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A transient post-translational modification of active site cysteine alters binding properties of the parkinsonism protein DJ-1

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ABSTRACT

Mutations in the human protein DJ-1 cause early onset of Parkinson's disease. A reactive cysteine residue (Cys^{106}) of DJ-1 is crucial for its protective function, although the underlying mechanisms are unclear. Here we show that a fraction of bacterially expressed polyhistidine-tagged human DJ-1 could not be eluted from a Ni-nitrilotriacetate (Ni-NTA) column with 150 mM imidazole. This unusually tight binding was accompanied by the appearance of blue violet color on the Ni-NTA column. We demonstrate by X-ray crystallography that Cys^{106} is carboxymethylated in a fraction of DJ-1 tightly bound to Ni-NTA and that the replacement of Cys^{106} by serine abrogates the tight binding and the appearance of blue violet color. However, carboxymethylation of purified DJ-1 is insufficient to confer the tight binding to Ni-NTA. Moreover, when eluted protein was re-applied to the Ni-NTA column, no tight binding was observed, indicating that the formation of high affinity complex with Ni-NTA depends on a transient modification of Cys^{106} that transforms into a Cys^{106}-carboxymethyl adduct upon elution from Ni-NTA. We conclude that an unknown metabolite reacts with Cys^{106} of DJ-1 to result in a transient post-translational modification. This modification is distinct from simple oxidation to sulfinic or sulfenic acids and confers altered binding properties to DJ-1 suggesting that it could serve as a signal for sensing oxidant stress.

1. Introduction

Parkinson's disease (PD), the second-most common neurodegenerative disorder [1], is caused by a loss of dopaminergic neurons due to a cascade of events likely triggered by oxidant stress in mitochondria and mediated by the oxidation of dopamine [2]. Genetic studies have identified *PARK7*, a gene which encodes the DJ-1 protein, as one of the causal genes for hereditary recessive PD [3]. Although most data indicate that DJ-1 protects cellular structures, particularly mitochondria, from oxidant stress [4,5], the biochemical basis of this protection remains unclear. A reactive cysteine (Cys^{106} in human DJ-1) is essential for the function of DJ-1 since its replacement results in a loss of protective effects in a number of experimental systems [5–7]. A facile oxidation of Cys^{106} to sulfenic, sulfinic, and sulfonic acid [5–9] has prompted speculation that oxidation of Cys^{106} may serve as a sensor for oxidant stress [10]. However, the oxidation propensity of Cys^{106} could merely reflect its high reactivity given the lack of evidence for any binding partner that preferentially recognizes oxidized forms of DJ-1.

The increased reactivity of Cys^{106} is caused at least in part by lowering of its pK_a by a side chain of glutamic acid Glu^{18} [11] since several mutations of Glu^{18} increase pK_a of Cys^{106} by 0.3–0.7 units and alter its oxidative propensity [6]. One such mutation, E18D, resists oxidation of Cys^{106} to sulfinic acid and fails to protect cells from oxidant stress leading to the hypothesis that oxidation of Cys^{106} to sulfinic acid is critical for the protective function of DJ-1. Nonetheless, it is possible that the E18D mutation disrupts a hitherto unknown function of DJ-1 by altering the reactivity of Cys^{106}.

Abbreviations used: LC-MS/MS, liquid chromatography tandem mass-spectrometry; NTA, nitrilotriactetic acid; PD, Parkinson's disease.

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Another mutation, E18N, oxidizes more readily than wild type DJ-1 and protects cells from oxidant stress similarly to a wild type DJ-1 [6]. However, increased oxidation sensitivity implies that the high reactivity of Cys^{106} remains largely unperturbed, and as a result, other DJ-1 functions dependent on high Cys^{106} reactivity are likely also unaffected, a possible explanation why the E18N mutation showed protective effects under oxidant stress. To summarize, in our opinion, whether oxidation or other post-translational modification of Cys^{106} mediate the protective function of DJ-1 remains an open question.

In the present study, we have identified and characterized a novel post-translational modification of Cys^{106} in DJ-1. We suggest that this modification is a part of an as yet uncharacterized metabolic pathway that mediates the protective function of DJ-1 in neurons.

2. Materials and methods

Protein expression and purification

The human PARK7 gene was cloned into a pHisParallel vector [12] using the NcoI/SalI cloning sites. BL21(DE3) RIPL cells were transformed with PARK7 expression constructs and plated on LB agar plates containing 100 μg/ml ampicillin and 34 μg/ml chloramphenicol. DJ-1 was purified as described in Ref. [13]. For carboxymethylation of DJ-1, the protein was purified on Ni-NTA and eluted in lysis buffer containing 150 mM imidazole and 10 mM 2-mercaptoethanol, and further diluted and purified on a 1 ml Q-Sepharose column. Protein concentration was determined spectrophotometrically at 280 nm. Prior to treatment with equimolar concentration of iodoacetic acid the pH was adjusted to 6.0 in order to suppress carboxymethylation of Cys^{46} and Cys^{53} which are expected to have much higher pK_a values than Cys^{106} [11]. For ammonium sulfate precipitation, 7 ml of *E. coli* cell lysate was diluted to 15 mL with distilled water, and crystals of ammonium sulfate were added to achieve 80% saturation.

