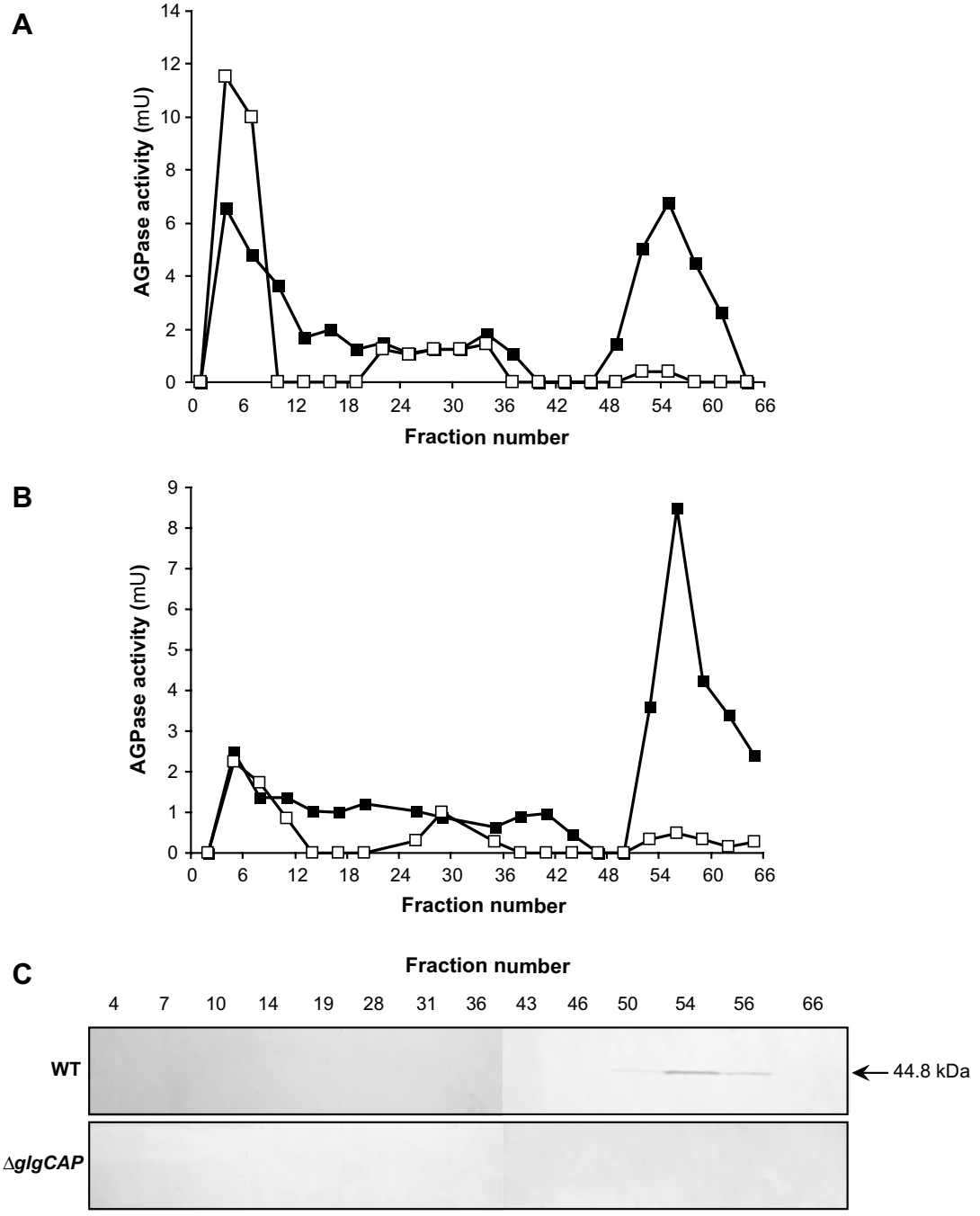

Fig. 4. Occurrence of enzyme(s), other than GlgC, catalyzing AGPase activity in enterobacteria. Three liters of cultures of WT (■) and Δ*glgCAP* (□) *S. enterica* LT2 cells entering the stationary phase were harvested by centrifugation at 10000 × *g* during 10 min, resuspended in 10 ml of 40 mM Tris/HCl (pH 7.5), disrupted by sonication and centrifuged at 30000 × *g* for 30 min. The supernatant thus obtained was subjected to Q-sepharose chromatography. The figure represents the G1P and ATP producing AGPase activities in the eluted fractions (A and B, respectively) and western blot analyses using antisera raised against *E. coli* GlgC and 10 μg each of the eluted fractions (C). GlgC-antisera was produced as described in [39].

