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Glyceraldehyde-3-phosphate dehydrogenase is required for efficient repair of cytotoxic DNA lesions in *Escherichia coli*

Elaine Ferreira [1], Rosa Giménez [1], María Alexandra Cañas, Laura Aguilera, Juan Aguilar, Josefa Badia, Laura Baldomà *

Departament de Bioquímica i Biología Molecular, Institut de Biomedicina de la Universitat de Barcelona, Facultat de Farmàcia, Universitat de Barcelona, E-08028 Barcelona, Spain

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ABSTRACT

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a multifunctional protein with diverse biological functions in human cells. In bacteria, moonlighting GAPDH functions have only been described for the secreted protein in pathogens or probiotics. At the intracellular level, we previously reported the interaction of *Escherichia coli* GAPDH with phosphoglycolate phosphatase, a protein involved in the metabolism of the DNA repair product 2-phosphoglycolate, thus suggesting a putative role of GAPDH in DNA repair processes. Here, we provide evidence that GAPDH is required for the efficient repair of DNA lesions in *E. coli*. We show that GAPDH-deficient cells are more sensitive to bleomycin or methyl methanesulfonate. In cells challenged with these genotoxic agents, GAPDH deficiency results in reduced cell viability and filamentous growth. In addition, the *gapA* knockout mutant accumulates a higher number of spontaneous abasic sites and displays higher spontaneous mutation frequencies than the parental strain. Pull-down experiments in different genetic backgrounds show interaction between GAPDH and enzymes of the base excision repair pathway, namely the AP-endonuclease Endo IV and uracil DNA glycosylase. This finding suggests that GAPDH is a component of a protein complex dedicated to the maintenance of genomic DNA integrity. Our results also show interaction of GAPDH with the single-stranded DNA binding protein. This interaction may recruit GAPDH to the repair sites and implicates GAPDH in DNA repair pathways activated by profuse DNA damage, such as homologous recombination or the SOS response.

1. Introduction

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12), a key glycolytic enzyme, is a moonlighting protein with additional functions that are unrelated to its original metabolic role. The multifunctionality of GAPDH is extensively documented in human cells, where it is involved in numerous processes (Sirover, 2011). The various moonlighting functions of GAPDH depend on specific post-translational modifications, oligomeric state and/or subcellular localization, which determine the binding partners (Copley, 2012; Sirover, 2005, 2011). Each function requires binding into specific protein complexes.

In bacteria, GAPDH moonlighting functions are mainly associated with its extracellular location. This protein is secreted and exposed on the bacterial surface, enabling pathogens to colonize and manipulate host cells (Pancholi and Chhatwal, 2003). In enteropathogenic and enterohemorrhagic *Escherichia coli*, secreted GAPDH interacts with human plasminogen and fibrinogen and remains associated with Caco-2 cells upon infection (Egea et al., 2007). In addition, the secreted protein can act as a target of oxidation and protect bacteria against the host oxidative response (Aguilera et al., 2009). GAPDH has also been identified in the secretome of probiotic strains (Aguilera et al., 2012; Sánchez et al., 2009a) and shown to interact with mucin, thus contributing to intestinal colonization (Kinoshita et al., 2008; Sánchez et al., 2009b).

Studies dealing with new GAPDH intracellular functions are scarce in bacteria. Following a proteomic approach aimed at identifying proteins that interact with GAPDH in *E. coli*, we identified

Abbreviations: AP sites, abasic sites (apurinic/apyrimidinic); BER, base excision repair; BM, bleomycin; CAA, casein acid hydrolysate; CFUs, colony-forming units; Endo, endonuclease; Exo, exonuclease; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Gph, phosphoglycolate phosphatase; GST, glutathione-*S*-transferase; HR, homologous recombination; IPTG, isopropyl β-D-1-thiogalactopyranoside; LB, Luria-Bertani broth; MMS, methyl methanesulfonate; NIR, nucleotide incision repair; NTA, nitrilotriacetic acid; PTasRNA, paired termini antisense RNA; Rifampicin, Rif; SSB, single-stranded DNA binding protein; SM, minimal medium; UDG, uracil DNA glycosylase.

