Parkinsonism-associated Protein DJ-1/Park7 Is a Major Protein Deglycase That Repairs Methylglyoxal- and Glyoxal-glycated Cysteine, Arginine, and Lysine Residues

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Background: Protein glycation is a nonenzymatic covalent reaction between proteins and carbonyl groups resulting in protein denaturation.

Results: DJ-1 is the first protein deglycase that repairs proteins from glycation by glyoxals, which constitutes most glycation damage.

Conclusion: DJ-1 is a novel protein repair enzyme that protects proteins against glycation.

Significance: DJ-1 deglycase activity changes our view on glycation/deglycation and DJ-1-associated Parkinsonism.

Glycation is an inevitable nonenzymatic covalent reaction between proteins and endogenous reducing sugars or dicarbonyls (methylglyoxal, glyoxal) that result in protein inactivation. DJ-1 was reported to be a multifunctional oxidative stress response protein with poorly defined function. Here, we show that human DJ-1 is a protein deglycase that repairs methylglyoxal- and glyoxal-glycated amino acids and proteins by acting on early glycation intermediates and releases repaired proteins and lactate or glycolate, respectively. DJ-1 deglycates cysteines, arginines, and lysines (the three major glycated amino acids) of serum albumin, glyceraldehyde-3-phosphate dehydrogenase, aldolase, and aspartate aminotransferase and thus reactivates these proteins. DJ-1 prevented protein glycation in an Escherichia coli mutant deficient in the DJ-1 homolog YajL and restored cell viability in glucose-containing media. These results suggest that DJ-1-associated Parkinsonism results from excessive protein glycation and establishes DJ-1 as a major anti-glycation and anti-aging protein.

The Parkinsonism-associated protein DJ-1/Park7 is described as a multifunctional oxidative stress response protein that belongs to the Pfpl/Hsp31/DJ-1 superfamily, whose members possess a conserved exposed cysteine involved in environmental stress resistance (1–3). DJ-1 has been reported to function as a chaperone for synuclein (4, 5), a covalent chaperone for the thiol proteome (6, 7), a peroxidase (8), a protease (9), a glyoxalase (10), a stabilizer of Nrf2 (11), an apoptosis inhibitor via interaction with Daxx (12), and a translational regulator (13, 14).

We reported recently that DJ-1 formed mixed disulfides with sulfonylated proteins and released repaired proteins following the reduction of mixed disulfides by low molecular weight thiols (6, 7). The ability of DJ-1 to repair covalently modified proteins, the fact that its presumed chaperone, protease, and glyoxalase activities are weaker than those of major chaperones, proteases, and glyoxalases (3, 9, 10), the finding that enzymes involved in protein repair, such as thioredoxins and peptidyl prolyl isomerases, display chaperone properties (15–17), and the co-expression of the Bacillus subtilis DJ-1 homolog YraA with the aldoketoreductase AdhA, which is involved in carboxyl stress resistance (18), suggested to us that DJ-1 might be involved in glycedated protein repair.

The nonenzymatic reaction between sugar carbonyl groups and amino acids was discovered by the French chemist Louis Camille Maillard in 1912 (19). This reaction also occurs in vivo (20) and involves the spontaneous reaction of endogenous and exogenous carbonyls with the thiol and amino groups of proteins, nucleic acids and amino lipids (21). The condensation reaction between carbonyls and amino acids begins with the rapid formation of a hemithiocetal with cysteines and formation of aminocarbinols with arginines and lysines (21, 22), after which a series of dehydrations, oxidations, and rearrangements leads to a myriad of products, including Schiff bases, Amadori products, advanced glycation end products (AGEs),2 and protein cross-links (21). Mechanisms to protect the cells against carbonyl/electrophile stress involve aldoketoreductases, which reduce carbonyls into alcohols (23); glyoxalases, which degrade glyoxals into acid-alcohols (24, 25); detoxification systems that form and export electrophile-glutathione conjugates (26), and fructosamine-3-kinases (FN3Ks), which, by phosphorylating lysine-fructosamines formed after Amadori rearrangement of protein lysines glycated by glucose, increase their rate of deglycation by a factor of three and release repaired proteins and proteins by a factor of three and release repaired proteins.

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2 The abbreviations used are: AGE, advanced glycation end product; MGO, methylglyoxal; GO, glyoxal; CML, carboxymethyl lysine; G-H1, N-(5-hydro-4-imidazolon-2-yl)ornithine; MG-H1, N-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithine; FN3K, fructosamine-3-kinase; FBP, fructose-1,6-biphosphate; RP, reverse phase; DTNB, 5,5′-dithiobis(nitrobenzoic acid).
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3-deoxyglucosone (27). Moreover, FN3K-related enzymes deglycate lysines glycated by ribose-5-phosphate and erythrose-4-phosphate, and prokaryotic amadoriases and fructosamine-6-kinases deglycate free lysine-fructosamines for metabolic purposes (26). Here, we show that DJ-1 is a major protein deglycase that repairs methylglyoxal- and glyoxal-glycated amino acids and proteins and releases deglycated proteins and lactate or glycolate, respectively.

EXPERIMENTAL PROCEDURES

DJ-1 Expression and Purification—The DJ-1 gene was amplified by PCR from a human kidney cDNA library and inserted into the expression plasmid pET-21a, and this recombinant plasmid was introduced into Escherichia coli strain BL21 (DE3) (7, 9). DJ-1 was purified as described previously (28). The C106S, C53S, and C46S DJ-1 mutants were expressed using recombinant pET-21a plasmids (7, 9) and purified as described previously (28).

