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Inactivation of Glyceraldehyde-3-phosphate Dehydrogenase by the Dopamine Metabolite, 3,4-Dihydroxyphenylacetaldehyde

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Abstract

Background—The aldehyde metabolite of dopamine, 3,4-dihydroxyphenylacetaldehyde (DOPAL) is an endogenous neurotoxin implicated in Parkinson's Disease. Elucidating protein targets of DOPAL is essential in understanding its pathology. The enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a target of DOPAL.

Methods—GAPDH activity was measured via reduction of NAD⁺ cofactor (340 nm). Protein aggregation was assessed with SDS-PAGE methods and specific modification via chemical probes.

Results—Low micromolar levels of DOPAL caused extensive GAPDH aggregation and irreversibly inhibited enzyme activity. The inactivation of GAPDH was dependent on both the catechol and aldehyde moieties of DOPAL. It is suggested that Cys are modified and oxidized by DOPAL.

Conclusions—The mechanism by which DOPAL modifies GAPDH can serve as a mechanistic explanation to the pathological events in Parkinson's Disease.

Keywords

3,4-dihydroxyphenylacetaldehyde; biogenic aldehyde; glyceraldehyde-3-phosphate dehydrogenase; Parkinson's Disease

1. Introduction

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the reversible conversion of glyceraldehyde-3-phosphate (G3P) to 1,3-bisphosphoglycerate in glycolysis[1]. GAPDH is a highly abundant and constitutively expressed enzyme and is utilized as an internal standard for quantitative protein comparison. However, recent work has shown that deficiencies in GAPDH activity or expression are possible factors in neurodegenerative

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disorders such as Alzheimer's and Parkinson's Disease (PD) [2]. In the PD rotenone mouse model, GAPDH activity was overexpressed and activity was potently inhibited [3]. Several neurodegenerative diseases are characterized by the accumulation of protein aggregates [4]. In particular, PD involves the selective loss of dopaminergic neurons in the substantia nigra and formation of proteinaceous Lewy bodies [5]. Accumulating evidence suggests that GAPDH is central to the formation of abnormal toxic protein aggregates or Lewy bodies in PD pathology. GAPDH promoted formation of Lewy bodies and co-localized with alpha-synuclein [6] and *in vivo* study showed that Lewy bodies in post-mortem PD brains were immunoreactive for GAPDH [7]. Evidence has demonstrated that GAPDH may even promote *in vivo* formation of these toxic protein aggregates [8]. The neurons affected in PD produce dopamine (DA), a neurotransmitter involved in vital physiological functions, including coordination of voluntary movement mood regulation [9].

The vulnerability of dopaminergic neurons in PD is currently unknown. Elucidating mechanistic links and protein targets are of high interest as potential biomarkers of PD pathogenesis. To account for the selective neurodegeneration observed in PD, it is hypothesized that a toxin endogenous to DA neurons is involved in the degeneration of these neurons [10, 11]. The major route of DA metabolism is deamination by monoamine oxidase (MAO), to form hydrogen peroxide and 3,4-dihydroxyphenylacetaldehyde (DOPAL). Metabolism of DOPAL is by aldehyde dehydrogenase (ALDH2) to 3,4-dihydroxyphenylacetic acid (DOPAC) and by cytosolic aldehyde reductase (AR) to 3,4-dihydroxyphenyletanol (DOPET) [10]. Due to its high electrophilicity, DOPAL modifies proteins [12–14] and is toxic to neurons [12, 15–17]. In fact, DOPAL is several of magnitudes more toxic than DA *in vitro* and *in vivo* [10, 18]. Toxicity of DOPAL is also significantly increased by auto-oxidation of the catechol to the quinone and subsequent formation of radical species [19]. DOPAL auto-oxidation greatly enhances protein cross-linking and produces superoxide radicals. Due to the presence of redox-sensitive thiols on GAPDH, it is utilized as a model for cysteine modification by electrophiles. It was extensively modified by dopamine-quinone (DAQ) [20], 4-hydroxy-2-nonenal [21]. In light of the structural similarity between DA, DAQ and DOPAL, it was hypothesized that DOPAL would similarly modify and inactivate GAPDH. We have previously used GAPDH as a protein model for crosslinking with DOPAL [14, 19]. The goals of this work are to elucidate the interaction between DOPAL and GAPDH, measure enzyme activity, mode of binding, and determine the specific sites of modification by DOPAL.

