Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Further characterization of the Maillard deglycase DJ-1 and its prokaryotic homologs, deglycase 1/Hsp31, deglycase 2/YhbO, and deglycase 3/YajL

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ARTICLE INFO

*Article history:*
Received 5 June 2018
Accepted 13 June 2018
Available online xxx

*Keywords:*
Electrophile stress
Carbonyl stress
Glycation
Maillard reaction
Protein/nucleotide repair

ABSTRACT

We reported recently that the Parkinsonism-associated protein DJ-1 and its bacterial homologs Hsp31, YhbO and YajL function as deglycases that repair proteins and nucleotides from endogeneous glycation by glyoxal and methylglyoxal, two reactive by-products of glucose metabolism responsible for up to 60% of glycation damage. Here, we show that DJ-1, deglycase 1 and deglycase 2 repair glyoxal- and methylglyoxal-glycated substrates, whereas deglycase 3 principally repairs glyoxal-glycated substrates. Moreover, deglycase 1 and 2 are overexpressed in stationary phase, whereas deglycase 3 is steadily expressed throughout bacterial growth. Finally, deglycase mutants overexpress glyoxalases, aldoketoreductases, glutathione-*S*-transferase and efflux pumps to alleviate carbonyl stress.

In the discussion, we present an overview of the multiple functions of DJ-1 proteins. Our thourough work on deglycases provides compelling evidence that their previously reported glyoxalase III activity merely reflects their deglycase activity. Moreover, for their deglycase activity the Maillard deglycases likely recruit: i) their chaperone activity to interact with glycated proteins, ii) glyoxalase 1 activity to catalyze the rearrangement of Maillard products (aminocarbinols and hemithioacetals) into amides and thioesters, respectively, iii) their protease activity to cleave amide bonds of glycated arginine, lysine and guanine, and iv) glyoxalase 2 activity to cleave thioester bonds of glycated cysteine. Finally, because glycation affects many cellular processes, the discovery of the Maillard deglycases, awaited since 1912, likely constitutes a major advance for medical research, including ageing, cancer, atherosclerosis, neurodegenerative, post-diabetic, renal and autoimmune diseases.



## 1. Introduction

We reported recently that the Parkinsonism-associated protein DJ-1/Park7, generally described as an oxidative stress response protein [1], functions as a protein and nucleic acid deglycase that repairs methylglyoxal- and glyoxal-glycated cysteines, arginines, lysines and guanines [2,3]. Methylglyoxal ($CH_3$-CO-CHO) and glyoxal (CHO-CHO) are potent endogenous glycating agents, principally formed as glycolytic by-products, and responsible for up to 60% of glycation damage. Glycation starts by the Maillard reaction [4], which involves the spontaneous covalent addition of a reactive carbonyl group from sugars or glyoxals, the glycating agents, with thiol and amino groups of proteins and nucleic acids [5]. The condensation reaction between amino acids or nucleotides and carbonyls begins with the formation of a hemithioacetal with cysteines (Cys-S-CHOH-R), and formation of aminocarbinols with arginines, lysines and guanines (Arg/Lys/Gua-NH-CHOH-R), which are transformed into Schiff bases, Amadori products, advanced glycation end-products (AGEs) and protein crosslinks [2,5]. By degrading hemithioacetals and aminocarbinols, deglycases prevent AGE formation, and protein and nucleic acid inactivation.

*Abbreviations:* MGO, methylglyoxal; GO, glyoxal; AGE, advanced glycation end product.

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https://doi.org/10.1016/j.bbrc.2018.06.064
0006-291X/

Deglycases repair glycated substrates, and, like glyoxalases, release lactate (from methylglyoxal-glycated substrates) or glycolate (from glyoxal-glycated substrates), so that they have been inappropriately characterized as glyoxalases III [2–3, 6–14, see discussion]. Hsp31, YhbO and YajL from *Escherichia coli*, previously described as stress response proteins [12–15], and protease PfpI from *Pyrococccus furiosus* also function as deglycases [12–14,16], suggesting that most proteins of the PfpI/Hsp31/DJ-1 superfamily, especially those previously characterized as glyoxalases III, might function as deglycases [2,3,6–14,16]. In the present study, we characterize further the deglycase activities of DJ-1 and its prokaryotic homologs, and present an overview of the chaperone, peptidase, glyoxalase and deglycase activities of PfpI/Hsp31/DJ-1 superfamily members.

## 2. Materials and methods

### 2.1. Protein expression and purification

DJ-1, deglycases 1, 2 and 3 were expressed and purified as described [3].

### 2.2. Glycation/deglycation of guanine nucleotides

These experiments were performed as described [3].

### 2.3. Glycation/deglycation of N-acetyl-cysteine

Glycation/deglycation of NacCys by MGO was monitored by hemithioacetal absorbance at 288 nm [2]. Glycation/deglycation of NacCys by GO was monitored by RP-HPLC analysis of the hemithioacetal. Samples were injected into a Kromasil Eternity C18 column at 40 °C equilibrated in 25 mM monobasic sodium phosphate, 0.3 mM of the ion-pairing agent 1-octane sulfonic acid, 4% (v/v) methanol (pH 2.7). Products were monitored spectrophotometrically at 210 nm [2].