* Corresponding author. Tel.: +34 934034496; fax: +34 934024520.
E-mail address: lbaldoma@ub.edu (L. Baldomà).

[1] These authors contributed equally to this work.

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phosphoglycolate phosphatase (Gph) (Ferreira et al., 2013). This enzyme is involved in the metabolism of 2-phosphoglycolate formed in the DNA repair of 3′-phosphoglycolate ends generated by bleomycin (BM) (Pellicer et al., 2003). Interaction between Gph and GAPDH increases in BM-treated cells, thus implicating GAPDH in processes linked to DNA repair (Ferreira et al., 2013).

Involvement of GAPDH in the repair of BM-generated DNA lesions has been reported in human cells. GAPDH interacts with and modulates the activity of endonuclease APE1, an enzyme involved in the repair of abasic sites (AP sites) (Azam et al., 2008). AP sites are generated when cells are exposed to genotoxic agents such as BM or alkylating agents like methyl methanesulfonate (MMS).

BM is a radiomimetic drug that promotes site-specific free radical attack on deoxyribose moieties, generating AP sites as well as single- and double-strand breaks with mainly 3′-phosphoglycolate ends (Povirk, 1996). The AP sites are repaired through the base-excision repair (BER) pathway by the sequential action of AP-endonucleases (Endo IV and Exo III), DNA polymerase I and DNA ligase (Morita et al., 2010). The 3′-phosphoglycolate ends are removed as free 2-phosphoglycolate by Endo IV and Exo III before the strand breaks are repaired by the homologous recombination pathway (Fig. S1). MMS mainly produces methylations in several positions of purine bases (Sedgwick, 2004; Sedgwick et al., 2007). The major base modifications are N^7-methylguanine or N^3-methyladenine. These lesions are an important source of AP sites, which are mainly generated during their repair by the DNA glycosylases Tag or/and AlkA. O^6-methylated guanines, also produced as minor products, are directly repaired by the alkyltransferases Ogt and Ada (Fig. S1).

The BER pathway is also involved in the processing of AP sites generated during the repair of spontaneous DNA lesions. One source of such AP sites is the action of uracil DNA glycosylases (UDG). These enzymes remove uracil present in DNA either as a product of cytosine deamination or mistaken incorporation of dUMP during replication (Morita et al., 2010).

Here, we report that *E. coli* GAPDH is required for the efficient repair of DNA lesions induced by BM or MMS, as well as of spontaneous AP sites. Deficiency in GAPDH leads to increased sensitivity to these agents and changes in cell morphology, which are consistent with the induction of the SOS response. Using pull-down assays with recombinant proteins expressed in different mutant backgrounds, we show interaction between GAPDH and proteins of the BER pathway, specifically Endo IV and UDG, as well as between GAPDH and SSB.

2. Materials and methods

2.1. Bacterial strains, growth conditions and preparation of cell extracts

The *E. coli* strains and plasmids used are listed in Table 1. Bacterial cells were routinely grown at 37 °C in Luria-Bertani broth (LB), except the *gapA* mutant strains, which were grown in minimal medium (SM) with malate and glycerol as carbon sources (Ganter and Plückthun, 1990). In drug challenge experiments, cells were grown in SM with 0.5% casein acid hydrolysate (CAA) as carbon source (Pellicer et al., 2003). For *gapA* mutants, this medium was supplemented with 20 mM glycerol Growth was monitored by measuring the optical density at 600 nm (OD_{600}). When required, tetracycline (12.5 μg/ml), chloramphenicol (30 μg/ml), ampicillin (100 μg/ml) or rifampicin (Rif) (50 μg/ml) was added to the medium. Genetic crosses were performed by P1 transduction (Miller, 1992).