2. Materials and Methods

2.1 Chemicals

DOPAL was biosynthesized via rat liver MAO procedure as previously established [15]. Stock concentrations were determined using an ALDH assay with nicotinamide adenine dinucleotide (NAD⁺) and HPLC [16]. MOPAL was biosynthesized as previously outlined [14]. The following compounds: DA, DOPAC, GAPDH from rabbit muscle, G3P, acetonitrile (ACN), glutathione (GSH) and all other chemicals were acquired from Sigma-Aldrich (St. Louis, MO), unless otherwise noted. The DOPAL analogs, 3,4-

Rabbit muscle GAPDH (0.3 mg/mL) was incubated with DOPAL (50 mM sodium phosphate buffer, pH 7.4, 37 °C) at the given time periods. Protein samples were denatured by addition of 6X gel loading buffer (11 mM EDTA, 3.3 mM Tris-HCl, 0.017% SDS, 0.015% bromophenol blue) and heated to 80 °C for 3 min. Briefly, 9 µg of protein was loaded and 10% polyacrylamide gel was used to separate protein bands via SDS-PAGE. Protein bands were stained with Coomassie blue.

Protein samples were stained with the redox-cycling sensitive dye nitroblue tetrazolium (NBT). GAPDH and DOPAL or MOPAL or DHPAN were incubated as stated before for 4 h. GAPDH samples (5 μ g) were subjected to 10% acrylamide SDS-PAGE and transferred to nitrocellulose membrane as previously described [14, 23, 24]. The membrane was placed in 0.24 mM NBT with 2 M potassium glycine buffer (pH 10) and incubated overnight at 4 °C.

GAPDH enzymatic activity was measured by NADH production at 340 nm for 3 min [25]. After incubation of GAPDH (0.3 mg/mL) with and without DOPAL or DOPAL analogs, 100- μ L aliquots were transferred to a 96-well plate with a final volume of 200- μ L, containing: 50 mM sodium phosphate buffer at either pH 7.4 or 8.0, 1 mM NAD⁺, and 1 mM EDTA. The reaction was initiated by adding 0.8 mM G3P to each well. Enzyme activity in the presence of DOPAL was expressed as the percentage of GAPDH activity without DOPAL.

GAPDH was incubated with DOPAL as previously described in *activity assay*. Following incubation, unbound DOPAL was removed by filtration with size-exclusion spin columns (Micro Bio-Spin) according to manual (Bio-Rad laboratories) and spun for 3 min at 3000 × g. Aliquots (100-μl) were transferred from mixtures with and without filtration, and activity was measured as described in *activity assay*. Activity at each DOPAL concentration was measured with and without filtration to control for binding of enzyme to column resin. To measure inhibition recovery as a product of GAPDH thiols, GSH (0.5 mM) was briefly added to each incubation mixture prior to activity measurement.

GAPDH (1 µg) was loaded in 10% polyacrylamide SDS-PAGE. After electrophoresis, protein was transferred to nitrocellulose membrane at 20V for 45 minutes and blocked overnight in 5% BSA+TBST. Primary rabbit anti-GAPDH antibody was diluted to 1:10,000 (Sigma Aldrich) for 2 h at room temperature. The nitrocellulose membranes were washed with 0.05 M Tris, 0.9% NaCl containing 0.05% Tween-20 (TBS-T). A horseradish-peroxidase-conjugated secondary goat-anti-rabbit antibody (Santa Cruz Biotechnology,

Santa Cruz, CA, USA) was diluted to 1:20,000 and incubated for 1.5 h at room temperature. Protein bands were detected with an Amersham ECL-plus Western Blotting Detection kit, according to manufacturing instructions.

2.7 Analysis of Free Thiols

GAPDH (0.5 mg/mL) was incubated with 25 or 125 μ M DOPAL in 50 mM sodium phosphate buffer pH 7.4 for 2 or 4 h. After incubation, samples were titrated with 1 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) for 20 min at room temperature, and heated for 3 min at 80 °C, samples were then centrifuged at 1000g for 3 min and 100- μ L of supernatant was transferred to a 96-well plate. Absorbance was measured for the reduced 2-nitro-5-benzoic acid product at 412 nm, which directly correlates amount of free thiols in solution.