### 2.4. Bacterial strains, preparation of bacterial extracts and immunoblotting

The wild type *E. coli* strain MG1655 was grown in Luria broth (LB) [2]. At different times, a volume of the bacterial culture equivalent to 1 ml at $OD_{600} = 4$ was centrifuged, resuspended in 1 ml of LB and treated with 5% trichloracetic acid. After centrifugation at 13,000 g, the pellet was resuspended in 400 μl of acetone, and the 13,000 g acetone pellet was dissolved in protein solubilization buffer. Whole cell fractions were separated by SDS-PAGE, transferred to a nitrocellulose membrane and probed with anti-Hsp31, anti-YhbO and anti-YajL antibodies, following the manufacturer's protocol (Cell Biolabs Inc.).

### 2.5. DNA microarray measurements

These experiments were performed as described [15]. The microarray data were deposited in the Gene Expression Omnibus (GEO) database under accession no. GSE42702.

## 3. Results

### 3.1. Nucleotide repair by the deglycases

Guanine glycation by methylglyoxal (Fig. 1A) starts with rapid formation of an aminocarbinol (Fig. 1B) that transforms itself into cyclic imidazopurinones dG-MG [3] (Fig. 1D) and carboxyethyl-deoxyguanosine CEdG (Fig. 1F). Guanine glycation by glyoxal leads to the formation of an aminocarbinol (similar to that shown in Fig. 1B), that transforms itself into cyclic imidazopurinones dG-G (Fig. 1C) and carboxymethyl-deoxyguanosine CMdG (Fig. 1E). In our experimental conditions, aminocarbinol formation occurred within minutes, and imidazopurinones within hours [3]. Glycation may also occur on the N3 and N7 guanine nitrogens, and may generate diglycated guanines [3].

Nucleotide sanitization (also called preventive DNA repair) provides a major contribution to DNA damage repair [17]. Altered deoxyribonucleotides induce mutations after being incorporated into DNA and transcriptional mutagenesis by directing misincorporation of ribonucleotides into RNA [17]. Altered ribonucleotides are responsible for translational defects and unequal availability of nucleoside diphosphates for ribonucleotide reductase [17]. We reported previously that DJ-1 repairs MGO-glycated dGTP, GTP, GDP and GMP, and GO-glycated GTP, and that *E. coli* deglycases 1 and 2, but not deglycase 3, efficiently repair MGO-glycated GTP (3).

In the present study, we investigated further the nucleotide sanitization activities of deglycases. MGO-glycated dGTP, GDP and GMP were repaired by DJ-1, deglycases 1 and 2, but not by deglycase 3 (Fig. 1 **H-K**), whereas GO-glycated GTP, GDP, dGTP and dGMP were repaired by DJ-1 and deglycases 1 to 3 (Fig. 1 **G, L-O**) (in Fig. 1G, glycated GDP (eluting at 4.54 and 4.81 min) is decreased in the presence of deglycase 3). Under our experimental conditions, 40%–90% sanitization of glycated nucleotides was obtained within 1 h, and almost complete sanitization within 2 h. Except for the negligible activity of deglycase 3 for MGO-glycated nucleotides, DJ-1 and deglycases 1, 2, or 3 displayed relatively similar sanitization activities for all nucleotides tested.

Interestingly, the apparent MGO glyoxalase III activities reported for bacterial deglycases [8] rank in the same order, Hsp31 > YhbO ≫ YajL, as their deglycase activities for MGO-glycated GTP, and their apparent GO glyoxalase III activities rank in the same order, YhbO > YajL > Hsp31, as their deglycase activities for GO-glycated nucleotides.

Thus, whereas deglycase 3 efficiently repairs only GO-glycated nucleotides, DJ-1, deglycases 1 and 2 repair both GO- and MGO-glycated nucleotides. Interestingly, human DJ-1 repairs all glycated guanine nucleotides, in contrast with MTH1, MTH2, MTH3 and NUDT5, that repair 8-oxoguanines, but exhibit distinct preferences for 8-oxoguanine-containing ribo-or deoxyribonucleosides, tri- or diphosphates [3]. Finally, whereas Maillard deglycases repair glycated nucleotides to intact nucleotides, sanitization of 8-oxo-guanine nucleotides involves the degradation of triphosphate and diphosphate nucleotides to monophosphate nucleotides.

### 3.2. Cysteine repair by the deglycases

The hemithioacetal formed by *N*-acetyl-cysteine and MGO was detected by its absorbance at 288 nm [2,13]. DJ-1, deglycases 1 and 2, degraded the hemithioacetal as previously reported [2,13,14], whereas deglycase 3 did not (Fig. 2A). We investigated by RP-HPLC the formation of the *N*-acetyl-cysteine/GO hemithioacetal. Whereas, *N*-acetylcysteine migrated as a single peak eluting at 5.88 min (not shown), incubation of 1 mM *N*-acetylcysteine with 4 mM glyoxal led to the formation of the hemithioacetal migrating at 5.55 min. Hemithioacetal accumulation was prevented by DJ-1 (Fig. 2B), and by deglycases 1, 2 and 3 (Fig. 2C).

Thus, the four deglycases repair GO-glycated cysteine, whereas DJ-1, deglycases 1 and 2, but not deglycase 3, repair MGO-glycated cysteine. Although it is unable to repair cysteine and guanine, deglycase 3 repairs MGO-glycated glyceraldehyde-3-phosphate dehydrogenase, serum albumin and collagen, although less efficiently than deglycases 1 and 2 [13,14]. Moreover, deglycase 3

Please cite this article in press as: G. Richarme, et al., Further characterization of the Maillard deglycase DJ-1 and its prokaryotic homologs, deglycase 1/Hsp31, deglycase 2/YhbO, and deglycase 3/YajL, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.06.064