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Thermodynamic and Structural Characterization of the Specific Binding of Zn(II) to Human Protein DJ-1

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Supporting Information

ABSTRACT: Mutations of *DJ-1* cause familial Parkinson's disease (PD), although the role of *DJ-1* in PD remains unresolved. Very recent reports have shown that DJ-1 interacts with copper ions. This evidence opens new avenues to understanding the function of DJ-1 and its role in PD. Herein, we report that Zn(II) binds to DJ-1 with great selectivity among the other metals examined: Mn(II), Fe(II), Co(II), Ni(II), and Cu(II). High-resolution X-ray crystallography (1.18 Å resolution) shows Zn(II) is coordinated to the protein by the key residues Cys106 and Glu18. These results suggest that DJ-1 may be regulated and/or stabilized by Zn(II).

Parkinson's disease (PD) is a progressive and devastating neurological disorder for which there is no cure or effective treatment.[1] Mutations of *DJ-1* (*PARK7*) lead to an early onset familial form of Parkinsonism.[2] Although the gene product DJ-1 has been extensively studied from both structural and biological viewpoints, its exact function and its relationship to the progression of PD remain elusive.[3] Very recently, three separate reports have demonstrated the binding of metal ions Cu(I) and Cu(II) to human DJ-1.[4−6] Because the concentration of transition metals is altered in the serum of PD patients with respect to basal levels,[7] we sought to examine the interaction of metals belonging to the Irving−Williams series[8] with DJ-1 using high-resolution techniques.

The binding to DJ-1 of Mn(II), Fe(III), Co(II), Ni(II), Cu(II), and Zn(II) was first evaluated by differential scanning fluorimetry (DSF).[9] Among all the metals examined, only Zn(II) induced a robust increase in the melting (unfolding) temperature of the protein (ΔT_M = +0.6 °C) (Figure 1, Table 1, and Figure S1 of the Supporting Information). Other metals had no effect on T_M, with the exception of Cu(II), which greatly destabilized DJ-1 (ΔT_M = −6.6 °C), reflecting unspecific aggregation at high temperatures.[10]

Binding of metals to DJ-1 was also examined by isothermal titration calorimetry (ITC). As described above, only Zn(II) showed clear evidence of specific binding to DJ-1 (Figure 1b). Fitting of the sigmoid isotherm to a one-site binding model yields a dissociation constant in the submicromolar range (K_D = 0.8 ± 0.3 μM). Zn(II) interacts with DJ-1 at a molar ratio of 1:1, in contrast to previous crystals structures of DJ-1 for which molar ratios of 2:3 [with Cu(II) bound] and 1:2 [with Cu(I) bound] were reported.[4,5] Among the other metals tested, only Ni(II) showed signs of interaction with DJ-1 (K_D = 400 ± 270 μM), although this affinity seems too low to be physiologically relevant (Figure 1, Table 1, and Figure S2 of the Supporting Information). We did not detect binding of Cu(II) to DJ-1 at the protein concentration used in the ITC experiment (40 μM) (Figure S2 of the Supporting Information).

To identify the exact location of the binding pocket of Zn(II), we determined the crystal structure of the DJ-1·Zn(II) complex under two different crystallization conditions (Table

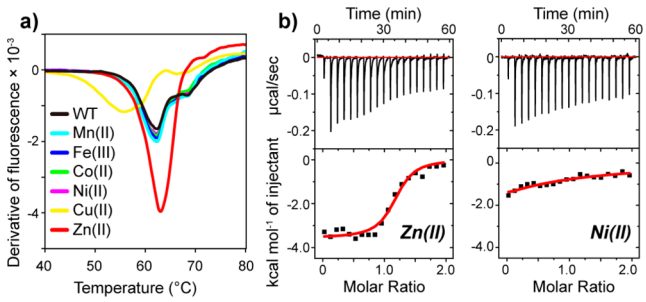

Figure 1. Metal screening. (a) DSF assay in the presence of metals. (b) Representative titrations of DJ-1 with Zn(II) and Ni(II).

Table 1. Summary of the Metal Screening (DSF and ITC)

metal	ΔT_M (°C) (DSF)[a]	K_D (μM) (ITC)
Zn(II)	+0.6	0.8 ± 0.3
Cu(II)	−6.6	ND[b]
Ni(II)	0	400 ± 270
Co(II)	0	ND[b]
Fe(III)	0	ND[b]
Mn(II)	0	ND[b]

[a]Instrumental error of ±0.2 °C. [b]Not determined.