2.8 LC-MS Identification of Oxidized Peptides

GAPDH (0.3 mg/mL) and GAPDH-DOPAL (0.3 mg/mL and 50 μ M) were incubated in glass vials for 4 h at 37 °C. Excess DOPAL was removed by filtration columns. Final concentrations of NaCNBH₃ (1 mM) and ACN (0.1%) v/v were added, samples were briefly heated for 3 min at 70° C, and trypsin digested (Promega) in solution for 8h at 37 °C at a 1:50 ratio. A 10- μ L aliquot was taken and diluted 1:10 with 50 mM ammonium carbonate and 15 μ L of sample was injected onto a Phenomenex Aeris Widespore XB-C18 column (150 $\times$ 2.1 mm, 3.6 μ m). Mobile phase A was 0.1% formic acid in water, mobile phase B was 0.1% formic acid in ACN. The gradient elution was 0 min: 5% B, 5–15 min: 5–50% B, 15–30 min: 50–90% B, 30–35 min: 90%, 36 min: 50%, 37 min: 5%. A column oven (CTO-20A, Shimadzu Scientific) was set to 40 °C. Total flow was set to 0.2 mL/min. The MS instrument was a Shimadzu LCMS– IT-TOF (Shimadzu Scientific Instruments, Columbia, MD) using electrospray ionization (ESI) in positive ion detection mode. The ESI interface temperature and heat block were set at 200 °C and the nebulizing gas flow at 1.5 L/min with makeup gas on. The instrument scanned from 200–2000 m/z with a 0.3 sec loop time. MS spectra were analyzed by comparison to UCSF Prospector *Digest* command which matched the experimental m/z masses to theoretical sequenced GAPDH peptides. The database was set to assume trypsin digest, maximum missed cleavages as 3, peptide mass 200–5000 and ion mass tolerance of 1.00 Da. Variable modifications were set to Gln>pyro-Glu (N-term Q) and oxidation of methionine. Mass shifts pertaining to oxidations: one-three oxygens (16, 32, 48 Da) were scanned among the peptides.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 5.0 (Graph Pad Software, San Diego, CA). All enzyme samples treated with DOPAL were compared to the controls and significant differences ($p < 0.05$) were determined using an ANOVA with a Tukey post-test, except for the GSH activity assay and DTNB analyses were statistically analyzed via t-test.

3. Results

3.1 GAPDH is covalently modified by DOPAL

GAPDH was incubated with DA, DOPAL, DOPAC (0, 50 μ M) for 4h. Although DA, DOPAL and DOPAC covalently modified GAPDH as evident by NBT staining (data not

shown), only DOPAL caused significant GAPDH aggregation. The monomer band at 37 kDa was intact in the untreated GAPDH. There was some loss of the monomer band density with DA and DOPAC (Fig. 1A), however, it was to a lesser extent compared to the loss observed with DOPAL. Inversely, the band density in the high molecular weight region greatly increased with DOPAL, and negligibly changed with DA and DOPAC. Since NAD⁺ is a cofactor for GAPDH, NAD⁺ (1 mM) was co-incubated along with DOPAL to measure differences in modification. The presence of NAD⁺ did not significantly affect aggregation with DOPAL or metabolites.

3.2 GAPDH modification by DOPAL is time and dose-dependent

As shown in Fig 1B, the GAPDH monomer band density decreased at higher DOPAL concentrations, and higher molecular weight bands formed at 50 and 100 μ M. This trend was augmented at longer incubation times (4h versus 2h). Distinct trimer GAPDH bands were observed at ~111 kDa. At 4h, the monomer band was almost absent and the corresponding crosslinked protein was too large to enter the resolving gel portion (>200 kDa).

3.3 DOPAL analogs lacking the aldehyde or catechol do not inhibit GAPDH

The two tested DOPAL analogs were DHPAN, containing a catechol but not the aldehyde, and MOPAL, with the aldehyde but not the catechol. Neither analog affected GAPDH activity (Fig. 2B) and correlated to the lack of protein modification and catechol adducts (Fig. 2C).

