Crystal structure of DJ-1/RS and implication on familial Parkinson's disease[1]

Qing Huai[a], Yingjie Sun[a], Huanchen Wang[a], Lih-Shen Chin[b], Lian Li[b], Howard Robinson[c], Hengming Ke[a,*]

[a] *Department of Biochemistry and Biophysics and Lineberger Comprehensive Cancer Center, The University of North Carolina, Chapel Hill, NC 27599-7260, USA*
[b] *Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30332-3090, USA*
[c] *Biology Department, Brookhaven National Laboratory, Upton, NY 11973-5000, USA*

Received 4 June 2003; revised 30 June 2003; accepted 30 June 2003

First published online 25 July 2003

Edited by Hans Eklund

Abstract DJ-1 is a protein involved in multiple physiological processes, including cancer, Parkinson's disease, and male fertility. It is unknown how DJ-1 functions in the apparently different systems. The crystal structure of DJ-1 at 1.6 Å resolution shows that DJ-1 is a helix-strand-helix sandwich and forms a dimer. The DJ-1 structure is similar to the members of the intracellular protease PfpI family. However, the catalytic triad of Cys–His–Glu is not strictly conserved in DJ-1, implying that DJ-1 has a different catalytic mechanism if it acts as a protease or DJ-1 serves as a regulatory protein in the physiological processes. The structure shows that Leu166 positions in the middle of a helix and thus predicts that the L166P mutation will bend the helix and impact the dimerization of DJ-1. As a result, the conformational changes may diminish the DJ-1 binding with its partner, leading to the familial Parkinson's disease caused by the single L166P mutation.

Key words: Crystal structure; Breast cancer; Parkinson's disease; Male fertility; Protein inhibitor of activated STAT; Androgen receptor

1. Introduction

DJ-1 is a ubiquitously expressed and highly conserved protein that plays important roles in several biological processes. First, *DJ-1* was reported as a novel oncogene showing a cooperative transforming activity with H-*ras* [1] and encoded a circulating tumor antigen in breast cancer [2,3]. Second, DJ-1 was reported to play an essential role in fertility. Treatment of male rats with sperm toxicants such as ornidazole induced infertility within 10–14 days [4–6]. The infertility was associated with the release of CAP1 (contraception-associated protein 1, a DJ-1 homolog in rat) from the spermatozoa [7–9], depletion of SP22 (CAP1 splicing isoform) [10], or decrease of DJ-1 level in sperm [11]. Third, DJ-1 was identified as a regulatory subunit (RS) of an RNA-binding protein complex and inhibits the RNA-binding activity of RBP [12]. Finally, DJ-1 was implicated in Parkinson's disease (PD). Point mutation of Leu166 to Pro in DJ-1 protein was directly linked to an early-onset, autosomal recessive form of familial PD [13]. The mechanism by which DJ-1 mutation causes PD is unknown, but oxidative stress has been implicated in the pathogenesis of a wide variety of human diseases such as cancer and PD [13–18]. We reported here the crystal structure of human DJ-1 and discuss the structural implications on PD and regulation of androgen receptor (AR).

2. Materials and methods

2.1. Subcloning, overexpression and purification of DJ-1

The gene of DJ-1 (GenBank Acc. No. AF021819) was subcloned into vector pET3E. For amplification of the DJ-1 coding region by polymerase chain reaction (PCR), a pair of oligonucleotide primers was synthesized: CGACCATGGCTTCCAAAAGAGCTC for 5′-end of the DJ-1 gene and TTCCTCGAGCTAGTCTTTAAGAACAAG-TG for 3′-end. The *Nco*I and *Xho*I restriction sites were introduced respectively into each primer. The amplified DJ-1 cDNA and the expression vector pET3E were separately digested by the restriction enzymes *Nco*I and *Xho*I, purified from agarose gel, and then ligated by T4 DNA ligase. The plasmid pET-DJ1 was transformed into *Escherichia coli* strain BL21(CodonPlus) for overexpression.