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S1 of the Supporting Information). The cocrystal of DJ-1 and Zn(II) was generated in the crystallization solution reported in the complex of DJ-1 with Cu(I), except that we used Zn(II).[4] A second crystal of the DJ-1·Zn(II) complex, diffracting to high resolution (1.18 Å), was obtained by soaking the metal into a preformed crystal of DJ-1.[11] Both structures are virtually identical to each other [root-mean-square deviation (rmsd) of 0.15 Å] (Figure 2 and Figure S3 of the Supporting Information) and to other structures of DJ-1 (rmsd of ≤0.15 Å).[4,5,11]

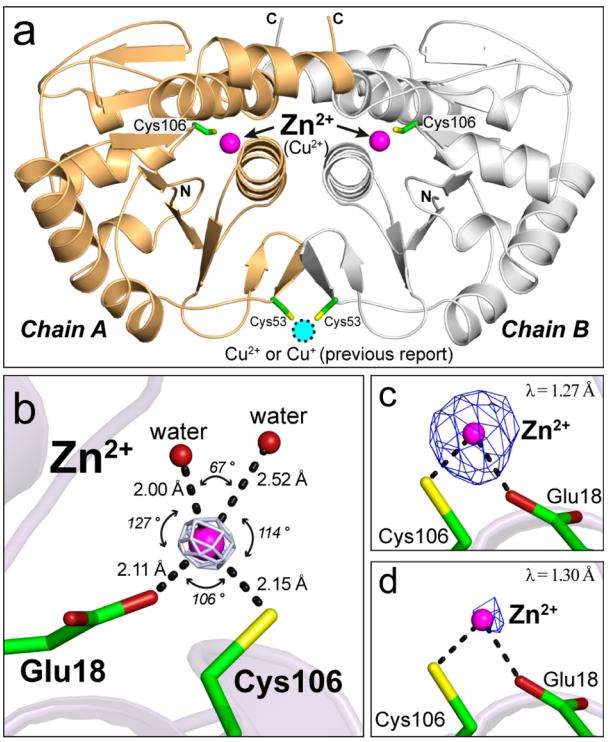

Figure 2. Crystal structure of Zn(II) bound to DJ-1. (a) Overall view of the DJ-1 dimer highlighting the location of Zn(II) (magenta sphere). The approximate locations of Cu(I) and Cu(II) as reported earlier are also indicated.[4,5] (b) Coordination geometry of Zn(II). The mesh around the metal corresponds to the σ-A-weighted map contoured at 11σ. No other electron density peak at this contouring level (11σ) is observed within 7 Å of Zn(II). The resolution was 1.18 Å. (c and d) Anomalous difference electron density maps calculated at the metal binding site (σ = 3.5), with data collected at the (c) high-energy (1.27 Å) and (d) low-energy (1.30 Å) sides of the absorption edge of Zn(II).

The electron density maps of our two crystal structures reveal a large peak in the immediate vicinity of the key residue Cys106 of DJ-1. This peak is not observed in crystals of apo-DJ-1,[11,12] suggesting the presence of Zn(II) at this position (Figure 2b and Figure S3 of the Supporting Information). To confirm the identity of Zn(II), we employed X-ray anomalous dispersion analysis.[13] The large difference between the anomalous difference electron density in two data sets collected before (1.30 Å) and after (1.27 Å) the anomalous edge of zinc determines unambiguously its identity (Figure 2c,d).

Zn(II) is coordinated by two residues of DJ-1 (Cys106 and Glu18) and by two water molecules (Figure 2), an unusual composition of ligands for a coordination sphere of Zn(II).[14] The distances between the coordinating atoms of Cys106 and Zn(II) and between those of Glu18 and Zn(II) are 2.15 ± 0.06 and 2.11 ± 0.06 Å, respectively. Overall, the coordination of Zn(II) resembles a distorted tetrahedron. In particular, the angle between the two coordinating water molecules (67°) is far from the ideal angle of 109.5°. The coordination geometry was favorably validated in the CheckMyMetal server in seven of the eight parameters examined.[15] The Zn(II) binding pocket is located in the same position as that reported very recently for Cu(II).[5] Distances between the coordinating residues and metals are also very similar, even though Cu(II) displays a planar trigonal coordination different from that of Zn(II).

The occupancy of Zn(II) in the binding pocket is ∼50%, as determined from the structure at higher resolution (1.18 Å), because of the oxidation of the sulfur atom of Cys106 to sulfenic acid during crystallization (Figure S3 of the Supporting Information). The progressive oxidation of Cys106 is an essential aspect of the biological function of DJ-1 and has been reported in previous crystal structures of the apoprotein.[12,16] The oxidation of Cys106 to its sulfenic acid form was confirmed in identical crystals, but depleted of Zn(II) [resolution of 1.55 Å (Figure S3 of the Supporting Information)]. Remarkably, the occupancy of the sulfenic acid form was also ∼50%. Crystals aged for 2 months were completely oxidized to the sulfinic acid form as observed at a resolution of 1.35 Å (Figure S3 of the Supporting Information).