3.4 DOPAL inhibits GAPDH activity

As a dehydrogenase, GAPDH activity was measured by reduction of cofactor NAD⁺ to NADH at 340 nm. All tested DOPAL concentrations (5–25 μ M) inhibited GAPDH activity, and a loss of ~50% of activity was achieved at only 1h with 25 μ M DOPAL. At 4h, the GAPDH activity with 25 μ M DOPAL was nearly zero. Therefore, DOPAL inhibits GAPDH in a time and dose-dependent manner (Fig. 3A–B). Additional studies showed complete enzyme inactivation after 4h at higher DOPAL doses and 8h at lower DOPAL doses (not shown). The presence of physiological concentration of NAD⁺ (100 μ M) cofactor in the incubation mixture attenuated enzyme inhibition by DOPAL (Fig. 3C–D).

3.5 DOPAL inhibition of GAPDH is irreversible

As shown in Fig. 4A, filtered GAPDH-DOPAL regained some activity at 1h and 2h, compared to non-filtered GAPDH-DOPAL. However, at 4h, there was no significant recovery in activity. There was no difference in activity between filtered and non-filtered GAPDH, accounting for negligible loss during filtration and centrifugation. The addition of 1 mM GSH to samples containing 5 or 50 μ M DOPAL did not reverse inhibition (Fig. 4C) or DOPAL binding (Fig. 4B) compared to DOPAL-only treated enzyme.

3.6 DOPAL modification of –Met and –Cys residues

The modified status of Cys were titrated by DTNB. In the presence of DOPAL, the number of free Cys residues, per GAPDH monomer, decreased in a dose-dependent manner (Fig

S1A). These results indicate that DOPAL does affect Cys thiol status, although it is not known if the loss of free Cys is due to covalent modification by DOPAL or spontaneous oxidation due to oxidative conditions. Higher DOPAL concentrations were used (50, 125 μ M) since a higher protein concentration (0.5 mg/mL) was needed for sufficient assay sensitivity. The GAPDH-DOPAL sample contained multiple shifts in mass of +16, +32 and +48 kDa, pertaining to thiol (Cys, Met) oxidation. For Met, oxidation would yield a sulfoxide or sulfone (+16 or +32 kDa, respectively), while Cys oxidation will produce a sulfenic, sulfinic or sulfonic acid (+16, +32, +48 kDa, respectively). There were 3 peptides containing Met that had a mass shift of 16 kDa, corresponding to sulfoxide formation. Although Met oxidation is a common product in LC/MS samples, these 3 mass shifts were specific to GAPDH samples treated with DOPAL and not seen in the control (Fig S1B). Peptides containing Cys exhibited a shift in masses corresponding to sulfenic, sulfinic and sulfonic acids.

4. Discussion

It is proposed that the presence of DA is a contributing factor for the heightened vulnerability of dopaminergic neurons during PD pathology [26]. A previous study showed cell treatment with DA directly caused GAPDH aggregation and cellular death [27]. In this current study, it is proposed that the downstream metabolite of DA, DOPAL may also be contributing to neurotoxicity, given its protein reactivity and ability to cause protein aggregation. This work demonstrates that both catechol and aldehyde are needed to modify GAPDH and cause inhibition. Previously, the reactivity of DOPAL for other proteins was also dependent on both the catechol and aldehyde [12, 14]. In regards to redox cycling and catechol formation, DOPAL induces catechol formation on proteins. Cell lysate treated with an ALDH inhibitor (4-HNE) produced catechol staining, however, staining was reversed with the MAO inhibitor, pargyline [23]. This suggests that significant protein modification is dependent on DOPAL catechol formation, as the MAO inhibitor will prevent DA to DOPAL conversion.

DOPAL causes aggregation of relevant proteins, such as alpha-synuclein (AS), forming AS dimers in a time and DOPAL concentration-dependent manner [28]. The concentration-dependent oligomerization of AS is analogous to what is observed with GAPDH oligomerization. Furthermore, DA, DOPAC, DOPET [29] and homovanillic acid [30] did not significantly affect AS aggregation, these results correlated well with the lack of GAPDH aggregation in the presence of these metabolites. Only DOPAL caused a time-dependent increase of oligomerization of both AS and GAPDH, and was further determined by the attenuation of AS oligomerization in SH-SY5Y cells by inhibition of MAO.