The *E. coli* cell carrying pET-DJ1 was grown in Luria–Bertani (LB) medium at 37°C to absorption $A_{600} = 0.7$ and then 0.1 mM isopropyl β-D-thiogalactopyranoside (IPTG) was added for further growth at 37°C for 5 h. The recombinant DJ-1 was fractionated by ammonium sulfate precipitation and then purified by the chromatographic columns of diethylaminoethyl (DEAE) Sepharose CL-6B (Pharmacia) and Sephacryl S300 (Amersham-Biosciences). The purified protein has >95% purity as judged on basis of the sodium dodecyl sulfate (SDS) gel and shows a single band in the native polyacrylamide gel electrophoresis (PAGE). A typical batch of purification yielded about 50 mg DJ-1 from 2 l of cell culture.

2.2. Preparation of selenomethionyl DJ-1 (SeDJ-1)

The method outlined by Doublie [19] was used for expression of *E. coli* SeDJ-1. The recombinant *E. coli* BL21 cell carrying the pET-DJ1 plasmid was grown in 50 ml of LB medium at 37°C overnight. The cells were spun down and inoculated into 1 l of M9 minimal medium containing 0.4% glucose, 50–100 mg/l of lysine, selenomethionine, threonine, phenylalanine, leucine, isoleucine, and valine. After the cell was grown to the mid-log phase (A_{600} about 0.7), the overexpression of the SeDJ-1 was induced by 0.1 mM IPTG and the cells were grown at 30°C for 16 h. The SeDJ-1 protein was purified using the same method as that for the wild-type DJ-1.

*Corresponding author. Fax: (1)-919-966 2852.
E-mail address: hke@med.unc.edu (H. Ke).

[1] Atomic coordinates have been deposited in PDB with entry number 1Q2U.

Abbreviations: PD, Parkinson's disease; CAP1, contraception-associated protein 1; SeDJ-1, selenomethionyl DJ-1; DJBP, DJ-1-binding protein; PIAS, protein inhibitor of activated STAT; AR, androgen receptor; SUMO, small ubiquitin-related modifier

0014-5793/03/$22.00 © 2003 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00764-6