Deglycation of N-Acetyl cysteine, N-Acetylgarginine, and N-Acetyllysine—Experiments were performed at 22 °C in N2-gassed 50 mM sodium phosphate buffer, pH 7.0. Glycation of NacCys by MGO and its deglycation by DJ-1 were monitored by hemithioacetal absorbance at 288 nm (29) and reverse phase (RP)-HPLC. Glycation/deglycation of NacArg and NacLys was followed by measurement of kinetic constants, and elution was achieved with a 4–10% methanol gradient. Col- and 4% (v/v) methanol at pH 2.7 adjusted with 85% phosphoric acid dehydrogenase. For RP-HPLC, samples were injected into a Kromasil Eternity C18 column at 40 °C (for Cys and Lys) or a Kromasil C106 column at 40 °C (for Arg). Mobile phase for isocratic elution was 80 mM NacArg was incubated at 22 °C in 150 μl of buffer with 80 mM MGO. After 40 min, the mixture was incubated with 4 μM DJ-1. Lactate was analyzed with d- and l-lactate dehydrogenases. For RP-HPLC, samples were injected into a Kromasil Eternity C18 column at 40 °C (for Cys and Lys) or a Kromasil C106 column (for Arg). Mobile phase for isocratic elution (Cys and Lys) consisted of 25 mM monobasic sodium phosphate, 0.3 mM of the ion-pairing agent 1-octane sulfonic acid, and 4% (v/v) methanol at pH 2.7 adjusted with 85% phosphoric acid. For Arg experiments, the same mobile phase was used, and elution was achieved with a 410% methanol gradient. Column chromatography experiments were performed twice and produced similar results. For the measurement of kinetic constants, glycated N-acetylcysteine, N-acetylgarginine (from the 1.9-min peak in Fig. 1F), and N-acetyllysine were prepared by RP-HPLC and used immediately for kcat determination. The results are the mean values of three experiments (S.E. was less than 15%).

Deglycation of BSA, GAPDH, Fructose 1,6-Biphosphate Aldolase, and Aspartate Aminotransferase—Experiments were performed in N2-gassed 50 mM sodium phosphate buffer (pH 7.0). Glycation/deglycation of BSA cysteine was followed by DTNB titration (30). BSA (700 μM) was incubated with 100 mM MGO for 110 min at 37 °C, separated by gel filtration on a Bio-Gel P2, and incubated at 22 °C in the absence or presence of DJ-1. Glycation/deglycation of GAPDH was followed by measurement of GAPDH activity (30) and titration of thiol groups. GAPDH from rabbit muscle (300 μM monomers) was incubated with 5 mM MGO at 22 °C for 40 min, separated from MGO by gel filtration, and incubated in the absence or presence of DJ-1. In experiments where DJ-1 was added to the initial glycation mixtures, GAPDH activity was measured by adding 5 μl of a 100-fold diluted glycation mixture to a cuvette containing 150 μl of substrates. Glycation/deglycation of BSA lysines was followed by measuring the absorbance at 333 nm (31). BSA was incubated in buffer at 22 °C in the absence or presence of 6 mM MGO and DJ-1, as indicated. MGO concentrations were measured with 2,4-dinitrophenylhydrazine (10), and lactate concentrations were determined using L- or D-lactate dehydrogenase. BSA arginines were measured using phenanthrenequinone (32). Glycation/deglycation of fructose bisphosphate aldolase A (from rabbit muscle; EC 4.1.2.13; Sigma) and aspartate aminotransferase (from pig heart, cytoplasmic; EC 2.6.1.1; Sigma) were followed by measuring enzymatic activities (33, 34), by detecting the glycated forms with anti-AGE antibodies (Cell Biolabs Inc.), and by mass spectrometry. Deglycation experiments were performed at least three times, and representative results are shown.

Measurement of MGO or GO Levels and Lactate or Glycolate Formation—DJ-1, MGO, or GO was added to N2-gassed 50 mM sodium phosphate buffer, pH 7.0, at 22 °C in a total volume of 70 μl. To measure the disappearance of MGO or GO, the reaction was stopped by the addition of 10 μl of reaction mixture to 120 μl of 0.1% 2,4-dinitrophenylhydrazine solution. The solution was incubated for 15 min at 22 °C, 160 μl of 10% NaOH was added, and absorbance was measured after 15 min at 540 nm for MGO and 350 nm for GO. The appearance of lactate was measured using L- or D-lactate dehydrogenase (35). Glycolate formation was measured by RP-HPLC (10). The results are the mean values of three experiments (S.E. was less than 15%).

Bacterial Strains, Bacterial Extracts, and Immunoblotting—The wild-type E. coli strain BL21 (DE3), the yajL mutant, and these two strains supplemented with the plasmids pCA24N-yajL and pET21a-DJ-1 (7) were grown overnight in Luria broth supplemented with 0.6% glucose. YajL and DJ-1 were induced with 1 mM isopropyl β-D-thiogalactopyranoside. Bacterial lysates were prepared by ultrasonic disruption of bacteria resuspended at A600 = 100 in 50 mM sodium phosphate, pH 7.0, and 50 mM NaCl. For immunoblotting with anti-AGE antibodies, 3,000 g of supernatants of bacterial lysates (10 μg) or purified proteins (0.2 μg, unless otherwise indicated) were separated by SDS-PAGE. Proteins were transferred to a nitrocellulose membrane and probed with anti-AGE antibodies, following the manufacturer’s protocol (Cell Biolabs Inc.). Rabbit anti-YajL antibodies were prepared by Eurogentec, using purified YajL from our laboratory, and rabbit anti-DJ-1 antibodies were obtained from Abcam. For measuring cell viability, cultures were serially diluted from 102 to 106 and spotted (4 μl) onto LB plates, and growth was observed after 20 h of incubation at 30 °C. For the fluorescent analysis of total E. coli protein, the yajL mutant and the yajL mutant transformed with pET21a-DJ-1 (induced with isopropyl β-D-thiogalactopyranoside at A600 = 0.8) were grown at 37 °C in LB medium. At A600 = 1.2 (late exponential phase), MGO was added to half of the samples, and bacterial lysates were prepared at the indicated times after treatment. Lysates were dialyzed for 3 h against 50 mM sodium phosphate buffer, pH 7.0, and incubated for 48 h at 22 °C, and fluorescence (excitation at 350 nm, emission at 440 nm) was measured at a protein concentration of 0.5 mg/ml using an F-2000 Hitachi fluorimeter. Bovine serum albumin at 0.5 mg/ml was used as a standard. Viability experiments, AGE...
detection experiments, and protein fluorescence analysis were performed three times; representative results are shown.