DOPAL is a potent inhibitor of GAPDH at low micromolar doses (5–25 μ M). This finding is contrasted by the mid-high millimolar concentrations of other electrophiles (acrylamide, acrolein, 4-HNE), needed to inhibit GAPDH[31]. Given that GAPDH is a dehydrogenase and the native substrate is an aldehyde (G3P), it was hypothesized a similar molecular weight sized aldehyde such as DOPAL would also interact at the active site. However, it was unknown whether this would be a reversible or irreversible reaction. When DOPAL was removed by filtration, the subsequent activity assay confirmed irreversibility since there was

no regain of activity with removal of DOPAL. Tyrosine hydroxylase is a protein target of DOPAL; however, the inhibition mode is semi-irreversible[13]. Maintaining proper GAPDH function and avoiding enzyme oxidation may be crucial in neuronal viability, such that specific proteins [32] which selectively bind and effectively block GAPDH oxidation produced neuroprotection.

The reversibility of thiol modification was suggested by a transarylation reaction with 1,2-naphthoquinone, and seemed like a relevant possibility for DOPAL, given its ability to oxidize to the reactive quinone. With 1,2-naphthoquinone, GSH reversed GAPDH modification, producing a glutathione-electrophile conjugate[33]. However, addition of GSH at the end of the DOPAL incubation did not reverse GAPDH oligomerization nor recover enzyme inhibition.

Another pertinent question was to identify which amino acids are modified by DOPAL. Previous analyses via LC-MS has demonstrated DOPAL modification of amines (Lys/Arg) but not of Cys. Here, Cys modification was analyzed via the thiol-sensitive reagent (DTNB) and it was demonstrated that DOPAL yielded a loss of free thiols. Given that the catechol of DOPAL is a source of reactive oxygen species (ROS)[19], it is possible that DOPAL oxidized the free thiols to disulfide or sulfinic/sulfenic acids, as evidenced by mass shifts of +16, +32, +48 Da for peptides containing Met or Cys. GAPDH Cys can form sulfenic acids in the presence of ROS donors such as nitric oxide [34]. In agreement with Cys oligomerization, GAPDH mutants containing C149A were unreactive to ROS donors and did not aggregate and S284C increased GAPDH aggregation and induced neuronal death[35].

This work shows that GAPDH is a novel target for DOPAL, leading to changes in global protein structure and enzyme inhibition. Aggregation of GAPDH by DOPAL may lead to detrimental pathological effects such as toxic Lewy body formation. Future work may further validate GAPDH as a target in PD therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ALDH2	aldehyde dehydrogenase 2
AR	aldehyde or aldose reductase
AS	alpha-synuclein

DA	dopamine
DAQ	dopamine quinone
DHPAN	3,4-dihydroxyphenylacetonitrile
DOPAC	3,4-dihydroxyphenylacetic acid
DOPAL	3,4-dihydroxyphenylacetaldehyde
DOPET	3,4-dihydroxyphenylethanol
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
G3P	glyceraldehyde-3-phosphate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GSH	glutathione
MAO	monoamine oxidase
MOPAL	3-methoxyphenylacetaldehyde
NAL	n-acetyl lysine
NBT	nitroblue tetrazolium