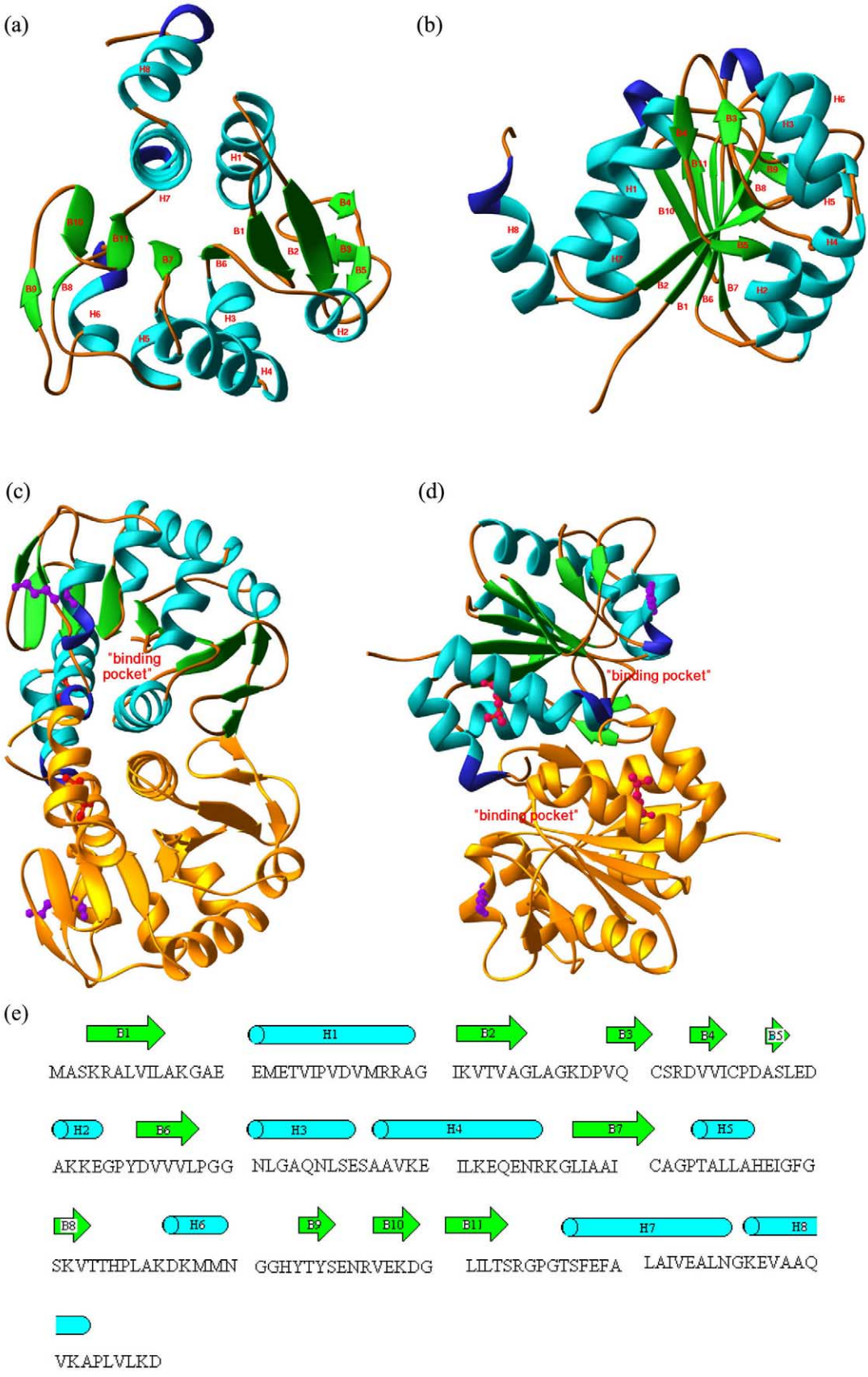

Fig. 1. Ribbon representation of monomeric DJ-1 in two different views (a and b), and DJ-1 dimer (c and d). The red balls in c and d represent Leu166 that is located in the middle of helix H7. The purple balls are Lys130, (e) the secondary structure and sequence.

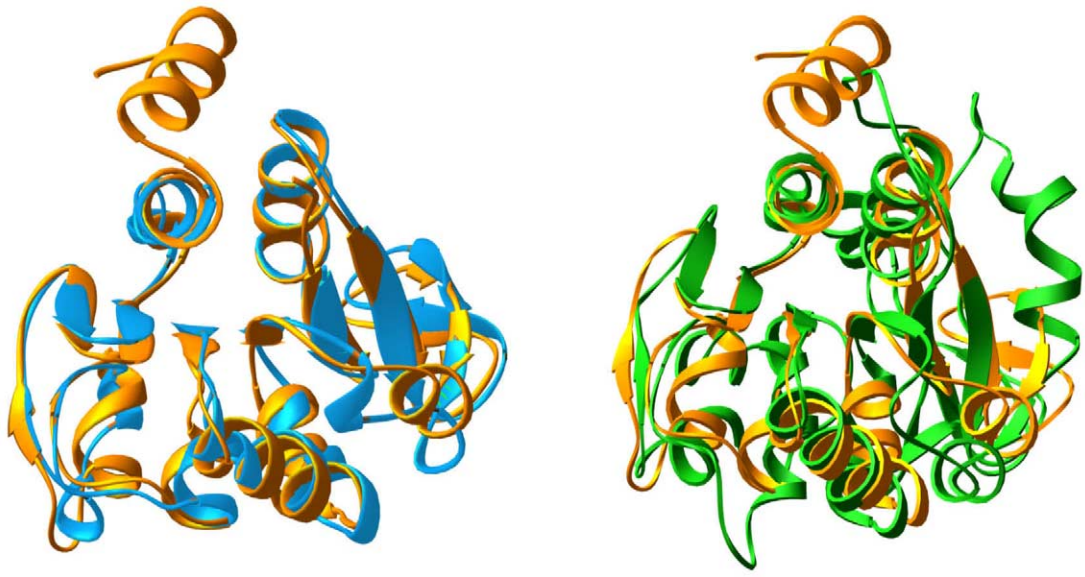

Fig. 2. Superposition of DJ-1 (golden) over intracellular protease PH1704 (cyan, left) and *E. coli* heat shock protein HSP31 (green, right).