**RESULTS**

Methylglyoxal and Glyoxal Metabolism

In addition to being an environmental toxin, methylglyoxal forms spontaneously as a glycolytic by-product and is detoxified by the sequential action of glyoxalases 1 and 2. It forms covalent adducts with cysteines, arginines, and lysines (hemithioacetals with cysteines and aminocarbinols with arginines and lysines) and also forms imidazolidines with the arginine guanidino group. Hemithioacetals, aminocarbinols, and imidazolidines are transformed into intermediate glycation products (such as Schiff bases) and AGEs, including MG-H1 and carboxyethyllysine (CEL), and are also transformed into cross-link products such as methylglyoxal-lysine dimers (MG-DLD; not shown). DJ-1, as described in this paper, transforms hemithioacetal (via a thioester) into cysteine and lactate, and aminocarbinol (via an amide) into arginine/lysine and lactate, and in this way prevents the formation of Schiff bases and AGEs. Glyoxal (CHO-CHO) is formed by lipid peroxidation and the degradation of saccharides and glycated proteins and is detoxified by glyoxalases (not shown). Glyoxal and methylglyoxal form similar glycation adducts, and the main glyoxal-derived AGEs are G-H1, carboxymethyllysine and glyoxal-lysine dimers (GOLD) (not shown). DJ-1 also repairs glyoxal-glycated proteins and produces glycolate (CH$_2$OH-COOH) instead of lactate.

**Methylglyoxal and Glyoxal Metabolism**

In addition to being an environmental toxin, methylglyoxal forms spontaneously from triosephosphates in all organisms (Scheme 1) and in minor amounts from threonine and acetone. Glyoxal is formed by lipid peroxidation and the degradation of saccharides and glycated proteins (21). The cellular concentrations of methylglyoxal and glyoxal are in the ranges 1–5 and 0.1–1 $\mu$M, respectively (21). Methylglyoxal and glyoxal are detoxified into lactate and glycolate, respectively, by the sequential action of glyoxalases 1 and 2 (24, 25) (Scheme 1), as well as by aldoketoreductases and efflux pumps (23, 26).

Methylglyoxal and glyoxal are potent glycating agents that form covalent adducts with cysteines, arginines, and lysines. With regard to the activity of methylglyoxal, reaction with a cysteine residue results in the rapid formation of a hemithioacetal, reaction with a lysine residue produces the aminocarbinol
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lysinemethylglyoxal glycosylamine, and reaction with an arginine residue forms the aminocarbinol arginine methylglyoxal glycosylamine and a 4,5-dihydroxy-5-methylimidazolidine derivative (Scheme 1). The lysine and arginine derivatives undergo dehydratation and/or rearrangement, which slowly lead to the formation of intermediate and AGEs, including Schiff bases, MG-H1 (derived from arginine), and carboxyethyllysine (Scheme 1). Protein glycation by glyoxal proceeds via similar reactions, producing the AGEs G-H1 (derived from arginine) and carboxymethyllysine (Lys-NH-CH$_2$-COOH; not shown). The bifunctional carboxyls of methylglyoxal and glyoxal form cross-linked products, such as MOLD (methylglyoxal-lysine dimer) and GOLD (glyoxal-l-lysine dimer). Degradation of hemithioacetals and aminocarbinols by DJ-1 (Scheme 1), as described in the present work, prevents the formation of these AGEs.

**DJ-1 Deglycates Cysteine, Arginine, and Lysine**

* N-Acetylcysteine Deglycation—We incubated equimolar concentrations of methylglyoxal and N-acetylcysteine for 2 min to form the hemithioacetal adduct (the product of the first reaction described below), which is detectable by its absorbance at 288 nm (29). We investigated hemithioacetal degradation in the presence of DJ-1 by measuring the decrease in absorbance at 288 nm and analyzing the reactants on a C18 RP-HPLC column (Fig. 1, A–C). The hemithioacetal was stable but exhibited a half-time of 30 min in the presence of 4 μM DJ-1 (Fig. 1, A and B). The $K_m$ and $k_{cat}$ of cysteine glycation at 22 °C were 0.3 mm and 0.4 s$^{-1}$ respectively (Table 1). Hemithioacetal degradation resulted in the quantitative formation of N-acetylcysteine and lactate (Fig. 1, B–D). Lactate analysis with l- and d-lactate dehydrogenases showed that NacCys deglycation produced 67% l-lactate and 33% d-lactate (Fig. 1D). The following reactions are likely to have occurred.

NacCys-SH + CHO-CO-CH$_3$ → NacCys-S-CHOH-CO-CH$_3$

(Spontaneous hemithioacetal formation)

NacCys-S-CHOH-CO-CH$_3$ → NacCys-S-CO-CHOH-CH$_3$

(H migration is catalyzed by DJ-1, reminiscent of glyoxalase 1)

NacCys-S-CO-CHOH-CH$_3$ → NacCys-SH + COOH-CHOH-CH$_3$

(Thioester hydrolysis is catalyzed by DJ-1, reminiscent of glyoxalase 2.)

**REACTIONS 1–3**

Thus, DJ-1 degrades the hemithioacetal formed between N-acetylcysteine and methylglyoxal into N-acetylcysteine and a 67/33 mixture of l- and d-lactate.

* N-Acetyllysine Deglycation—We next incubated equimolar concentrations of NacLys and methylglyoxal for 40 min to form glycated NacLys, which is composed primarily of the aminocarbinol NacLys methylglyoxal glycosylamine, the product of the first reaction written below (29). RP-HPLC analysis of the glycation mixture after incubation shows the NacLys peak and the glycated NacLys peak. When the glycation mixture was incubated with 4 μM DJ-1, the glycated NacLys peak decreased, whereas the NacLys peak increased (Fig. 1, E and F), and quantitative formation of l-lactate occurred (Fig. 1, D and F). Thus, DJ-1 deglycates glycated N-acetyllysine into N-acetyllysine and lactate. The reactions likely to have occurred are described below in the paragraph dealing with NacArg.