cheon *Thermococcus litoralis* that catalyzes the reversible conversion of trehalose and ADP into ADPG and glucose. To explore the possible occurrence of a trehalose-dependent machinery producing ADPG linked to glycogen biosynthesis in enterobacteria we produced and characterized *glgA*-expressing Δ*glgCAP*Δ*otsBA* double deletion mutants of *S. enterica* LT2. These cells lack both *glgCAP* and the *otsBA* operon encoding the principal enzymes involved in trehalose biosynthesis [28]. As illustrated in Supplementary Fig. 4, *glgA*-expressing Δ*glgCAP* Δ*otsBA* cells displayed a normal glycogen content phenotype, indicating that trehalose glucosyltransferase-dependent conversion of trehalose into ADPG linked to glycogen biosynthesis does not occur in enterobacteria.

GalU is a nucleotide-sugar pyrophosphorylase that is considered to specifically recognize G1P and UTP to produce UDPglucose (UDPG) [29]. However, various reports have shown that this enzyme can also produce ADPG from G1P and ATP [30,31]. This, and the fact that (a) heterologous expression of *E. coli galU* in *Corynebacterim glutamicum* is accompanied by significant increases in cellular glycogen levels [32] and (b) *E. coli* Δ*galU* deletion mutants display a glycogen-deficient phenotype [2], suggest that GalU may act as an important source of ADPG linked to glycogen biosynthesis in enterobacteria. Certainly, further investigations will be necessary to confirm this point.

Mounting evidences have shown that glycogen biosynthesis occurs by the ADPG pathway in most bacteria [1,3,33–36]. To date, the main exception to this rule is *Prevotella bryantii*, a Gram-negative ruminal bacterium that lacks AGPase and whose GlgA exclusively recognizes UDPG as glucosyl donor [37]. Therefore, we cannot totally exclude the possibility that nucleotide-sugars other than ADPG may also act to some extent as precursors of glycogen biosynthesis in *glgA*-expressing Δ*glgCAP* cells of *E. coli* and *S. enterica* (this work). However, this is highly unlikely since enterobacterial GlgA does not employ UDPG, GDPglucose, TDPglucose and CDPglucose as relevant substrates for glycogen biosynthesis [34,35, Morán-Zorzano et al., unpublished].

Our results apparently conflict with the occurrence of glycogen-less *glgC*$^{-}$ mutants of *E. coli* such as AC70R1-504 [38]. However, we have found that AC70R1-504 cells accumulate both ADPG and glycogen [39], the overall data further confirming that GlgC enteric bacteria possess various important sources of ADPG linked to glycogen biosynthesis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2007.08.017.

