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Structure of Cu(I)-Bound DJ-1 Reveals a Biscysteinate Metal Binding Site at the Homodimer Interface: Insights into Mutational Inactivation of DJ-1 in Parkinsonism

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Supporting Information

ABSTRACT: The Parkinsonism-associated protein DJ-1 has been suggested to activate the Cu−Zn superoxide dismutase (SOD1) by providing its copper cofactor. The structural and chemical means by which DJ-1 could support this function is unknown. In this study, we characterize the molecular interaction of DJ-1 with Cu(I). Mass spectrometric analysis indicates binding of one Cu(I) ion per DJ-1 homodimer. The crystal structure of DJ-1 bound to Cu(I) confirms metal coordination through a docking accessible biscysteinate site formed by juxtaposed cysteine residues at the homodimer interface. Spectroscopy *in crystallo* validates the identity and oxidation state of the bound metal. The measured subfemtomolar dissociation constant (K_d = 6.41 × 10^{-16} M) of DJ-1 for Cu(I) supports the physiological retention of the metal ion. Our results highlight the requirement of a stable homodimer for copper binding by DJ-1. Parkinsonism-linked mutations that weaken homodimer interactions will compromise this capability.

Copper is an essential cofactor in various biochemical processes. Its redox activity is used by a range of vital enzymes.[1] Paradoxically, due to a highly reactive nature, copper mishandling can also lead to cellular damage. To prevent this harmful effect, the cell maintains a negligible pool of free copper ions and employs copper chaperone proteins to deliver the metal ion.[2] The Cu−Zn superoxide dismutase (SOD1) is an antioxidant metalloenzyme that catalyzes the conversion of superoxide to oxygen and hydrogen peroxide.[3] Its active site requires a copper ion for catalysis while a zinc ion plays a structural role.[4] The copper cofactor is mainly provided by the dedicated copper chaperone CCS.[5] However, an alternative mechanism for copper insertion is known to exist as CCS knockout mice retain 15%−20% of SOD1 activity and in *C. elegans* SOD1 copper maturation is entirely CCS-independent.[6,7]

DJ-1 is a ubiquitously expressed homodimeric protein with multifunctional roles including transcriptional regulation, chaperone activity, oncogenesis, and protection against mitochondrial toxins (recently reviewed by Ariga et al., 2013).[8] DJ-1 is also protective against oxidative stress[9] which is one of the main pathological features of brain tissue from patients with Parkinson's disease.[10,11] Early onset forms of Parkinsonism can result from mutations in DJ-1.[12] A number of these Parkinsonism-linked mutations abrogate the DJ-1 dimer and lead to a loss of antioxidant function.[13,14] It is crucial to understand how DJ-1 responds to oxidative stress to understand the pathways that may lead to onset of the disease. A recent report suggested DJ-1 could activate SOD1 by providing copper as a cofactor.[15] Despite the etiological relevance of this copper chaperone activity, the structural and chemical means through which DJ-1 could carry out this function is lacking. We characterized the interaction of DJ-1 with copper using spectroscopic analysis, mass spectrometry, X-ray crystallography and metal affinity estimation.

Cu(I) is the commonly transported oxidation state of copper inside the cell and the form received by SOD1.[16] We thus characterized the interaction of DJ-1 with Cu(I) in an anaerobic environment to prevent the facile oxidation of the metal ion. Using electronic absorption spectroscopy Cu(I) binding to DJ-1 was monitored. Incubation of an equimolar amount of Cu(I) with DJ-1 in solution gave rise to an envelope peak centered at 254 nm, a spectral feature shared by copper chaperones using thiolate moieties to bind copper (Figure 1a).[17] The absorbance corresponds to a charge transfer transition between a cysteine Sγ and a Cu(I) center.[17] Cu(I) makes thermodynamically stable complexes with highly polarizable soft ligands preferentially, in proteins, with cysteine Sγ's found in metal binding motifs such as CXC and CXXC of previously characterized copper chaperones.[16] Remarkably, these sequence motifs are not present in DJ-1.

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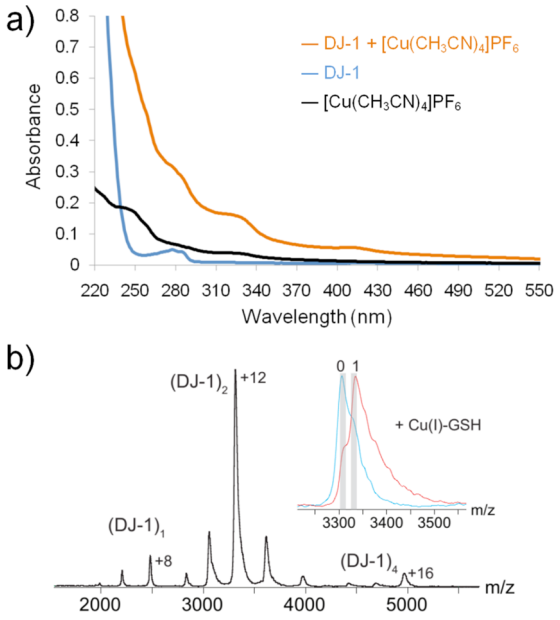

