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Parkinsonism-associated protein DJ-1 is a *bona fide* deglycase

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ABSTRACT

We discovered recently that Parkinsonism-associated DJ-1 and its bacterial homologs function as protein deglycases that repair glyoxal- and methylglyoxal-glycated proteins. Protein glycation levels are 2- to 10-fold increased in deglycase-depleted cells, and deglycase mutants display up to 500-fold loss of viability in methylglyoxal or glucose-containing media, suggesting that these deglycases play important roles in protecting cells against electrophile and carbonyl stress. Although the deglycase activity of DJ-1 is well supported by extensive biochemical work, Pfaff et al. (J. Biol. Chem. *in press* http://dx.doi.org/10.1074/jbc.M116.743823) claimed in a recent study that deglycation of the hemithioacetal formed upon cysteine glycation by methylglyoxal results from a Tris buffer artefact. Here, we show that this is not the case, and that DJ-1 and its homologs are the *bona fide* deglycases awaited since the Maillard discovery.

1. Introduction

The Parkinsonism-associated protein DJ-1/Park7, an oxidative stress responsive protein endowed with chaperone, peptidase, and glutathione-independent glyoxalase activities [1,2], functions as a protein deglycase that repairs glyoxal- and methylglyoxal-glycated proteins, and releases repaired proteins and glycolate or lactate, respectively [3]. The spontaneous reaction between amino acids and carbonyl groups was discovered by the French chemist Louis Camille Maillard [4]. It involves the spontaneous reaction of carbonyls with the thiol and amino groups of proteins, nucleic acids and amino lipids [5]. Glyoxal (CHO–CHO) and methylglyoxal (CH_3–CO–CHO) are potent endogenous glycating agents which are responsible for up to 60% of glycation damage [5], and lead to protein and DNA modification, and cell dysfunction. The condensation between amino acids and carbonyls begins with the formation of a hemithioacetal with cysteines, and of aminocarbinols with arginines and lysines [3,5], that are metabolized into Schiff bases, Amadori products, advanced glycation end-products (AGEs), and protein crosslinks [5]. Defense against carbonyl stress involves glyoxalases [6,7], aldoketoreductases [8] and efflux pumps [9] which scavenge reactive carbonyls, and fructosamine-3-kinases [10] and DJ-1 family deglycases [3,11], repairing proteins that have been glycated by glucose and glyoxals, respectively.

The discovery of the DJ-1 family deglycases constitutes a milestone in glycation research, since they degrade the early glycation adducts formed by the Maillard reaction, and prevent the formation of advanced glycation endproducts (AGEs) [3]. Although the deglycase activity of DJ-1 is well supported by extensive biochemical results, Pfaff et al. claimed in a recent study [12] that deglycation of the hemithioacetal formed upon cysteine glycation by methylglyoxal resulted from a Tris artefact. They did not observe any deglycation activity of their preparation of DJ-1, either in Tris or phosphate buffer, but observed a decrease in hemithioacetal levels in the presence of undefined Tris concentrations [12]. Since our DJ-1 preparation was eluted from the last hydroxyapatite column in N_2-gassed sodium phosphate buffer pH 8.0, and since our Hsp31, YhbO and YajL deglycases, prepared in buffer containing 20 mM Tris, 20 mM NaCl, 1 mM DTT, were dialyzed against N_2-gassed 50 mM sodium phosphate buffer pH 7.0 before use, this is not the case. However, we show here that the addition to reaction mixtures of a 1–2% volume of a deglycase prepared in 20 mM Tris buffer results in submillimolar Tris concentrations that don't affect the results, and that DJ-1 is active in both Tris and phosphate buffers. Tris ($(CH_2OH)_3$–C–NH_2) reversibly reacts with carbonyl substrates via its amino group, and for example the use of 50 mM Tris buffer mediates an increase of the Ki values for erythrose 4-phosphate with glucose phosphate isomerase [13]. Because Tris can affect

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

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the cysteine/MGO/hemithioacetal equilibrium, our studies were performed in sodium phosphate buffer. In this study, we confirm that the Maillard deglycases are *bona fide* deglycases, and that the Tris effect described by Pfaff et al., in which final Tris concentrations are not provided and human DJ-1 deglycase is inactive (in contrast with our DJ-1 preparation), is not representative of our results and of those concerning the related glutathione-independent glyoxalases [14].

2. Materials and methods

2.1. DJ-purification

DJ-1 was overexpressed in *E. coli* from a pET21a-DJ-1 plasmid [15]. Bacteria were lysed by ultrasonic disruption. The crude bacterial extract was centrifuged for 1 h at 120,000 ×*g*, and the supernatant was used for DJ-1 purification. DJ-1 was purified on a DEAE-Sephacel column equilibrated in 30 mM Tris pH 8.0, 20 mM NaCl, 1 mM DTT, and a hydroxyapatite column equilibrated in the same buffer, and eluted with a linear gradient of N_2-gassed 0–200 mM sodium phosphate pH 8.0. For the Tris/phosphate experiments, DJ-1 was dialyzed against N_2-gassed Tris or sodium phosphate buffers (20 mM, pH 8.0).

2.2. Deglycation of N-acetyl cysteine

The glycation of NacCys by MGO and its deglycation by DJ-1 was monitored by hemithioacetal absorbance at 288 nm [3,16]. 1–6 μl of DJ-1 (104 or 202 μM) in 20 mM Tris pH 8.0 or 20 mM sodium phosphate pH 8.0 were added to 120 μl of 50 mM sodium phosphate pH 7.0, and absorbance was followed at 288 nm.