References

[1] Preiss, J. and Romeo, T. (1994) Molecular biology and regulatory aspects of glycogen biosynthesis in bacteria. Prog. Nucleic Acid Res. Mol. Biol. 47, 299–329.
[2] Eydallin, G., Viale, A.M., Morán-Zorzano, M.T., Muñoz, F.J., Montero, M., Baroja-Fernández, E. and Pozueta-Romero, J. (2007) Genome-wide screening of genes affecting glycogen metabolism in *Escherichia coli*. FEBS Lett. 581, 2947–2953.
[3] Ballicora, M.A., Iglesias, A.A. and Preiss, J. (2003) ADP-glucose pyrophosphorylase, a regulatory enzyme for bacterial glycogen synthesis. Microbiol. Mol. Biol. Rev. 67, 213–225.
[4] Dietzler, D.N. and Leckie, M.P. (1977) Regulation of ADP-glucose synthetase, the rate-limiting enzyme of bacterial glycogen synthesis, by the pleiotropic nucleotides ppGpp and pppGpp. Biochem. Biophys. Res. Commun. 77, 1459–1466.
[5] Moreno-Bruna, B., Baroja-Fernández, E., Muñoz, F.J., Bastarrica-Berasategui, A., Zandueta-Criado, A., Rodríguez-López, M., Lasa, I., Akazawa, T. and Pozueta-Romero, J. (2001) Adenosine diphosphate sugar pyrophosphatase prevents glycogen biosynthesis in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 98, 8128–8132.
[6] Morán-Zorzano, M.T., Viale, A.M., Muñoz, F.J., Alonso-Casajús, N., Eydallin, G.G., Zugasti, B., Baroja-Fernández, E. and Pozueta-Romero, J. (2007) *Escherichia coli* AspP activity is enhanced by molecular crowding and by both glucose-1,6-bisphosphate and nucleotide-sugars. FEBS Lett. 581, 1035–1040.
[7] Romeo, T. and Preiss, J. (1989) Genetic regulation of glycogen biosynthesis in *Escherichia coli*: in vitro effects of cyclic AMP and guanosine 5′-diphosphate 3′-diphosphate and analysis of in vivo transcripts. J. Bacteriol. 171, 2773–2782.
[8] Hengge-Aronis, R. and Fischer, D. (1992) Identification and molecular analysis of *glgS*, a novel growth-phase-regulated and *rpoS*-dependent gene involved in glycogen synthesis in *Escherichia coli*. Mol. Microbiol. 6, 1877–1886.
[9] Baker, C.S., Morozov, I., Suzuki, K., Romeo, T. and Babitzke, P. (2002) CsrA regulates glycogen biosynthesis by preventing translation of *glgC* in *Escherichia coli*. Mol. Microbiol. 44, 1599–1610.
[10] Kozlov, G., Elias, D., Cygler, M. and Gehring, K. (2004) Structure of GlgS from *Escherichia coli* suggests a role in protein–protein interactions. BMC Biol. 2, 10.
[11] Alonso-Casajús, N., Dauvillée, D., Viale, A.M., Muñoz, F.J., Baroja-Fernández, E., Morán-Zorzano, M.T., Eydallin, G., Ball, S. and Pozueta-Romero, J. (2006) Glycogen phosphorylase, the product of the *glgP* gene, catalyzes glycogen breakdown by removing glucose units from the non-reducing ends in *Escherichia coli*. J. Bacteriol. 188, 5266–5272.
[12] Recondo, E. and Leloir, L.F. (1961) Adenosine diphosphate glucose and starch synthesis. Biochem. Biophys. Res. Commun. 6, 85–88.
[13] Preiss, J., Shen, L., Greenberg, E. and Gentner, N. (1966) Biosynthesis of bacterial glycogen. IV. Activation and inhibition of the adenosine diphosphate glucose pyrophosphorylase of *Escherichia coli*. Biochemistry 5, 1833–1845.
[14] Muñoz, F.J., Baroja-Fernández, E., Morán-Zorzano, M.T., Viale, A.M., Etxeberria, E., Alonso-Casajús, N. and Pozueta-Romero, J. (2005) Sucrose synthase controls the intracellular levels of ADPglucose linked to transitory starch biosynthesis in source leaves. Plant Cell Physiol. 46, 1366–1376.
[15] Baroja-Fernández, E., Muñoz, F.J., Saikusa, T., Rodríguez-López, M., Akazawa, T. and Pozueta-Romero, J. (2003) Sucrose synthase catalyzes the de novo production of ADPglucose linked to starch biosynthesis in heterotrophic tissues of plants. Plant Cell Physiol. 44, 500–509.
[16] Murata, T., Sugiyama, T., Minamikawa, T. and Akazawa, T. (1966) Enzymic mechanism of starch synthesis in ripening rice grains. Mechanism of the sucrose–starch conversion. Arch. Biochem. Biophys. 113, 34–44.
[17] Delmer, D.P. (1972) The purification and properties of sucrose synthase from etiolated *Phaseolus aureus* seedlings. J. Biol. Chem. 247, 3822–3828.
[18] Zervosen, A., Römer, U. and Elling, L. (1998) Application of recombinant sucrose synthase-large scale synthesis of ADP-glucose. J. Mol. Catal. B: Enzym. 5, 25–28.
[19] Römer, U., Schrader, H., Gunther, N., Nettelstroth, N., Frommer, W.B. and Elling, L. (2004) Expression, purification and