Figure 1. DJ-1 binds copper as a $Cu^{I}(DJ\text{-}1)_2$ complex. (a) Absorption spectrum of DJ-1 with Cu(I) (as $[Cu(CH_3CN)_4]PF_6$); Wavelength scans of the reduced DJ-1 apoprotein (10 μM dimer) without Cu(I) (blue) and with an equimolar amount of Cu(I) (orange). Cu(I) titrant (10 μM, black). (b) Mass spectrum of DJ-1; DJ-1 (1 μM dimer, black). Calculated masses 19 865 ± 1 Da (monomer), 39 842 ± 10 Da (dimer) and 79 571 ± 19 Da (tetramer). Inset shows the +12 charge state of dimeric DJ-1 when incubated in a 2-fold (blue) and 4-fold (red) molar excess of Cu(I)-GSH. For full mass spectra, see Supplementary Figure 1.

Nanoflow electrospray ionization mass spectrometry was employed to study DJ-1:Cu(I) stoichiometry. The DJ-1 apoenzyme spectrum is dominated by a dimeric species (Figure 1b) showing conditions sustain the physiologically relevant, dimerized DJ-1. When incubated with a molar excess of Cu(I) stabilized with glutathione, Cu(I)-GSH, the dimer increased in mass by 374 Da (Figure 1b). This suggests a single copper (63 Da) bound to a DJ-1 dimer, and a further glutathione species (305 Da). Mass spectrometry may indicate the formation of a mixed disulfide, gluthathione adduct; an m/z increase of 302 Da is observed for a portion of the monomeric species (Supplementary Figure 1). However, the greater proportion of the DJ-1 monomer does not exhibit this increase. Thus, we cannot discount glutathione participating as a third ligand for Cu(I) with the dimeric DJ-1 as in other copper-binding proteins.[18] Tricoordinate copper complexes have been characterized and proposed as a transfer mechanism between chaperones and target proteins.[19,20] Glutathione is also responsible for CCS-independent insertion of copper into SOD1 in *S. cerevisiae*.[21]

To map the copper binding site, the X-ray structure of a DJ-1 crystal soaked in Cu(I)-GSH was solved. This 1.38 Å structure revealed a copper ion coordinated by the Sγ of juxtaposed cysteine 53 residues, one from each monomer, forming a solvent-exposed, docking-accessible metal binding site at the dimer interface (Figure 2a; see Supplementary Table 1 for data collection and refinement statistics). Copper binding does not induce a gross change in the structure of DJ-1 (the Cα positional RMSD from the apoprotein is less than 0.2 Å).The copper−biscysteinate complex exhibits a S−Cu−S angle of 163.2° and Cu−S distances of 1.9 Å. Diffraction data were collected using X-rays near the copper K-edge (λ = 1.3772 Å). An anomalous difference map shows a peak of 39 σ at the copper ion position that validates its identity (Figure 2b). Contrary to the mass spectrometry, glutathione neither coordinates Cu(I) in the crystal nor is present as an adduct. Crystal packing may prevent glutathione coordination/adduct formation, and thus we cannot determine if glutathione plays a role in copper coordination. To ensure the oxidation state of the copper ion was assigned correctly in the structure, we performed various spectroscopic techniques *in crystallo*. A single crystal electronic absorption spectrum of the Cu(I)-bound protein produced the same absorption band at 254 nm (Figure