To investigate the importance of Cys106 and Glu18 for the coordination of Zn(II), we performed ITC experiments with wild-type DJ-1 and muteins (Figure 3). This new set of

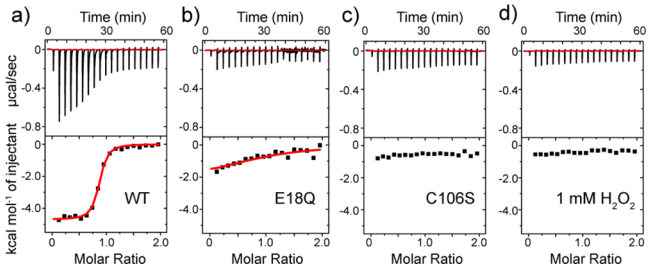

Figure 3. Mutational analysis. Titration of (a) wild-type, (b) E18Q, (c) C106S, and (d) preoxidized DJ-1 (100 μM) with Zn(II) (1 mM).

experiments, performed at a higher concentration of protein and metal, afforded accurate thermodynamic parameters of the DJ-1·Zn(II) complex (Table 2). The dissociation constant in

Table 2. Thermodynamic Parameters for the Binding of Zn(II) to DJ-1

protein	K_D (μM)	$\Delta H°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)	n
wild type	0.6 ± 0.1	−4.7 ± 0.1	−3.8 ± 0.1	0.83 ± 0.01
E18Q	49 ± 59	−2.2 ± 0.4	−3.7 ± 0.6	1[a]
C106S	ND[b]	ND[b]	ND[b]	ND[b]
H_2O_2	ND[b]	ND[b]	ND[b]	ND[b]

[a]Parameters of E18Q were calculated assuming n = 1. [b]Not determined.

wild-type DJ-1 showed submicromolar affinity as observed in the screening of metals described above (K_D = 0.6 ± 0.1 μM). Moreover, the interaction between DJ-1 and the metal is driven by comparable contributions that are enthalpic (ΔH = −4.7 ± 0.1 kcal/mol) and entropic ($-T\Delta S$ = −3.8 kcal/mol) in nature.

Titration experiments with mutated versions of Cys106 and Glu18 corroborated the importance of the protein−metal

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interactions for the capture of Zn(II). The binding affinity was reduced ~80-fold in mutein E18Q, whereas the mutation of C106S completely abolished the binding of the metal (Figure 3). Moreover, the oxidation of Cys106 with H_2O_2 also abrogated the binding of Zn(II) (we note H_2O_2 was exhaustively washed out prior to the ITC experiment).

In summary, we report for the first time the binding of Zn(II) to human DJ-1. Our study is accompanied by a rigorous dissection of the thermodynamic and structural features of their interaction. It is shown that Cys106 and Glu18 are the coordinating residues of Zn(II). The pocket of DJ-1 is selective for Zn(II) among the metals of the Irving–Williams series examined (Table 1). The selectivity may arise from the tetrahedral coordination geometry, which in general favors the coordination of Zn(II) with respect to other metals.[17] The number and the nature of the ligands suggest that Zn(II) could act as a weak Lewis acid playing some catalytic role.[14]

The Zn(II) ion is located in the same binding pocket as the Cu(II) ion, although the latter metal displays a planar trigonal coordination different from that of Zn(II).[5] Intriguingly, we did not detect binding of Cu(II) to DJ-1 under our experimental conditions, probably because the reported affinity of Cu(II) for DJ-1 is low (K_D ~ 0.4 mM).[5]

Because Glu18 and the reactive Cys106 (easily oxidized to sulfenic acid and sulfinic acid forms) are well-conserved residues and essential for the protective function of DJ-1, we suggest that Zn(II) could be involved in the regulation of DJ-1.[12,18] However, the dissociation constant determined by ITC [K_D = 0.6 ± 0.1 μM (Table 2)] indicates that this affinity is insufficient for Zn(II) to bind significantly to DJ-1 in the cytoplasm, where the concentration of free metal is several orders of magnitude lower than the K_D.[19] Because DJ-1 has been reported to be present in the extracellular medium[20,21] where the concentration of free Zn(II) reaches micromolar concentration levels,[22] we suggest a putative role of Zn(II) in modulating the function of DJ-1 outside the cell. Under these environmental conditions, a majority of DJ-1 is expected to form a complex with Zn(II) (Figure S4 of the Supporting Information). Looking ahead, we believe it will be necessary to evaluate the significance of these findings for the function of DJ-1 and in connection with Parkinson's disease in a more biological environment.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods, crystallographic data, raw DSF data, metal screening (ITC), structural figures, and fractions of bound versus unbound DJ-1 at endogenous levels of protein. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

Coordinates and structure factors have been deposited in the Protein Data Bank as entries 4P35 [DJ-1·Zn(II) complex, crystal I], 4P36 [DJ-1·Zn(II) complex, crystal II], 4P34 (DJ-1 sulfenic), and 4P2G (DJ-1 sulfinic).