2.3. Crystallization of DJ-1 and SeDJ-1

Crystals of DJ-1 were grown by hanging drop. The full-length DJ-1 (residues 1–189) at 8 mg/ml in a buffer of 20 mM Tris base (pH 7.5), 50 mM NaCl, 1 mM ethylenediamine tetraacetic acid (EDTA), and 1 mM β-mercaptoethanol (β-ME), was mixed in a 2:2 ratio with a well buffer of 12% PEG3350, 0.1 M Tris base, pH 8.5, 5% MPD at room temperature. The SeDJ-1 crystals were grown under similar conditions as the wild type of DJ-1, except for replacement of 5% MPD with 5% glycerol. The diamond-shaped crystals formed in a few hours and reached a typical size of $0.3\times0.3\times0.3$ mm in a couple of days. The crystals have the space group of $P3_121$ with cell dimensions of $a=b=75.2$, $c=75.3$ Å.

2.4. Structure determination

Diffraction data of the native DJ-1 and the MAD data of SeDJ-1 were collected on beamline x26c of National Synchrotron Light Source and processed by program HKL [20]. Four selenium sites were automatically located by the program SOLVE [21] and yielded the 'figure of merit' of 0.81 for 16438 reflections at 30–2.04 Å resolution. The MAD phases were improved by solvent flattening as implemented in the density modification package of CCP4 [22]. The models of the structures were built by program O [23] and refined by program CNS [24]. The statistics of the diffraction data and structure refinement are shown in Table 1.

3. Results and discussion

3.1. Architecture of the structure

DJ-1 contains 189 amino acids and has a calculated molecular mass of 19.8 kDa [12]. The electron density maps from the MAD phases clearly revealed the trace of full-length DJ-1, except for slightly weaker density for the N- and C-terminal residues. The monomeric DJ-1 contains eight α-helices and 11 β-strands that are organized into a helix-strand-helix sandwich (Fig. 1). A seven-stranded sheet (B1, B2, B5–B7, and B10, B11) forms the central core of the molecule (Fig. 1), and is flanked by three helices (H1, H7, H8) on one side and five helices (H2–H6) on another side. Two two-stranded sheets (B3, B4 and B8, B9) lace the sandwich on right and left sides. An analysis on the backbone conformation showed that most residues in the DJ-1 structure have the energy-favored conformations, except for Cys106 that has the energy-disallowed backbone conformation of $\varphi=66°$ and $\psi=-106°$. This unusual conformation was verified by the omitted maps and may be compensated by two hydrogen bonds between the backbone nitrogen of Cys106 and carbonyl oxygen of Ser155 and between carbonyl oxygen of Cys106 and the backbone nitrogen of His126.

Although the crystallographic asymmetric unit contains one molecule, DJ-1 is tightly associated into a dimer in the crystals. The dimeric interface is formed between helices H1, H7, H8 and strands B3 and B4 (Fig. 1). It contains eight pairs of hydrogen bonds (Glu15 Oε1–Asp24 Oδ2, Glu15 Oε1–Arg28 NH2, Arg27 NH1–Arg48 O, Val51 O–Cys53 N, His126 Nε2–Pro184 O, Arg145 NH1–Val186 O, Arg145 NH2–Val186 O,