3. Results

3.1. DJ-1 purification

The expression and purification procedure led to soluble and pure DJ-1 (Fig. 1A).

3.2. Deglycation of N-acetyl cysteine in tris and phosphate buffers

We incubated equimolar concentrations (2.5 mM) of MGO and NacCys for 2 min in order to form the hemithioacetal adduct (the product of the first reaction written below) which is detectable by its absorbance at 288 nm [3]. We investigated hemithioacetal degradation by measuring the decrease in absorbance at 288 nm. Hemithioacetal was stable (Fig. 1B, *curve 1*), and the addition of 2, 4 or 6 μl of 20 mM Tris (final concentrations 0.3, 0.6 or 0.9 mM, respectively) did not result in any decrease in absorbance at 288 nm (Fig. 1B, *curves 2–4*). In contrast, the addition of 6 μl of DJ-1 (dialyzed against 20 mM Tris) led to a decrease in absorbance (Fig. 1B, *curve 5*), and the addition of 2, 4 or 6 μl of 104 μM DJ-1 (dialyzed against 20 mM Tris) led to decreases in absorbance that were proportional to enzyme concentration (Fig. 1C, *curves 1–3*). The addition of 10 μl of 20 mM Tris led to a detectable decrease in absorbance (the Tris effect, Fig. 1D, *curve 2*), which was considerably smaller than the decrease observed with 10 μl of 104 μM DJ-1 in 20 mM Tris (Fig. 1D, *curve 3*). Finally, the successive addition of 3 μl of 202 μM DJ-1 prepared in sodium phosphate buffer led to decreases in absorbance that were proportional to enzyme concentration (Fig. 1E). As previously reported, hemithioacetal degradation resulted in the quantitative formation of lactate (CH_3–CHOH–COOH) (data not shown) according to the mechanism of action of the deglycase:

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 catalyzed by DJ-1)

NacCys-S–CO–CHOH–CH_3 → NacCys-SH + COOH–CHOH–CH_3 (thioester hydrolysis by DJ-1)

Similar reactions also occur between methylglyoxal and the amino groups of arginine and lysine, and DJ-1 deglycates the aminocarbinols (NacArg/Lys–NH–CHOH–CO–CH_3) following the same mechanism (except that an amide bond is cleaved instead of a thioester). Of course, such formation of lactate does not occur with Tris, which, by competing with cysteine for methylglyoxal, induces only a displacement of the NacCys/MGO/hemithioacetal equilibrium.

We then used the non optimal conditions in which Pfaff et al. did not detect any deglycase activity for *Drosophila* and human DJ-1. We incubated equimolar concentrations (20 mM instead of 2 mM) of MGO and NacCys for 10 min in order to form the hemithioacetal adduct. Absorbance rose to 3.6 (sic), which is not an adequate value to perform enzymatic studies (1/4000 of incident light is transmitted), and which was beyond the range of our spectrophotometer. We diluted the mixture 2-fold and obtained a more acceptable absorbance of 1.4 (<1.8 because dilution displaces the NacCys/MGO/hemithioacetal equilibrium towards dissociation). As shown in Fig. 2, the absorbance was stable, and remained stable after the addition of 5 μl of 20 mM Tris in a 120 μl reaction mixture (0.8 mM final Tris concentration). In the Pfaff et al. experiments, the addition of an undefined volume of 20 mM Tris (which does not allow to calculate *its final concentration*) led to a decrease in absorbance at 288 nm, probably because an important volume of Tris resulted in the Tris effect. When the authors added their *Drosophila* or *human* DJ-1 preparation, there was no additional decrease in absorbance over the Tris effect. In contrast, when we added 5 μl of our human DJ-1 preparation (prepared in phosphate buffer), we observed a clear decrease in hemithioacetal absorbance (a kcat of 0.4 s^{-1}, like that previously reported by us, was calculated). Thus, our DJ-1 *deglycase* is active, whereas the DJ-1 preparations of Pfaff et al. were not. As a matter of fact, during their purification process, the major part of DJ-1 was aggregated, and a tiny fraction of their « soluble fraction » was retained on a nickel-affinity chromatography column, suggesting that the preparation was mostly composed of aggregates, that generally display low, negligible or null enzymatic activity. Researchers working on DJ-1 reported excellent expression/purification processes [2], and in our hands, DJ-1 behaves as a soluble protein whose purification is easy. From incomplete experiments (undefined final Tris concentration, inactive enzymes) Pfaff et al. conclude that the deglycase activity of DJ-1 does not exist, implying that the related glutathione-independent glyoxalases activities described by several groups [14,17,19,20,22] do not exist either.

Our results demonstrate that the unsupported hypothesis of Pfaff et al. is wrong, and their DJ-1 preparation inactive.

4. Discussion

Our experiments clearly show that DJ-1 is a *bona fide* deglycase, and that the Tris effect described by Pfaff et al. does not apply to our study [3], or to similar studies performed in phosphate buffer containing submillimolar Tris concentrations.

The Tris effect of Pfaff et al. is not supported: i) the presence of Tris in our DJ-1 preparation is not published anywhere [15,23], and the final step of the purification procedure occurred in phosphate

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