characterization of recombinant sucrose synthase 1 from *Solanum tuberosum* L. for carbohydrate engineering. J. Biotechnol. 107, 135–149.
[20] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.G. and Struhl, K. (2001) Current Protocols in Molecular Biology, vol. 2, Wiley Interscience, New York.
[21] Datsenko, K.A. and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA 97, 6640–6645.
[22] Baroja-Fernández, E., Muñoz, F.J., Zandueta-Criado, A., Morán-Zorzano, M.T., Viale, A.M., Alonso-Casajús, N. and Pozueta-Romero, J. (2004) Most of ADPglucose linked to starch biosynthesis occurs outside the chloroplast in source leaves. Proc. Natl. Acad. Sci. USA 101, 13080–13085.
[23] Govons, S., Vinopal, R., Ingraham, J. and Preiss, J. (1969) Isolation of mutants of *Escherichia coli* B altered in their ability to synthesize glycogen. J. Bacteriol. 97, 970–972.
[24] Bonafonte, M.A., Solano, C., Sesma, B., Alvarez, M., Montuenga, L., García-Ros, D. and Gamazo, C. (2000) The relationship between glycogen synthesis, biofilm formation and virulence in *Salmonella enteritidis*. FEMS Microbiol. Lett. 191, 31–36.
[25] Martin, M.C., Schneider, D., Bruton, C.J., Chater, K.F. and Hardisson, C. (1997) A *glgC* gene essential only for the first two spatially distinct phases of glycogen synthesis in *Streptomyces coelicolor*. J. Bacteriol. 179, 7784–7789.
[26] Makinoshima, H., Aizawa, S.I., Hayashi, H., Miki, T., Nishimura, A. and Ishihama, A. (2003) Growth phase-coupled alterations in cell structure and function of *Escherichia coli*. J. Bacteriol. 185, 1338–1345.
[27] Qu, Q., Lee, S.-J. and Boos, W. (2004) TreT, a novel trehalose glycosyltransferring synthase of the hyperthermophilic archaeon *Thermococcus litoralis*. J. Biol. Chem. 279, 47890–47897.
[28] Hengge-Aronis, R., Klein, W., Lange, R., Rimmele, M. and Boos, W. (1991) Trehalose synthesis genes are controlled by the putative sigma factor encoded by *rpoS* and are involved in stationary-phase thermotolerance in *Escherichia coli*. J. Bacteriol. 173, 7918–7924.
[29] Nakae, T. and Nikaido, H. (1971) Multiple molecular forms of uridine diphosphate glucose pyrophosphorylase from *Salmonella typhimurium*. J. Biol. Chem. 246, 4397–4403.
[30] Ritter, J.E., Berlin, C. and Elling, L. (1996) A continuous microtiter plate assay for screening nucleotide sugar-synthesizing nucleotidyltransferases. Anal. Biochem. 234, 74–82.
[31] Zea, C.J. and Pohl, N.L. (2004) General assay for sugar nucleotidyltransferases using electrospray ionization mass spectrometry. Anal. Biochem. 328, 196–202.
[32] Padilla, L., Morbach, S., Krämer, R. and Agosin, E. (2004) Impact of heterologous expression of *Escherichia coli* UDP-glucose pyrophosphorylase on trehalose and glycogen synthesis in *Corynebacterium glutamicum*. Appl. Environ. Microbiol. 70, 3845–3854.
[33] Greenberg, E. and Preiss, J. (1964) The occurrence of adenosine diphosphate glucose:glycogen transglucosylase in bacteria. J. Biol. Chem. 239, 4314–4315.
[34] Preiss, J. and Greenberg, E. (1965) Biosynthesis of bacterial glycogen. III. The adenosine diphosphate-glucose:α-4-glucosyl transferase of *Escherichia coli* B. Biochemistry 4, 2328–2334.
[35] Greenberg, E. and Preiss, J. (1965) Biosynthesis of bacterial glycogen. II. Purification and properties of the adenosine diphosphoglucose: glycogen transglucosylase of *Arthrobacter* species NRRL B1973. J. Biol. Chem. 240, 2341–2348.
[36] Dietzler, D.N. and Strominger, J.L. (1973) Purification and properties of the adenosine diphosphoglucose:glycogen transglucosylase of *Pasteurella pseudotuberculosis*. J. Bacteriol. 113, 946–952.
[37] Lou, J., Dawson, K.A. and Strobel, H.J. (1997) Glycogen biosynthesis via UDP-glucose in the ruminal bacterium *Prevotella bryantii* B1(4). Appl. Environm. Microbiol. 63, 4355–4359.
[38] Greene, T.W., Chantler, S.E., Kahn, M.L., Barry, G.F., Preiss, J. and Okita, T.W. (1996) Mutagenesis of the potato ADPglucose pyrophosphorylase and characterization of an allosteric mutant defective in 3-phosphoglycerate activation. Proc. Natl. Acad. Sci. USA 93, 1509–1513.
[39] Eydallin, G., Morán-Zorzano, M.T., Muñoz, F.J., Baroja-Fernández, E., Montero, M., Alonso-Casajús, N., Viale, A.M., Pozueta-Romero, J. An *Escherichia coli* mutant producing a truncated inactive form of GlgC synthesizes glycogen: further evidence for the occurrence of various important sources of ADPglucose in enterobacteria. FEBS Lett., in press, doi:10.1016/j.febslet.2007.08.016.