Table 1
Statistics on the diffraction data and structure of DJ-1

Crystals	Wavelength (Å)	Unit cell (*a*, *c*)	Resolution (Å)	Reflections		R_{merge}[a]	I/σ[a]	Completeness[a] (%)
				Total	Unique			
SeDJ-1	0.9800	75.53, 75.52	2.0	326808	16349	0.070(0.173)	23.0(25.0)	95.2(51.2)
SeDJ-1	0.9791	75.53, 75.50	2.0	320822	16363	0.078(0.149)	27.2(32.8)	95.6(54.9)
SeDJ-1	0.9611	75.50, 75.52	2.0	268318	17204	0.080(0.238)	17.9(15.8)	100.0(100.0)
Native	0.9790	75.23, 75.31	1.6	337226	33002	0.071(0.525)	12.4(5.0)	100.0(100.0)

Figure of merit from SOLVE: 0.81 for 16438 reflections at 30–2.04 Å resolution. Structure refinement: *R*-factor/*R*-free: 0.191/0.202; resolution: 1.6 Å; number of reflections: 31910 (3179); root mean square deviation for bonds and angles: 0.004 and 1.33°; average *B*-factors (Å^2): 16.0 for 1391 protein atoms, 27.9 for 195 water molecules.

[a]The numbers in the parentheses are for the highest resolution shell, 2.00–2.07 Å for SeDJ-1 and 1.60–1.66 Å for the native DJ-1. Space group is $P3_121$.

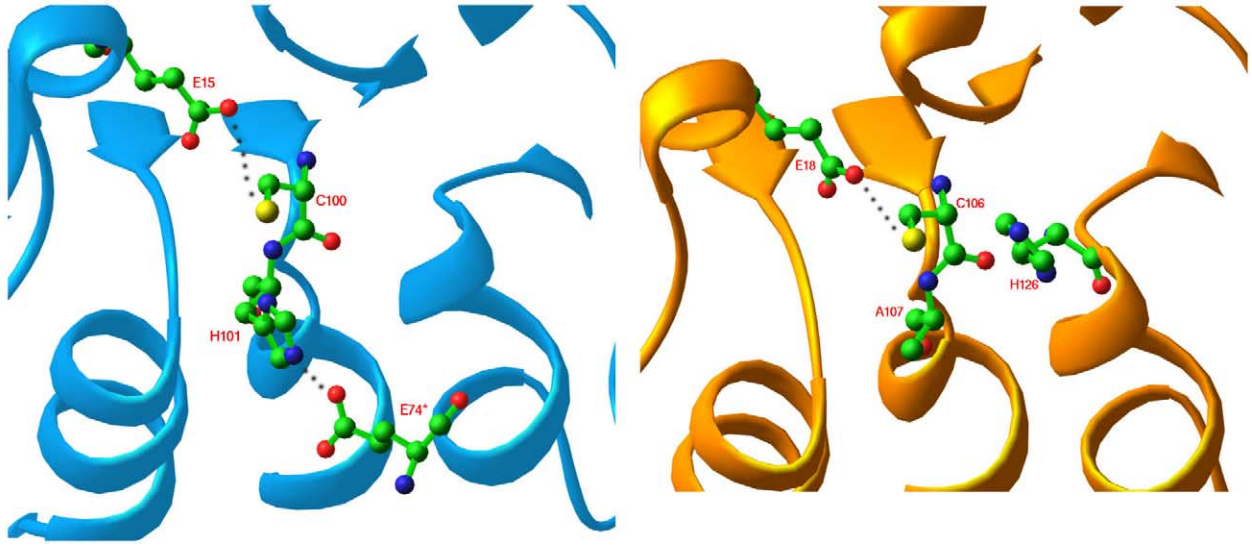

Fig. 3. The catalytic triad of Cys100–His101 and Glu74 at the active site of PH1704 (left). The dotted lines represent the hydrogen bonds. Residue Glu74 comes from the neighboring subunit in the hexamer of PH1704. Right, a putative binding pocket of DJ-1. The catalytic triad is not conserved in DJ-1, but the pocket may still be capable of binding with its substrate proteins.

and Gly159 N–Leu185 O) and numerous van der Waal's interactions.