**N-Acetyllarginine Deglycation**—To form glycated NacArg, we incubated equimolar concentrations of NacArg and methylglyoxal for 40 min. Under similar conditions, Lo et al. (29) reported formation of the aminocarbinol NacArg-methylglyoxal glycosylamine (the product of the first reaction written below) and the imidazolidine formed by reaction of the ketone group of the aminocarbinol with the arginine imino group (Scheme 1). RP-HPLC analysis (PrimeSep200) of the glycation mixture (Fig. 1H) revealed a NacArg peak, a major NacArgglc peak (at 30 min) that likely contains the aminocarbinol formed with the arginine NH$_2$ plus the corresponding imidazolidine, and a minor NacArgglc peak (at 19 min) that possibly contains the aminocarbinol formed with the arginine δ-NH group (see below). When the glycation mixture was incubated with 4 μM DJ-1, the two glycated NacArg peaks decreased, whereas the NacArg peak increased (Fig. 1, H and I), and quantitative formation of l-lactate occurred (Fig. 1, D and F). However, whereas degradation of the NacArgglc (aminocarbinol 2) eluting at 1.9 min was rapid (~1 h, as with the degradation of NacCysglc and NacLysglc; Fig. 1G), degradation of NacArgglc eluting at 3.0 min displayed biphasic kinetics, with a rapid initial phase (which likely corresponds to degradation of aminocarbinol 1), and a slow exponential phase lasting several hours ($t_{1/2} = 2.8$ h, corresponding to a catalytic constant of $0.7 \times 10^{-4}$ s$^{-1}$ at pH 7.4 and 37 °C (29). For the degradation of aminocarbinols, the following reactions are likely to have occurred.

For NacArg, an aminocarbinol can also form with the arginine δ-NH group and imidazolidines by further reaction of aminocarbinols with the arginine imino group (Scheme 1).

NacArg/Lys-NH$_2$ + CHO-CO-CH$_3$ → NacArg/Lys-NH-CHOH-CO-CH$_3$

(Spontaneous aminocarbinol formation occurs on the NH$_2$ group)

NacArg/Lys-NH-CHOH-CO-CH$_3$ → NacArg/Lys-NH-NH$_2$ + COOH-CHOH-CH$_3$

(H migration is catalyzed by DJ-1, reminiscent of glyoxalase 1)

NacArg/Lys-NH-NH$_2$ + COOH-CHOH-CH$_3$ → NacArg/Lys-NH-CHOH-CO-CH$_3$

(Amidolysis is catalyzed by DJ-1, reminiscent of peptidase activity.)

**REACTIONS 4–6**

These results suggest that DJ-1 degrades aminocarbinol intermediates formed upon arginine (or lysine) glycation by methylglyoxal, with the quantitative release of arginine (or lysine).
FIGURE 1. Deglycation of N-acetylcysteine, N-acetylariginine, and N-acetylysine. A, NacCys and MGO (2 mM each) were incubated for 2 min, resulting in formation of the hemithioacetal, which absorbs light at 288 nm. DJ-1 (2 μM) was added to one sample at 0, 300, and 1100 s. B, E, and H, NacCys and MGO (2 mM each; B), NacArg and MGO (80 mM each; E), or NacLys and MGO (80 mM each; H) were incubated for 2 min (B) and 40 min (E and H), respectively, after which 4 μM DJ-1 was added to the glycation mixture (B) or to the 4-fold diluted glycation mixture (E and H). The amounts of Nac amino acids and glycated Nac amino acids were analyzed by RP-HPLC at various time points after DJ-1 addition. C, F, and I, the amounts of Nac amino acids, glycated Nac amino acids, and lactate were determined during glycation/deglycation experiments. MGO was added to Nac amino acids at time 0, allowing glycation to occur, and 4 μM DJ-1 was added as indicated to initiate deglycation. Nac amino acids (open circles) and glycated Nac amino acids (filled circles) were determined by RP-HPLC (as described for B, E, and H), and lactate was assessed using lactate dehydrogenases. D, the relative amounts of L-lactate (black columns) and D-lactate (gray columns) produced during deglycation of NacCys, NacArg, and NacLys by DJ-1 were analyzed using L- and D-lactate dehydrogenases. G, degradation kinetics of NacArgglc (peak eluting at 1.9 min (open symbols) and peak eluting at 3.0 min (closed symbols)). J, dependence of the rate of deglycation of the hemithioacetal formed between N-acetylcysteine and MGO on its concentration. A value of 100 represents a rate of 1.26 μmol/min/mg of DJ-1. K, deglycase activity of DJ-1 and the C106S, C53S, and C46S mutants were assayed for deglycation of the hemithioacetal formed between N-acetylcysteine and MGO. L, glutathione and MGO (2 mM each) were incubated for 15 min, resulting in formation of a thiohemiacetal that absorbs light at 288 nm, after which 4 μM DJ-1 was added to one sample at time 0.
and 1-lactate. They also suggest that DJ-1, by displacing the imidazolidine-aminocarbinol equilibrium, allows indirect degradation of imidazolidine intermediates before they convert into irreversible AGES, such as MG-H1, MG-H2, and MG-H3. Thus, in contrast with known deglycases, which only deglycate lysines (27), DJ-1 deglycates cysteines, arginines, and lysines, the three major glycated amino acids.

**Catalytic Constants of DJ-1 and Importance of Cys106**

We measured the catalytic constants of the deglycation of glycated N-acetylcysteine, N-acetylarginine, and N-acetylysine by DJ-1 (Fig. 1A and Table 1). The $K_m$ and $k_{cat}$ of the three reactions were similar (~0.4 mM for the $K_m$ and 0.3 s$^{-1}$ for the $k_{cat}$ at 22 °C), suggesting that DJ-1 principally recognizes the glycated portion of these molecules. Moreover, these $k_{cat}$ values were in the same range as those of other enzymes involved in protein folding or repair, such as GroEL and thioredoxins (16, 17).

The crystal structure of DJ-1 combined with phylogenetic analysis and physiological studies underscored the importance of cysteine 106 in its function (1–3, 5), whereas Cys53 and Cys46 appear to play secondary roles. We investigated N-acetylcysteine deglycation by C106S, C53S, and C46S DJ-1 mutants and found that the C106S mutant was inactive, whereas the other two mutants displayed activities similar to those of wild-type DJ-1 (Fig. 1K). Thus, Cys106 is the nucleophile crucial for the deglycase activity of DJ-1.