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Notes

The authors declare no competing financial interests.

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■ REFERENCES

(1) Antony, P. M. A., Diederich, N. J., Kruger, R., and Balling, R. (2013) *FEBS J. 280*, 5981–5993.
(2) Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C. J., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J. W., Vanacore, N., van Swieten, J. C., Brice, A., Meco, G., van Duijn, C. M., Oostra, B. A., and Heutink, P. (2003) *Science 299*, 256–259.
(3) Cookson, M. R. (2012) *Cold Spring Harbor Perspect. Med. 2*, a009415.
(4) Puno, M. R., Patel, N. A., Moller, S. G., Robinson, C. V., Moody, P. C. E., and Odell, M. (2013) *J. Am. Chem. Soc. 135*, 15974–15977.
(5) Girotto, S., Cendron, L., Bisaglia, M., Tessari, I., Mammi, S., Zanotti, G., and Bubacco, L. (2014) *J. Biol. Chem.*, DOI: 10.1074/jbc.M113.535112.
(6) Bjorkblom, B., Adilbayeva, A., Maple-Grodem, J., Piston, D., Okvist, M., Xu, X. M., Brede, C., Larsen, J. P., and Moller, S. G. (2013) *J. Biol. Chem. 288*, 22809–22820.
(7) Dexter, D. T., Wells, F. R., Lees, A. J., Agid, F., Agid, Y., Jenner, P., and Marsden, C. D. (1989) *J. Neurochem. 52*, 1830–1836.
(8) Irving, H., and Williams, R. J. P. (1953) *J. Chem. Soc.*, 3192–3210.
(9) Kranz, J. K., and Schalk-Hihi, C. (2011) *Methods Enzymol. 493*, 277–298.
(10) Capanni, C., Taddei, N., Gabrielli, S., Messori, L., Orioli, P., Chiti, F., Stefani, M., and Ramponi, G. (2004) *Cell. Mol. Life Sci. 61*, 982–991.
(11) Wilson, M. A., Collins, J. L., Hod, Y., Ringe, D., and Petsko, G. A. (2003) *Proc. Natl. Acad. Sci. U.S.A. 100*, 9256–9261.
(12) Canet-Aviles, R. M., Wilson, M. A., Miller, D. W., Ahmad, R., McLendon, C., Bandyopadhyay, S., Baptista, M. J., Ringe, D., Petsko, G. A., and Cookson, M. R. (2004) *Proc. Natl. Acad. Sci. U.S.A. 101*, 9103–9108.
(13) Miyafusa, T., Caaveiro, J. M. M., Tanaka, Y., and Tsumoto, K. (2012) *Biochem. J. 443*, 671–680.
(14) Lee, Y. M., and Lim, C. (2008) *J. Mol. Biol. 379*, 545–553.
(15) Zheng, H. P., Chordia, M. D., Cooper, D. R., Chruszcz, M., Muller, P., Sheldrick, G. M., and Minor, W. (2014) *Nat. Protoc. 9*, 156–170.
(16) Zhou, W. B., Zhu, M., Wilson, M. A., Petsko, G. A., and Fink, A. L. (2006) *J. Mol. Biol. 356*, 1036–1048.
(17) Dudev, T., and Lim, C. (2014) *Chem. Rev. 114*, 538–556.
(18) Witt, A. C., Lakshminarasimhan, M., Remington, B. C., Hasim, S., Pozharski, E., and Wilson, M. A. (2008) *Biochemistry 47*, 7430–7440.
(19) Outten, C. E., and O'Halloran, T. V. (2001) *Science 292*, 2488–2492.
(20) Kim, J. M., Shin, H. I., Cha, S. S., Lee, C. S., Hong, B. S., Lim, S., Jang, H. J., Kim, J., Yang, Y. R., Kim, Y. H., Yun, S., Rijal, G., Lee-Kwon, W., Seo, J. K., Gho, Y. S., Ryu, S. H., Hur, E. M., and Suh, P. G. (2012) *Nat. Commun. 3*, 1296.
(21) Maita, C., Tsuji, S., Yabe, I., Hamada, S., Ogata, A., Maita, H., Iguchi-Ariga, S. M. M., Sasaki, H., and Ariga, H. (2008) *Neurosci. Lett. 431*, 86–89.
(22) De Leon-Rodriguez, L., Lubag, A. J. M., and Sherry, A. D. (2012) *Inorg. Chim. Acta 393*, 12–23.