3.2. A putative binding pocket in DJ-1

We used bioinformatic tools to explore the possible roles of DJ-1 in the biological systems. The structure comparison by program DALI [25] revealed that 355 structures in Protein Data Bank have Z-scores >2, presumably having a certain degree of structural similarity to DJ-1. The best comparable structure is an intracellular protease PH1704 from *Pyrococcus horikoshii* [27], as predicted on basis of the sequence alignment [13]. In addition, the recently determined structure of *E. coli* heat shock protein Hsp31 [26] also has high degree of structural similarity with DJ-1. The superposition showed the root mean square deviations of 1.73 Å for the backbone atoms of 160 residues between DJ-1 and PH1704, and 1.46 Å for the backbone atoms of 120 residues between DJ-1 and hsp31 (Fig. 2). On the other hand, the sequence alignment by program BLAST [28] showed that DJ-1 has significant sequence identity with the PfpI protease family (PH1704, PfpI, ThiJ), a catalase, and an enzyme involved in thiamine (4-methyl-5(B-hydroxyethyl)-thiazol monophosphate) biosynthesis.

On basis of the sequence and structure similarity, we carefully examined the possibility that DJ-1 serves as a protease in the biological processes. In the structure of protease PH1704, the 'catalytic triad' of Cys–His–Glu is located in the active site (Fig. 3) that is jointly formed between two neighboring subunits in the PH1704 hexamer [27]. The catalytic triad is the key catalytic element in various classes of enzymes, including proteases [29], hydrolases [30], transglutaminases [31], and amidotransferases [32]. DJ-1 contains a pocket with similar shape and size as the active site of PH1704, which is made up of residues Glu18, Lys32, Arg48, Asn76, Cys100, Thr110, and His126 from the same subunit and also residues Arg28 and Leu185 from the neighboring subunit of the DJ-1 dimer (Figs. 1 and 3). We speculate that this pocket serves as a binding site for protein substrates. However, the 'catalytic triad' is not strictly conserved in DJ-1. The structure comparison showed that Cys106 of DJ-1 has an energy-disallowed configuration, the same as Cys100 of PH1704, but the neighboring histidine is replaced with Ala107. To search a possible replacement of the histidine, we examined the structure in a graphic system and found His126 within 5 Å to Cys106. However, no glutamate or aspartate within the same monomer or from the molecules related by crystallographic symmetry is found to form hydrogen bonds with His126. Thus, the structural comparison suggests that DJ-1 does not function as a PH1704-like protease. However, one could not rule out the possibility that DJ-1 may use a different catalytic mechanism, e.g. the Cys–His diad, to carry out protease function.

3.3. Implication of the L166P mutation and PD

A single mutation of Leu166 to proline of DJ-1 has been reported to cause neurodegeneration and PD [13]. In transfected COS cells, V5-His-tagged wild-type DJ-1 exhibited a diffuse cytoplasmic and nuclear staining pattern, whereas V5-His-tagged L166P mutant DJ-1, although showing similar nuclear staining, was mostly localized to mitochondria [13]. The crystal structure of DJ-1 provides clues to how the L166P mutation causes DJ-1 to mislocalize to mitochondria. Leu166 is positioned in the middle of helix H7 in the DJ-1 structure

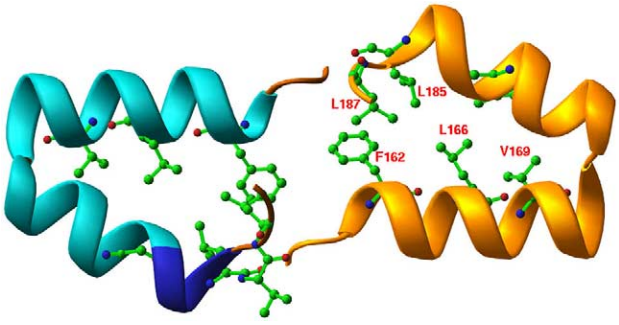

Fig. 4. The interfacial region of DJ-1 dimer and the position of Leu166. Two helices H7 and H8 are shown in the similar orientation as in Fig. 1d. The L166P mutation is expected to cause significant conformational changes and to impact the dimerization of DJ-1.