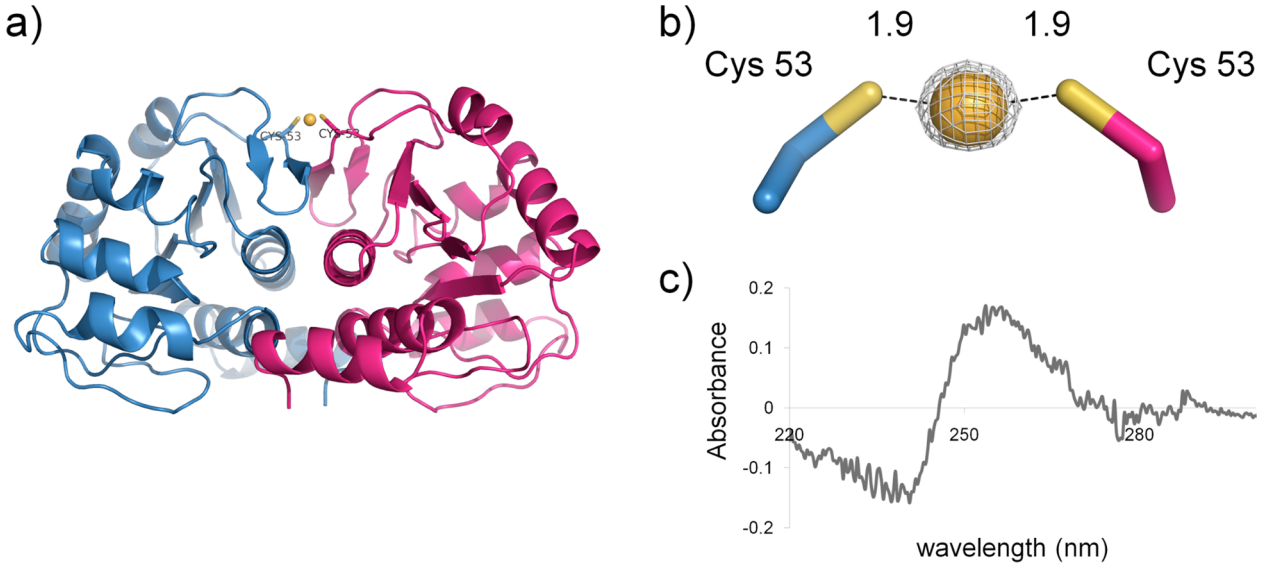

Figure 2. Structure of the Cu(I) binding site of DJ-1. (a) A backbone representation of the dimeric DJ-1 bound to Cu(I) shown as an orange sphere. (b) The anomalous difference map (gray, contoured at 10.0 σ) of the Cu(I) binding site with cysteine Sγ distances to the metal center in Å. (c) Electronic absorption spectrum *in crystallo* of DJ-1 soaked in a Cu(I)-GSH complex. Spectra of apoprotein and metal-loaded crystals (Supplementary Figure 2) were normalized, and the resulting difference spectrum is shown.

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2c) as the UV spectrum in solution. X-ray fluorescence spectroscopy confirms the incorporation of a copper ion, and the weak shoulder at 8983 eV correlates to the characteristic 1s→4p transition observed on excitation of Cu(I) (Supplementary Figure 3).[22]

Free copper ions present a cytotoxic hazard; thus, metallochaperones sequester and deliver them only to specific target proteins.[2] In order to achieve this, copper chaperones bind tightly to their metal ion. The affinity of DJ-1 for Cu(I) was determined using a competition assay with bicinchoninic acid (Bca), a Cu(I)-specific chelator that forms a 2:1 copper complex $[Cu^{I}(Bca)_2]^{3-}$ (λ_{max} = 562 nm; $\beta_2^{-2} = 10^{17.2}$ M^{-1}) (Supplementary Table 2).[23,24] At ∼50-fold molar excess of Bca, only 50% of the Cu(I) was extracted from the $Cu^{I}(DJ\text{-}1)_2$ complex indicating a tight affinity. The average dissociation constant, $K_d = 6.4 \times 10^{-16}$ M, is consistent with the subfemtomolar dissociation constants of other human Cu(I) binding proteins such as the metallochaperone Atox1 (3.9×10^{-18} M)[13] and the N-terminal metal-binding domains 5 and 6 of the Wilson disease protein WLN5-6 (2.5×10^{-18} M) consistent with physiological retention of Cu(I) by DJ-1 inside the cell.[23]

Using crystallographic and spectroscopic analyses, we have mapped the Cu(I)-binding site of human DJ-1. Uniquely, Cu(I) is held through a biscysteinate site formed by cysteine 53 residues from separate subunits of the homodimer instead of a canonical sequence motif, such as CXC or CXXC.[25] Biscysteinate metal binding sites fulfill the requirements for a good metal ion donor. They have a high metal affinity to acquire metal ions and to then prevent leakage. The low coordination number of biscysteinate sites also supports ligand-exchange/metal-transfer mechanisms. For this reason, they are prevalent in Cu(I)-transporting proteins, e.g. CCS, Hah1, and the proteins of Menkes disease and Wilson's disease.[16,25]

Cysteine 53 is conserved across all vertebrates but not the wider eukaryotic family (Supplementary Figure 4). Cysteines 53 and 106 both play a role in DJ-1's cytoprotective effects. Cysteine 106 is critical for mitochondrial localization,[26] chaperone activity,[27] and glyoxalase activity.[28] DJ-1 has been shown to protect cells from copper and mercury toxicity, where cysteine 106 was dispensable; however, preincubation with dopamine abrogated this protection.[29] Cysteine 53 is susceptible to S-nitrosylation in cultured cells[30] and is covalently modified by dopamine quinones that form in cytosolic accumulation of dopamine.[31] Our results show how DJ-1 can protect from copper toxicity and how modifications of cysteine 53 would render DJ-1 incapable of metal sequestration.

The structure of DJ-1 complexed with Cu(I) reveals the need for a stable homodimer to function as a copper carrier. A number of Parkinsonism-linked DJ-1 mutations abolish the protein's antioxidant capability and result in reduced protein stability and/or weaken dimer contacts (Supplementary Figure 5).[32−35] These mutations would prevent the formation of the Cu(I) binding site described here. Our study warrants further work on the role of DJ-1's copper binding capability in neurodegeneration.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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