References

1. Park JH, Meriwether BP, Clodfelder P, Cunningham LW. The hydrolysis of p-nitrophenyl acetate catalyzed by 3-phosphoglyceraldehyde dehydrogenase. *The Journal of biological chemistry*. 1961; 236:136–141. [PubMed: 13732829]
2. Mazzola JL, Sirover MA. Alteration of intracellular structure and function of glyceraldehyde-3-phosphate dehydrogenase: a common phenotype of neurodegenerative disorders? *Neurotoxicology*. 2002; 23:603–609. [PubMed: 12428732]
3. Huang J, Xiong N, Chen C, Xiong J, Jia M, Zhang Z, Cao X, Liang Z, Sun S, Lin Z, Wang T. Glyceraldehyde-3-phosphate dehydrogenase: activity inhibition and protein overexpression in rotenone models for Parkinson's disease. *Neuroscience*. 2011; 192:598–608. [PubMed: 21736921]
4. Ross CA, Poirier MA. Protein aggregation and neurodegenerative disease. *Nature medicine*. 2004; 10(Suppl):S10–17.
5. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature*. 1997; 388:839–840. [PubMed: 9278044]
6. Olah J, Tokesi N, Vincze O, Horvath I, Lehotzky A, Erdei A, Szajli E, Medzihradszky KF, Orosz F, Kovacs GG, Ovadi J. Interaction of TPPP/p25 protein with glyceraldehyde-3-phosphate dehydrogenase and their co-localization in Lewy bodies. *FEBS letters*. 2006; 580:5807–5814. [PubMed: 17027006]
7. Tatton NA. Increased caspase 3 and Bax immunoreactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson's disease. *Experimental neurology*. 2000; 166:29–43. [PubMed: 11031081]
8. Tsuchiya K, Tajima H, Kuwae T, Takeshima T, Nakano T, Tanaka M, Sunaga K, Fukuhara Y, Nakashima K, Ohama E, Mochizuki H, Mizuno Y, Katsube N, Ishitani R. Pro-apoptotic protein glyceraldehyde-3-phosphate dehydrogenase promotes the formation of Lewy body-like inclusions. *Eur J Neurosci*. 2005; 21:317–326. [PubMed: 15673432]

9. Marsden CA. Dopamine: the rewarding years. *Br J Pharmacol.* 2006; 147(Suppl 1):S136–144. [PubMed: 16402097]
10. Burke WJ, Li SW, Chung HD, Ruggiero DA, Kristal BS, Johnson EM, Lampe P, Kumar VB, Franko M, Williams EA, Zahm DS. Neurotoxicity of MAO metabolites of catecholamine neurotransmitters: role in neurodegenerative diseases. *Neurotoxicology.* 2004; 25:101–115. [PubMed: 14697885]
11. Goldstein DS, Kopin IJ, Sharabi Y. Catecholamine autotoxicity. Implications for pharmacology and therapeutics of Parkinson disease and related disorders. *Pharmacol Ther.* 2014; 144:268–282. [PubMed: 24945828]
12. Vermeer LM, Florang VR, Doorn JA. Catechol and aldehyde moieties of 3,4-dihydroxyphenylacetaldehyde contribute to tyrosine hydroxylase inhibition and neurotoxicity. *Brain Res.* 2012; 1474:100–109. [PubMed: 22877852]
13. Mexas LM, Florang VR, Doorn JA. Inhibition and covalent modification of tyrosine hydroxylase by 3,4-dihydroxyphenylacetaldehyde, a toxic dopamine metabolite. *Neurotoxicology.* 2011; 32:471–477. [PubMed: 21514317]
14. Rees JN, Florang VR, Eckert LL, Doorn JA. Protein reactivity of 3,4-dihydroxyphenylacetaldehyde, a toxic dopamine metabolite, is dependent on both the aldehyde and the catechol. *Chem Res Toxicol.* 2009; 22:1256–1263. [PubMed: 19537779]
15. Nilsson GE, Tottmar O. Biogenic aldehydes in brain: on their preparation and reactions with rat brain tissue. *Journal of neurochemistry.* 1987; 48:1566–1572. [PubMed: 3559567]
16. Ungar F, Tabakoff B, Alivisatos SG. Inhibition of binding of aldehydes of biogenic amines in tissues. *Biochem Pharmacol.* 1973; 22:1905–1913. [PubMed: 4722459]
17. Helander A, Tottmar O. REACTIONS OF BIOGENIC ALDEHYDES WITH HEMOGLOBIN. *Alcohol.* 1989; 6:71–75. [PubMed: 2470392]
18. Burke WJ, Chung HD, Li SW. Quantitation of 3,4-dihydroxyphenylacetaldehyde and 3, 4-dihydroxyphenylglycolaldehyde, the monoamine oxidase metabolites of dopamine and noradrenaline, in human tissues by microcolumn high-performance liquid chromatography. *Analytical biochemistry.* 1999; 273:111–116. [PubMed: 10452806]
19. Anderson DG, Mariappan SV, Buettner GR, Doorn JA. Oxidation of 3,4-dihydroxyphenylacetaldehyde, a toxic dopaminergic metabolite, to a semiquinone radical and an ortho-quinone. *The Journal of biological chemistry.* 2011; 286:26978–26986. [PubMed: 21642436]
20. Akagawa M, Ishii Y, Ishii T, Shibata T, Yotsu-Yamashita M, Suyama K, Uchida K. Metal-catalyzed oxidation of protein-bound dopamine. *Biochemistry.* 2006; 45:15120–15128. [PubMed: 17154550]
21. Uchida K, Stadtman ER. Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reaction. *The Journal of biological chemistry.* 1993; 268:6388–6393. [PubMed: 8454610]
22. Rees JN, Florang VR, Eckert LL, Doorn JA. Protein Reactivity of 3,4-Dihydroxyphenylacetaldehyde, a Toxic Dopamine Metabolite, Is Dependent on Both the Aldehyde and the Catechol. *Chem Res Toxicol.* 2009; 22:1256–1263. [PubMed: 19537779]
23. Rees JN, Florang VR, Anderson DG, Doorn JA. Lipid peroxidation products inhibit dopamine catabolism yielding aberrant levels of a reactive intermediate. *Chemical research in toxicology.* 2007; 20:1536–1542. [PubMed: 17887726]
24. Paz MA, Fluckiger R, Boak A, Kagan HM, Gallop PM. Specific detection of quinoproteins by redox-cycling staining. *J Biol Chem.* 1991; 266:689–692. [PubMed: 1702437]
25. Teipel J, Koshland DE Jr. The effect of NAD⁺ on the catalytic efficiency of glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle. *Biochim Biophys Acta.* 1970; 198:183–191. [PubMed: 4313530]
26. Hastings TG, Zigmond MJ. Loss of dopaminergic neurons in parkinsonism: possible role of reactive dopamine metabolites. *Journal of neural transmission Supplementum.* 1997; 49:103–110. [PubMed: 9266419]