and forms, together with the residues from helices H7 and H8, a local hydrophobic core of the dimer interface (Fig. 4). Since Leu166 is not exposed to the surface of the DJ-1 dimer, it is unlikely that Leu166 directly interacts with binding partners. In crystal structures, a proline positioning in middle of a helix will cause kink or 20–30° bend of the helix axis [33]. Thus, the L166P mutation of DJ-1 is expected to change significantly the conformations on the region around H7 and potential reorientation of helix H8 due to the H7 bend. Since both helices H7 and H8 are key elements in formation of the DJ-1 dimer, the L166P mutation must also cause changes of dimerization or quaternary structure. As a result, the conformational changes may cause the mislocalization of DJ-1 to mitochondria or diminish interactions of DJ-1 with the binding partners, thus leading to development of PD.

3.4. DJ-1 and its potential regulation on AR

Two proteins in testis were reported to bind DJ-1: DJBP (DJ-1-binding protein) and PIASxα (protein inhibitor of activated STAT). Both DJBP and PIASxα bind to the DNA-binding domain of AR and inhibit in a testosterone-dependent manner the transcription activity of AR [34,35]. DJBP has 570 amino acids and contains three calmodulin-like domains as predicted by BLAST [28], but its exact function is unknown. PIAS is a family of proteins known as SUMO-1 (small ubiquitin-related modifier 1) ligases and regulates various transcription factors such as p53 and steroid hormone receptors [36,37]. DJ-1 is sumoylated at Lys130 by a SUMO ligase, presumably by PIAS [35]. The wild type of DJ-1 reverses the inhibition by binding to PIASxα [35]. However, the single K130R mutation or the quartic mutation (K130R, S57R, E96G, and H126Y) failed to bind PIASxα so as to be unable to reverse the inhibition of PIASxα on AR. The crystal structure showed that Lys130 is located on the surface of the DJ-1 molecule (Fig. 1). This residue is spatially available for sumoylation. However, it is not clear whether the loss of binding capacity of PIASxα is due to sumoylation or the amino acid replacement. On the other hand, residues Ser57, Glu96, and His126 are located at distal places of the DJ-1 surface and do not seem to structurally correlate with one another. Since the single mutation of K130R has a similar effect as the quartic mutation, the other residues might play a less critical role in the binding of PIASxα.

While DJ-1 has been shown to play important roles in cancer, PD, and male fertility, how DJ-1 functions in the diverse physiological processes is unknown. The crystal structure of DJ-1 shows that the catalytic triad is not conserved in DJ-1, implying that DJ-1 has a different mechanism if it acts as a protease. Most likely, DJ-1 serves as a regulatory protein in the various physiological processes, presumably via interactions with its binding partners such as BJBP and PIAS. The crystal structures of DJ-1 in complex with its partners will ultimately reveal the regulation mechanism in the multiple physiological processes.

4. Note added in proof

During the submission of our manuscript, the structure of DJ-1 was electronically published online of J. Biol. Chem. by two groups (Tao, X. and Tong, L. (2003) Crystal structure of human DJ-1, a protein associated with early-onset Parkinson's disease, J. Biol. Chem. May; Honbou, K., Suzuki, N.N., Horiuchi, M., Niki, T., Taira, T., Ariga, H. and Inagaki, F. (2003) The crystal structure of DJ-1, a protein related to male fertility and Parkinson's disease, J. Biol. Chem., June).

Acknowledgements: Diffraction data collection at the NSLS beamline x26c was supported by the NIH National Center for Research Resources and US Department of Energy/BER/BES. This work was supported in part by NIH grant GM59791 to H.K.