**DJ-1 Deglycates Glutathione and Protein Cysteines**

We incubated equimolar concentrations of methylglyoxal and glutathione for 15 min to form the hemithioacetal adduct, which was detectable by its absorbance at 288 nm. The hemithioacetal was stable in the absence of DJ-1 but disappeared with a half-time of 30 min in the presence of 4 mM DJ-1. In the absence of DJ-1, GAPDH activity decreased to 10% of its initial activity, whereas in its presence, it increased to 50% of its initial activity, with a half-time of 2 min. DTNB-titrated cysteines increased from 1.1 to 1.6 with similar kinetics. Thus, DJ-1 reactivates GAPDH by deglycating its active site cysteine. When 2 μM DJ-1 was added to the initial glycation mixture, GAPDH activity did not decrease, suggesting that DJ-1 deglycates GAPDH as glycation occurs, thereby preventing formation of deglycation-resistant intermediates. Moreover, when added to the initial glycation mixture, 2 μM DJ-1 fully protected 300 μM GAPDH against glycation by 5 mM glyoxal (Fig. 2C).

Remarkably, compared with *in vivo* concentrations, the concentrations of MGO and GO are >1000-fold higher (5 mM versus 1–5 μM for MGO and 0.1–1 μM for GO (25)), substrate protein concentrations are 9-fold lower (11 mg/ml GAPDH versus 100 mg/ml total cytoplasmic protein (25)), and DJ-1 concentrations are similar (2 μM in our experiment versus ~1 μM in skin, 10 μM in brain, and 40 μM in liver). Thus, DJ-1 efficiently deglycates proteins *in vitro*, even though the workload is markedly heavier than *in vivo*.

**DJ-1 Prevents Schiff Base Formation between Serum Albumin Lysines and MGO, but Does Not Degrade Schiff Bases**

When proteins react with methylglyoxal, they exhibit an increase in light absorption between 300 and 400 nm, which can be attributed to the formation of Schiff base linkages between lysine side chains and MGO. (The initially formed aminocarbinol Lys-NH-CHO-CO-CH$_3$ dehydrates in minutes to hours to form the Schiff base Lys-NH-C=O-CH$_3$ (31) (Scheme 1)). When 70 μM BSA was incubated with 6 mM MGO for 3 h, its absorption spectrum displayed a shoulder between 300 and 370 nm. Remarkably, the shoulder was considerably lower when 3 μM DJ-1 was present in the BSA/MGO mixture (Fig. 2D). In samples containing 70 μM BSA, 6 mM MGO, and 1.5 or 3 μM DJ-1, the rate of Schiff base formation was reduced by several-fold compared with samples without DJ-1 (Fig. 2E), whereas the concentration of MGO decreased by less than 12% in 60 min. This result suggests that the anti-glycation effect of DJ-1 does not result from MGO depletion but instead from its deglycase activity; moreover, in samples containing MGO and DJ-1 (or MGO and BSA), the MGO concentration did not decrease significantly, suggesting that MGO depletion correlates with BSA deglycation (see below).

In contrast with its ability to prevent Schiff base formation, DJ-1 did not degrade Schiff bases. We glycated 70 μM BSA by treatment with 6 mM MGO for 2 h and then incubated glycated BSA (separated from MGO by gel permeation chromatography) with 4 μM DJ-1. We observed no decrease in BSA absorbance at 333 nm over 90 min, suggesting that DJ-1 was unable to deglycate Schiff bases (not shown). The ability of DJ-1 to

**TABLE 1**

Catalytic constants of the deglycation by DJ-1 of glycated N-acetylcysteine, N-acetylarginine, and N-acetylysine at 22 °C in 50 mM sodium phosphate buffer, pH 7.0

| Catalytic constant | N-Acetylcysteine | N-Acetylarginine | N-Acetylysine |
|--------------------|------------------|------------------|---------------|
| $K_m$ (mM)         | 0.32             | 0.44             | 0.35          |
| $k_{cat}$ (s$^{-1}$) | 0.42             | 0.27             | 0.28          |

GAPDH performs covalent catalysis with its active site cysteine 150 (30). GAPDH activity decreased by 80% in the presence of 5 mM MGO, whereas DTNB-titrated cysteines decreased from 1.9 to 1.1, reflecting the covalent reaction of the active site cysteine with MGO (30) (Fig. 2B). We then separated GAPDH from MGO by gel permeation chromatography and incubated 60 μM GAPDH, either alone or in the presence of 2 μM DJ-1. In the absence of DJ-1, GAPDH activity decreased further, to 10% of its initial activity, whereas in its presence, it increased to 50% of its initial activity, with a half-time of 2 min. DTNB-titrated cysteines increased from 1.1 to 1.6 with similar kinetics. Thus, DJ-1 reactivates GAPDH by deglycating its active site cysteine.
prevent Schiff base formation, but not to degrade them, is consistent with its ability to degrade the aminocarbinol (Schiff base precursor). Consequently, the designation of Schiff bases (which form in hours) and Amadori products (which form in days) as early glycation intermediates should be revisited, because for DJ-1 these compounds represent late intermediates, whereas hemithioacetals and aminocarbinols, which form in seconds to minutes, represent early intermediates.

**DJ-1 Deglycates Serum Albumin Arginines**

Arginine is a major target of glycation by dicarbonyl compounds including methylglyoxal, with the initial formation of arginine-methylglyoxal glycosylamine, followed by dihydroxyimidazolidine, which is slowly dehydrated to produce arginine-derived hydroimidazolone MG-H1 (Scheme 1), the major methylglyoxal-derived AGE (25, 29). When 500 μM BSA was incubated at 37 °C with 20 mM MGO for 2 h, its titratable arginines decreased from 18.8 to 13.2 per BSA molecule as a consequence of arginine glycation, whereas they remained constant when 5 μM DJ-1 was added to the glycation mixture (Fig. 2F). Thus, DJ-1 efficiently deglycates BSA cysteines, lysines, and arginines.