27. Nakajima H, Amano W, Kubo T, Fukuhara A, Ihara H, Azuma YT, Tajima H, Inui T, Sawa A, Takeuchi T. Glyceraldehyde-3-phosphate dehydrogenase aggregate formation participates in oxidative stress-induced cell death. *J Biol Chem.* 2009; 284:34331–34341. [PubMed: 19837666]
28. Follmer C, Coelho-Cerqueira E, Yatabe-Franco DY, Araujo GD, Pinheiro AS, Domont GB, Eliezer D. Oligomerization and Membrane-binding Properties of Covalent Adducts Formed by the Interaction of alpha-Synuclein with the Toxic Dopamine Metabolite 3,4-Dihydroxyphenylacetaldehyde (DOPAL). *J Biol Chem.* 2015; 290:27660–27679. [PubMed: 26381411]
29. Jinsmaa Y, Sullivan P, Gross D, Cooney A, Sharabi Y, Goldstein DS. Divalent metal ions enhance DOPAL-induced oligomerization of alpha-synuclein. *Neuroscience letters.* 2014; 569:27–32. [PubMed: 24670480]
30. Burke WJ, Kumar VB, Pandey N, Panneton WM, Gan Q, Franko MW, O'Dell M, Li SW, Pan Y, Chung HD, Galvin JE. Aggregation of alpha-synuclein by DOPAL, the monoamine oxidase metabolite of dopamine. *Acta neuropathologica.* 2008; 115:193–203. [PubMed: 17965867]
31. Martyniuk CJ, Fang B, Koomen JM, Gavin T, Zhang L, Barber DS, Lopachin RM. Molecular mechanism of glyceraldehyde-3-phosphate dehydrogenase inactivation by alpha,beta-unsaturated carbonyl derivatives. *Chem Res Toxicol.* 2011; 24:2302–2311. [PubMed: 22084934]
32. Sen N, Hara MR, Ahmad AS, Cascio MB, Kamiya A, Ehmsen JT, Agrawal N, Hester L, Dore S, Snyder SH, Sawa A. GOSPEL: a neuroprotective protein that binds to GAPDH upon S-nitrosylation. *Neuron.* 2009; 63:81–91. [PubMed: 19607794]
33. Miura T, Kakehashi H, Shinkai Y, Egara Y, Hirose R, Cho AK, Kumagai Y. GSH-Mediated S-Transarylation of a Quinone Glyceraldehyde-3-Phosphate Dehydrogenase Conjugate. *Chem Res Toxicol.* 2011; 24:1836–1844. [PubMed: 21827172]
34. Ishii T, Sunami O, Nakajima H, Nishio H, Takeuchi T, Hata F. Critical role of sulfenic acid formation of thiols in the inactivation of glyceraldehyde-3-phosphate dehydrogenase by nitric oxide. *Biochem Pharmacol.* 1999; 58:133–143. [PubMed: 10403526]
35. Nakajima H, Amano W, Fukuhara A, Kubo T, Misaki S, Azuma YT, Inui T, Takeuchi T. An aggregate-prone mutant of human glyceraldehyde-3-phosphate dehydrogenase augments oxidative stress-induced cell death in SH-SY5Y cells. *Biochem Biophys Res Commun.* 2009; 390:1066–1071. [PubMed: 19874799]