2.2. Protein crystallization and structure determination

Proteins were treated with rTEV protease overnight with concomitant dialysis against lysis buffer and purified by size-exclusion chromatography. Crystallization setups were made with DJ-1 dissolved in 5 mM HEPES buffer containing 0.5 M NaCl. Crystals of tightly and loosely bound DJ-1 grew at room temperature from vapor diffusion setups consisting of equal volumes (500 nL) of the protein solution and a reservoir solution consisting of 0.1 M HEPES-Na and 1.5 M sodium citrate pH 7.5 and 0.1 M Bis-tris pH 5.5, 0.1 M KSCN, and 29% PEG MME 2000 respectively. Crystals were harvested in reservoir buffer and flash-cooled in liquid nitrogen. Single wavelength ($\lambda = 0.97872$ Å) X-ray diffraction data were collected at 100 K at the LS-CAT 21-ID-F beamline at the Advanced Photon Source, Argonne National Laboratory. Data were indexed, integrated, and scaled with HKL-3000 [14] in the anomalous mode using corrections for radiation decay and anisotropic diffraction (auto-corrections). R-free was monitored by setting aside 5% of reflections as a test set. Initial phase estimates were obtained by molecular replacement in HKL-3000 integrated with MOLREP using 1P5F as a search model [15]. The ACHESYM server [16] was used for standardized placement of the model in the unit cell. Structures were refined in HKL-3000 using REFMAC5 [17] in the restrained mode and hydrogen atoms in riding positions. Coot was used for the visualization of electron density maps, manual inspection, and correction of the atomic model. Structure quality assessment was performed with Coot, a standalone version of MolProbity [18], and the PDB validation tools [19]. Molstack [20], a web-based interactive publishing platform, was used to enable interactive inspection of the model and the respective electron density maps. The diffraction images are available on http://proteindiffraction.org/ [21].

2.3. Tandem mass-spectrometry of DJ-1

Protein bands were excised from the Coomassie-stained gel, digested with trypsin, and purified with ZipTip-C_{18} (Millipore). A trapping column set-up (AcclaimPepMap100C18 pre-column) and a Dionex HPLC pump were used for chromatography. Peptides were separated on an Acclaim Pep-Map RSLC column using a 75 min multistep acetonitrile gradient at a flow rate of 0.3 μl/min. The unmodified Captive Spray ion source (Capillary 1300 V, dry gas 3.0 l/min, dry temperature 150 °C) was used to interface the LC system to the Impact II mass spectrometer (Bruker). For quantification, full scan MS spectra were acquired at a spectra rate of 2.0 Hz followed by acquisition of one MS/MS spectrum. For data acquisition of the sample, the two most intense precursor ions were selected for fragmentation, resulting in a total cycle time of 3 s. One triple-charged peak (3+) precursor ion at m/z of 799.1213 was fragmented by MS/MS to produce spectrum with series of b- and y-ions.

3. Results

3.1. A portion of His-tagged DJ-1 binds to Ni-NTA with high affinity

When a Ni-NTA gravity column was loaded with bacterial cell lysate overexpressing a variant of human DJ-1 with a cleavable N-terminal hexahistidine tag and washed to remove impurities, the column changed color from the original light cyan to blue-violet (Fig. 1, WT). Elution with 10 column volumes of 150 mM imidazole did not restore the original color of the column. The blue violet color gradually disappeared as the column was eluted with progressively increasing concentrations of imidazole, with an abrupt and complete loss of the blue violet color after elution with 2.4 M imidazole. Analysis of the eluted fractions on polyacrylamide gels (Fig. 1, right) indicated that a significant fraction of DJ-1 resisted elution with 150 mM imidazole since large amounts of DJ-1 were detected in fractions eluted with higher concentrations of imidazole. The elution of DJ-1 was complete with 2.4 M imidazole since little protein was detected in the 3 M imidazole fraction.

The reactive cysteine, Cys^{106}, is implicated in most protective functions of DJ-1 [10]. Therefore, we tested whether this residue was involved in the enhanced binding to Ni-NTA. When we mutated Cys^{106} to isosteric serine and attempted purification of the C106S mutant, we did not observe the appearance of a blue violet color on the Ni-NTA column (Fig. 1, C106S). Analysis of eluted fractions showed that nearly all of the mutant protein eluted in the 150 mM imidazole fraction, as is usually observed for hexahistidine-tagged proteins, with only trace amounts present in fractions eluted with higher imidazole concentrations (Fig. 1, right) indicating that Cys^{106} is required for high affinity binding to Ni-NTA.

3.2. Cys^{106} is carboxymethylated in tightly bound DJ-1 eluted from Ni-NTA

Although tightly bound DJ-1 could be efficiently eluted with ~3 M imidazole, it precipitated after a few minutes. To avoid precipitation, tightly bound DJ-1 was eluted with 25 mM HCl or 100 mM acetic acid for further characterization. In order to exclude the possibility that tight binding of DJ-1 to the Ni-NTA column results from denaturation or that acidic elution conditions denature

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