**DJ-1 Deglycates FBP Aldolase and Aspartate Transaminase**

The active site of rabbit muscle FBP contains three lysines (Lys42, Lys108, and Lys230) and three arginines (Arg43, Arg149, and Arg303). As previously reported (33), 15 μM FBP was rapidly inactivated by 10 mM MGO (Fig. 3A). DJ-1 (4 μM) prevented (when added to the initial glycation mixture), stopped (when added after 10 min), or partially reversed (when added after 20 min) this inactivation (Fig. 3A). FBP was also inactivated by 5 mM GO, and 4 μM DJ-1 completely prevented this inactivation (Fig. 3B). As shown in Fig. 3C, in which aldolase was revealed with anti-AGE antibodies, DJ-1 afforded full protection against glycation of aldolase by glyoxal. 
Aldolase glycation was also analyzed by mass spectrometry. DJ-1 prevented glycation of Arg22, Lys28, Lys42, Arg43, Lys111, Cys202, Lys208, Lys230, Lys243, Arg259, Cys290, Lys318, Lys330, Arg331, Cys339, and Lys342 (Fig. 3D and Table 2); glycation ratios in the absence or presence of DJ-1 ranged from 1.9 for Cys290 and 2.6 for Lys230 (two buried residues) to 330 for Lys330 and 980 for Lys42. The median glycation ratio was 25, reflecting the efficiency of DJ-1 in the presence of high glyoxal concentrations. Lys42, Arg43, and Lys230 are located in the active site, with Lys32 and Arg43 being involved in substrate binding (mutation of Arg43 results in 14-fold decrease in activity) and Lys230 forming a Schiff base with the substrate (mutation of Lys230 results in complete loss of activity). These results confirm that DJ-1 prevents glycation of cysteines, arginines, and lysines.

The active site of cytoplasmic aspartate aminotransferase contains two arginines involved in aspartate binding and a...
lysine that forms a transient covalent bond with pyridoxal phosphate. As reported previously (34), aspartate aminotransferase (10 μM) activity was decreased by 20–90% by 1–5 mM methylglyoxal at 37 °C (Fig. 3E). The addition of 2 μM DJ-1, 30 min after the addition of MGO, rapidly restored (in 2 min) up to 90–100% of aspartate aminotransferase activity following 1–2 mM methylglyoxal stress and up to 60% after 5 mM methylglyoxal stress (Fig. 3E). As shown in Fig. 3C, DJ-1 afforded full protection against glycation by 2 mM methylglyoxal.

### DJ-1 Deglycation

We incubated seven proteins (β-casein, DnaK, BSA, DJ-1, GAPDH, fructose biphosphate aldolase, and aspartate aminotransferase; 0.8 μg each) separately with 5 mM MGO or 1 mM BSA (Fig. 4B). DJ-1 afforded full protection against glycation by 2 mM methylglyoxal.

### The Previously Reported Glyoxalase Activity of DJ-1 Reflects Its Deglycase Activity

DJ-1 has been reported to function as a glutathione-independent glyoxalase, displaying a 1,000-fold lower activity than the glutathione-dependent glyoxalases Glo1 and Glo2 (10). We demonstrate below that the apparent glyoxalase activity of DJ-1 reflects its deglycase activity. First, the kinetics of MGO degradation (at micromolar DJ-1 concentrations) displayed a lag, which was likely required for spontaneous formation of the substrate, glycated DJ-1 or glycated BSA. Duration of the lag ranged from 40 min at 1 μM DJ-1 to 5 min at 8 μM DJ-1 and 1 min in the presence of 15 μM BSA (Fig. 4A). Second, the apparent glyoxalase activity of DJ-1 increased in a linear fashion with the square of DJ-1 concentration, in accordance with DJ-1 being both an enzyme (deglycase) and a substrate (glycated DJ-1) (Fig. 4B). DJ-1 was dimeric at all concentrations tested, suggesting that this did not result from a monomer-dimer equilibrium with an active dimer (not shown) (37). Third, the negligible levels of apparent glyoxalase activity of DJ-1 (1.5 μM) were strongly stimulated by BSA, with an apparent Kₘ of ~5 μM BSA (Fig. 4C). Stimulation by BSA was also observed when glyoxal was used as a glycating agent (Fig. 4C), and the amounts of glycolate produced were similar to those of GO consumed (not shown). These results are consistent with DJ-1 substrates being glycated proteins (DJ-1 or BSA in this section) instead of glyoxals, in accordance with the results presented above.

### DJ-1 Protects Cells against Protein Glycation and Loss of Viability in Glucose-containing Media

*E. coli* contains three DJ-1 homologs, YajL, YhBo, and HchA, which are involved in resistance to environmental stresses (7, 28, 38–41) including carbonyl stress. YajL is the closest DJ-1 homolog, with YajL and DJ-1 displaying 40% sequence identity and a similar three-dimensional structure (42). In addition, the yajL mutant displayed the highest sensitivity to carbonyl stress. Glycation affects bacteria under standard laboratory conditions, leading to a decline in viability during the stationary phase, and the addition of excess glucose or methylglyoxal results in increased loss of viability (43). The yajL mutant (but not the parental strain) suffered from a 100-fold decrease in viability after incubation for 48 h in LB medium containing 0.6% glucose, and the mutant was rescued by YajL- and DJ-1-overproducing plasmids (Fig. 4D).

We also investigated the glycation status of protein extracts by probing immunoblots with anti-AGE antibodies. The wild-type strain displayed a small quantity of glycated proteins after overnight culture in LB medium supplemented with 0.6% glucose, whereas the yajL mutant displayed much higher levels of glycated proteins (Fig. 4, E and F). Protein glycation in both the wild-type strain and the yajL mutant was reduced when DJ-1 was expressed in these strains (Fig. 4, E and F).

We investigated further the effect of DJ-1 expression on glycation of the yajL mutant under basal and methylglyoxal stress conditions by examining the fluorescent properties of total protein isolated from *E. coli* cells (glycated proteins display

#### TABLE 2

Mass spectrometry analysis of fructose bisphosphate aldolase deglycation by DJ-1

The previously reported glyoxalase activity of DJ-1 reflects its deglycase activity. Three proteins were glycated for 1 h by 5 mM glyoxal in the absence of presence of 5 μM DJ-1, separated from glyoxal by gel permeation and analyzed by mass spectrometry. Glycation ratio is the ratio between glycation levels in the absence or presence of DJ-1.