To construct strain MC4100 Δ*gapA*, the Δ*gapA::tet* mutation present in strain W3CG was transduced into strain MC4100. Since GAPDH-deficient mutants do not grow in LB, strain W3CG was first transformed with plasmid pBAD-gapA (Ferreira et al., 2013). Expression of GAPDH-V5 from this recombinant plasmid restored GAPDH deficiency and allowed strain W3GC to grow in this medium. The P1 lysate obtained from this transformed strain was used to infect strain MC4100, and transductants were selected on SM-malate-glycerol plates containing tetracycline. The correct insertion of Δ*gapA::tet* in the transductants was assessed by negative growth on SM-glucose plates. One of these transductants, strain MC4100 Δ*gapA*, was selected and GAPDH deficiency was demonstrated by enzymatic activity and western blot analysis.

Cell extracts were obtained by sonic disruption of bacterial cells as described previously (Aguilera et al., 2012).

2.2. Recombinant DNA techniques

Bacterial genomic DNA was obtained using the Wizard Genomic DNA purification kit (Promega), and plasmid DNA was prepared using the Wizard Plus SV Midipreps DNA purification system (Promega). DNA manipulations were performed essentially as described elsewhere (Sambrook and Rusell, 2001). DNA fragments were amplified by PCR using *E. coli* chromosomal DNA as a template. Primers used in this work are listed in Table S1.

To obtain recombinant GST-Ogt, the *ogt* gene was amplified by PCR and cloned into *Bam*HI/*Eco*RI restriction sites of vector pGEX-3x (Amersham) yielding plasmid pGEX-Ogt. Recombinant His_6-Exo III was produced from plasmid pQE30-XthA, which was constructed by cloning the PCR-amplified *xthA* gene into *Bam*HI/*Hin*dIII restriction sites of vector pQE30 (Qiagen).

2.3. Cloning of gapA antisense RNA (asRNA) sequences into IPTG-inducible vectors for conditional gene silencing

To obtain *gapA* asRNA, we used a collection of IPTG-inducible expression vectors that allow conditional expression of the asRNA with paired termini (PTasRNA), thus conferring great RNA stability and high silencing efficacy (Nakashima et al., 2006; Nakashima and Tamura, 2009). Two sets of oligonucleotides were used to amplify two different *gapA* asRNA sequences (Table S1). Both asRNAs were complementary to the genomic region that encompasses the *gapA* ribosome binding site and the start codon. The amplified PCR products were cloned into the *Nco*I/*Xho*I restriction sites of the PTasRNA vectors pHN1009, pHN678 or pHN1242, which differ in their copy number (Table 1). Strain XL1-Blue was used for cloning and plasmid preparation. Strain MC4100 was used as a host for expressing *gapA* PTasRNA in silencing experiments. To this end, cells of strain MC4100 bearing the *gapA* PTasRNA constructs were grown overnight in the absence of IPTG. These cultures were diluted 1:200 with fresh medium containing 1 mM IPTG and cultured to mid- to late-logarithmic phase depending on the experiment. Knockdown GAPDH expression under these conditions was assessed by measurement of enzyme activity and western blot analysis in cell extracts.

2.4. Expression and purification of recombinant proteins

Recombinant wild-type GAPDH and the derived C149A and C153A mutants were expressed and purified using the glutathione-*S*-transferase (GST) gene fusion system with recognition sites for factor Xa cleavage, as described elsewhere (Egea et al., 2007; Aguilera et al., 2009). This expression system was also used to obtain recombinant Ogt. For this protein, induction and purification conditions were the same as those described for GST-GAPDH.

Proteins Endo IV (Nfo), single-stranded DNA binding protein (SSB), 6-methylguanine-DNA methyltransferase (Ada), 3-methyladenine DNA glycosylases I (Tag) and II (AlkA) and uracil