Highlights

- DOPAL caused GAPDH oligomerization in a time and dose-dependent manner.
- GAPDH activity was irreversibly inhibited at low micromolar levels of DOPAL.
- Inhibition of activity was dependent on the catechol and aldehyde of DOPAL.
- It is suggested that DOPAL modifies Cys residues by oxidation of free thiols.

Figure 1.

DOPAL causes GAPDH aggregation in a concentration and time-dependent manner. GAPDH (0.3 mg/mL) was incubated with: A, DA, DL, DC (50 μM) +/- NAD⁺ (1 mM) for 4h at 37 °C, subjected to SDS-PAGE (9 μg) and stained by Coomassie stain. Each band at 37 kDa was quantified on an UVP Imager. B, DOPAL (0–100 μM) for 2 or 4h at 37 °C. subjected to SDS-PAGE (9 μg) and stained by Coomassie. C, Scheme of DA metabolism. Bands at 37 kDa was quantified on an UVP Imager. unt = untreated, DOPAL=DL, DOPAC=DC, MAO=monoamine oxidase, AR=aldose reductase, ALDH=aldehyde dehydrogenase. Band densities are based on three Coomassie stain gels, n=3. Significance is denoted with (*), 1-way ANOVA analysis, p 0.05.

Figure 2.

Both aldehyde and catechol of DOPAL are required for producing catechol protein adducts.

A, Structures of DOPAL, and analogs: MOPAL and DHPAN. B, GAPDH activity was measured following incubation with 2.5 and 25 μ M of DOPAL, DHPAN and MOPAL, n=3.

C, NBT staining of redox-active catechols on GAPDH (12 μ g load).

Significance is denoted with (*), 1-way ANOVA analysis, p 0.05.

Figure 3. DOPAL inhibits GAPDH activity. GAPDH (0.3 mg/mL) incubated with DOPAL: 5, 10, 15 μ M for 4h in 50 mM sodium phosphate buffer at 37 $^{\circ}$ C. Values were calculated as the activity ratio of the DOPAL-treated sample, enzyme activity of G3P oxidation as the NADH absorbance at 340 nm. A, Time and DOPAL concentration dependence on GAPDH inhibition. B, GAPDH inhibition by DOPAL at 4h. C, GAPDH activity assayed containing 100 μ M NAD⁺ in incubation mixture. D, Comparison of the attenuated inhibition by DOPAL at 4h, when NAD⁺ cofactor is present in the incubation mixture. Panel B was analyzed by 1-way ANOVA, and panel D via 2-way ANOVA analysis, significance is denoted with (*), p 0.05, n=3.

Figure 4.

Inhibition of GAPDH by DOPAL is mainly irreversible and cannot be reversed by addition of GSH. A, Aliquots of GAPDH (0.3 mg/mL) were incubated with 25 μ M DOPAL and filtrated via size-exclusion columns, to remove unbound DOPAL. Non-filtered aliquots were assayed and compared to the filtered aliquots. B, Western blot GAPDH (1 μ g) with DOPAL with and without 0.5 mM GSH. C, GAPDH activity following DOPAL treatment $\pm$ GSH. Significance is denoted by (*). For activity experiments, n=3, p 0.05, significance was analyzed via t-test.