| Sequence of glycated peptide | Modification and position in peptide | Amino acid | Glycation ratio | Analysis of variance | Mascot score |
|-----------------------------|--------------------------------------|------------|----------------|---------------------|-------------|
| IAHRIVAPGKILAADE            | Glyoxal (Arg⁴)                        | Arg²²      | 102            | 3.70E-05           | 23.93       |
| LSIDIAHRIVAPGKILAADE        | Glyoxal (Lys¹)                        | Lys²²⁸     | 22             | 1.71E-06           | 40.01       |
| STGSIKRIQLQISTENTEE         | Glyoxal (Lys⁵)                        | Lys⁹⁸⁰     | 980            | 1.12E-05           | 43.52       |
| STGSIKRIQLQISTENTEE         | Glyoxal (Arg⁵)                        | Arg⁴³      | 11             | 2.73E-03           | 38.45       |
| KIVVPLAGTNGE                | Glyoxal (Lys⁴)                        | Lys¹¹      | 7.7            | 0.000105           | 75.07       |
| LKRCQQVTE                  | Glyoxal (Cy⁵)                         | Cy²⁰²      | 5.4            | 3.14E-05           | 22.75       |
| KVLAAVYFALSD               | Glyoxal (Lys⁴)                        | Lys⁵⁶⁰     | 56             | 0.00995            | 47.56       |
| GFLRPANMYTPGHACTQKSYEE      | Glyoxal (Lys¹⁵)                       | Lys²³⁰     | 2.6            | 7.51E-05           | 22.41       |
| IAMATVTALRRTTPAVGTYFCLSQQSEBEE | Glyoxal (Arg¹⁵)       | Arg²⁵⁹     | 27             | 0.000572           | 27.79       |
| ASINLNAIKCPLKLFWALTFSYRAL   | Glyoxal (Cy¹⁴)                       | Cy³⁹⁰      | 1.9            | 0.0286             | 21.28       |
| KVLAAVYFALSD               | Glyoxal (Lys¹⁴)                       | Lys²⁵⁰     | 25             | 0.0016             | 45.9        |
| IAMATVTALRRTTPAVGTYFCLSQQSEBEE | Glyoxal (Arg¹⁴)       | Arg³³¹     | 43             | 5.41E-05           | 22.83       |
| ASINLNAIKCPLKLFWALTFSYRAL   | Glyoxal (Cy¹⁵)                       | Cy³³⁹      | 40             | 0.00195            | 38.72       |
| KVLAAVYFALSD               | Glyoxal (Lys¹⁵)                       | Lys⁴⁴²     | 23             | 2.43E-06           | 62.1        |

3 G. Richarme, M. Mihoub, and V. Gautier, unpublished results.
increased fluorescence (Ex350 nm) in the 400–500-nm range (44). DJ-1 expression resulted in decreased protein glycation of the yajL mutant (Fig. 4G), both in the absence of exogenous carbonyl stress (DJ-1 alleviated the increase in protein glycation observed during the transition to the stationary phase of growth (44) (Fig. 4H)) and after the induction of stress with 2 mM methylglyoxal (Fig. 4I).

**DISCUSSION**

The literature on DJ-1 should be reconsidered in view of its deglycase activity. **DJ-1 Deglycase—**To execute its deglycase activity, DJ-1 recruits its previously reported biochemical functions: (i) chaperone activity (4, 5, 28) to interact with non-native glycated proteins and gain access to partially buried glycated sites; (ii) glyoxalase 1 activity (10, 21) to interact with glycated substrates and convert hemithioacetals into thioesters, and aminocarbamins into amides; (iii) reactive cysteine 106 with a depressed pKa of 5.4 (3, 7), to attack carbonyl groups of thioesters and amides; (iv) glyoxalase 2 activity (10, 21) to cut thioesters for cysteine deglycation; and (v) amidase/peptidase activity (9, 45) to cut amide bonds for lysine/arginine deglycation.
Glutathione and Electrophile Stress—Whereas glutathione performs multiple roles in electrophile stress resistance, acting as a co-enzyme of glyoxalases and glutathione-dependent aldehyde dehydrogenases, a covalent acceptor of carbonyl compounds, and a substrate of glutathione S-transferases and multidrug resistance transporters (as a glutathione-electrophile conjugate), it is surpassed by DJ-1 for protein deglycation: glutathione is rather inefficient at degrading glycosylamines (Schiff bases) by transglycating glucose to itself (47), and in our hands, it was unable to deglycate cysteine and BSA. DJ-1 does not require glutathione as a co-factor; however, glycated glutathione constitutes a DJ-1 substrate, suggesting that DJ-1 may be involved in sanitizing the glutathione pool.

Neurodegenerative Diseases—The deglycase activity of DJ-1 suggests that DJ-1-associated Parkinsonism results from excessive protein glycation, which affects dopaminergic transmission in the substantia nigra. It also strengthens the role of protein glycation in the etiology of neurodegenerative diseases (48). Protein glycation promotes the deposition of proteins, because of protein denaturation and formation of protease-resistant cross-links, and increases protein aggregation in Alzheimer, Parkinson, and Huntington diseases. The extent of Tau, β-amyloid, synuclein, and huntingtin glycation is correlated with the pathologies of the patients (48). Moreover, glycation is responsible, via the AGE receptor, for an increase in oxidative stress and inflammation through the formation of reactive oxygen species and activation of NF-κB (49). In Alzheimer disease, the AGE receptor also stimulates the transport of β-amyloid across the blood brain barrier into the brain (49).

Glycation/Deglycation—The discovery of the deglycase activity of DJ-1 is a major advance in glycation research. Whereas scavengers involved in degradation and export of electrophiles (aldoketoreductases, glyoxalases, glutathione/glutathione S-transferases/eﬄux pumps) are relatively efficient, FN3Ks display more moderate functions in electrophile stress resistance (21, 27). Compared with FN3Ks, DJ-1 appears to be an overachiever: (i) its specific activity is 20,000-fold higher ($k_{obs}$ expressed in s$^{-1}$ instead of d$^{-1}$); (ii) it deglycates cysteines, arginines, and lysines (instead of only lysines); (iii) it deglycates all proteins tested; (iv) it operates immediately after glycation onset (whereas FN3K acts after the Amadori rearrangement); (v) it releases innocuous products (lactate and glycolate) instead of dicarbonyl 3-deoxyglucosone; (v) it does not require any co-factors (whereas FN3K requires ATP); and (vi) in accordance with its efficiency, DJ-1$^{-/-}$ mice display Parkinson disease-type abnormalities and metabolic defects (3), whereas Fn3k$^{-/-}$ mice are healthy (although they do display higher levels of free and protein-bound fructosamines (27). Nevertheless, fructosamine-3-kinases and related enzymes are involved in defense against glycation by glucose, ribose-5-phosphate and erythrose-4-phosphate, whereas DJ-1 reverses glycation by glyoxalys, which suggests that these enzymes possess complementary functions in providing protection against carbonyl stress. However, because glyoxalys are by-products of carbohydrate metabolism, DJ-1 is also involved in preventing sugar-dependent glycation. Both sugars and dicarbonyls cause important glycation damage, with sugars being more concentrated than dicarbonyls (millimolar range instead of micromolar range) but less active (methylglyoxal is 24,000-fold more potent as a glycating agent than glucose (21)). If we consider the clinical urinary excretion of glycation free adducts by type 1 diabetic subjects, fructoselysine represents 22 nmol/mg creatinine, whereas adducts mainly derived from glyoxals-dependent glycation (carboxymethyllysine, carboxethyllysine, G-H1, and MG-H1) represent 42 nmol/mg creatinine (22).

Consideration of the characteristics of DJ-1 deglycase changes our views on glycation and deglycation. First, it shortens the time scale of deglycation. Because DJ-1 catches glycation in the act, it appears that everything is settled at the onset of glycation, when it is still time for DJ-1-catalyzed deglycation. Consequently, with respect to glycation by dicarbonyl compounds, “late” should describe minutes or hours after glycation onset (29, this work) rather than days, months, or years after, as is often assumed. Second, hemithioacetals and aminocarbinols (DJ-1 substrates) that form immediately upon glycation, which are tacitly evoked in many glycation reviews (however, see Ref. 29 for a description of their formation) could be upgraded from a merely implicit status to the rank of early glycation products. Schiff bases, which form in minutes to hours, and Amadori products, which form in ~2 days and are often referred to as “early glycation products,” could be reclassified as “intermediate glycation products.” Schiff bases, Amadori products, and AGEs might represent inherently irreversible (or quasi-irreversible) damage because of their stability, diversity, and/or weak chemical reactivity. This may explain the moderate deglycating efficiencies of fructosamine-3-kinases (27) and AGE breakers (50).

Oxidative Stress Resistance—The involvement of DJ-1 in oxidative stress resistance could be explained by its ability to induce or protect oxidative stress resistance enzymes (3) and protect the thiol proteome against sulfenylation (6). It might also result from interrelations between electrophile and oxidative stresses, as follows: (i) major oxidative stress-resistance proteins, including superoxide dismutases, thioredoxins, glutaredoxins, and peroxiredoxins are inactivated by electrophile stress; (ii) glutathione is hotly contested by proteins involved in electrophile and oxidative stress resistance; and (iii) glylated proteins increase the production of reactive oxygen species (51).

It is debated whether Cys$^{106}$ oxidation to sulfenic or sulfinic form. These important functions of DJ-1 in protein repair reduce the probability (although do not eliminate it) of an important role for cysteine 106 oxidation in signaling (3).

Cancer and Apoptosis—The relationship between DJ-1 and cancer is still unclear. A number of reports suggest that DJ-1 is overexpressed in lung, prostate, breast, and pancreatic carcinomas and could be used as a biomarker for tumor diagnosis. We suggest that increased DJ-1 expression in tumors may result from the increased aerobic glycolysis of cancer cells, necessary to meet the metabolic requirements of cell proliferation (52) (the Warburg effect, a characteristic trait of Ras and Myc can-
Protein Deglycase DJ-1/Park7

cers (53)). High glycolytic/HMP rates produce elevated amounts of methylglyoxal and glycated proteins (21), resulting in the overexpression of DJ-1 and avoidance of methylglyoxal-induced toxicity and growth arrest. Glyoxalase 1, thioredoxins, and Nrf2 are also overexpressed in many tumors, providing a growth advantage by increasing chemoresistance or enhancing tumor cell growth (46, 53–55). Such a role for DJ-1 in supporting the viability or proliferation of tumor cells would be sufficient reason to use it as a tumor marker and target for anticancer drug development.

However, several results suggest that DJ-1 may perform more specific functions in cancer or cellular apoptosis. DJ-1 has been described as an oncosgene that cooperates with H-Ras or Myc, as a positive effector of the AKT pathway (via PTEN inhibition) and a negative regulator of p53 transcriptional activity (3). DJ-1 was also described as an apoptosis inhibitor, acting by binding the pro-apoptotic proteins Daxx, BCL-XL, or FADD and by forming a mixed disulfide with the apoptotic regulating kinase ASK1 (3). Several of these results could be explained by the global functions of DJ-1 as a chaperone, a covalent chaperone, a major deglycase, and an antioxidant protein enhancing cell growth. Moreover, the levels of the proapoptotic or tumor-suppressor proteins ASK, PTEN, and p53, which contain active site cysteines (Cys250 (ASK), Cys124 (PTEN), Cys176, Cys238, and Cys242 (p53, zinc binding)) that may physiologically or artifically react with DJ-1, could also be modified when DJ-1 is overexpressed, consequently generating some of the reported results.

Miscellaneous—Numerous roles for DJ-1 in unrelated functions could be explained by its extensive involvement in protein repair because any protein constitutes a potential DJ-1 substrate. Moreover, the ability of DJ-1 to repair cysteines, lysines, and arginines (frequently encountered in active sites) and the greater sensitivity of active site amino acids to glycation because of their accessibility or shifted pKa likely increase the occurrence of glycation-induced cellular defects and their correction by DJ-1.

Considering the novel function of DJ-1 and the fact that deglycation obviously constitutes its primary function, we propose that it be renamed DJ-1 deglycase. Although aldoketoreductases, glyoxalases, and multidrug detoxification systems decrease electrophile stressor pools, DJ-1 is a major protein bodyguard, providing front line defense against protein glycation. Finally, the discovery of the deglycase function of DJ-1 may constitute a major advance in biotechnology and for medical research, including aging, neurodegenerative diseases, cancer, atherosclerosis, arterial hypertension, postdiabetic, renal, and autoimmune diseases.

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