References

[1] Nagakubo, D. et al. (1997) Biochem. Biophys. Res. Commun. 231, 509–513.
[2] Taira, T., Takahashi, K., Kitagawa, R., Iguchi-Ariga, S.M.M. and Ariga, H. (2001) Gene 263, 285–292.
[3] Le Naour, F. et al. (2001) Clin. Cancer Res. 7, 3328–3335.
[4] Oberlander, G., Young, C.H. and Cooper, T.G. (1994) J. Reprod. Fertil. 100, 551–559.
[5] Klinefelter, G.R., Laskey, J.W., Ferrell, J., Suarez, J.D. and Roberts, N.L. (1997) J. Androl. 18, 139–150.
[6] Klinefelter, G.R. et al. (2002) J. Androl. 23, 48–63.
[7] Wagenfeld, A., Yeung, C.H., Strupat, K. and Cooper, T.G. (1998) Biol. Reprod. 58, 1257–1265.
[8] Wagenfeld, A., Gromoll, J. and Cooper, T.G. (1998) Biochem. Biophys. Res. Commun. 251, 545–549.
[9] Wagenfeld, A. et al. (2000) J. Androl. 21, 954–963.
[10] Welch, J.E., Barbee, R.R., Roberts, N.L., Suarez, J.D. and Klinefelter, G.R. (1998) J. Androl. 19, 85–93.
[11] Okada, M. et al. (2002) Biol. Pharm. Bull. 25, 853–856.
[12] Hod, Y., Pentyala, S.N., Whyard, T.C. and El-Maghrabi, M.R. (1999) J. Cell Biol. 72, 435–444.
[13] Bonifati, V. et al. (2003) Science 299, 256–259.
[14] Trush, M.A. and Kensler, T.W. (1991) Free Radic. Biol. Med. 10, 201–209.
[15] Giasson, B.I., Ischiropoulos, H., Lee, V.M. and Trojanowski, J.Q. (2002) Free Radic. Biol. Med. 32, 1264–1275.
[16] Dawson, T.M. and Dawson, V.L. (2003) J. Clin. Invest. 111, 145–151.
[17] Mitsumoto, A. and Nakagawa, Y. (2001) Free Radic. Res. 35, 885–893.
[18] Mitsumoto, A. et al. (2001) Free Radic. Res. 35, 301–310.
[19] Doublie, S. (1997) Methods Enzymol. 276, 523–530.
[20] Otwinowski, Z. and Minor, W. (1997) Methods Enzymol. 276, 307–326.
[21] Terwilliger, T.C. and Berendzen, J. (1999) Acta Crystallogr. D55, 849–861.
[22] Collaborative Computational Project, Number 4 (1994) Acta Crystallogr. D50, 760–763.
[23] Jones, T.A., Zou, J.-Y., Cowan, S.W. and Kjeldgaard, M. (1991) Acta Crystallogr. A47, 110–119.
[24] Brünger, A.T. et al. (1998) Acta Crystallogr. D54, 905–921.
[25] Holm, L. and Sander, C. (1993) J. Mol. Biol. 233, 123–138.
[26] Quigley, P.M., Korotkov, K., Baneyx, F. and Hol, W.G.J. (2003) Proc. Natl. Acad. Sci. USA 100, 3137–3142.
[27] Du, X. et al. (2000) Proc. Natl. Acad. Sci. USA 97, 14079–14084.
[28] Altschul, S.F. et al. (1997) Nucleic Acids Res. 25, 3389–3402.
[29] Schmid, M.F. and Herriott, J.R. (1976) J. Mol. Biol. 103, 175–190.
[30] Ollis, D.L. et al. (1992) Protein Eng. 5, 197–211.
[31] Yee, V.C. et al. (1994) Proc. Natl. Acad. Sci. USA 91, 7296–7300.
[32] Tesmer, J.J., Klem, T.J., Deras, M.L., Davisson, V.J. and Smith, J.L. (1996) Nat. Struct. Biol. 3, 74–86.
[33] Visiers, I., Braunheim, B.B. and Weinstein, H. (2000) Protein Eng. 13, 603–606.
[34] Niki, T., Takahashi-Niki, K., Taira, T., Iguchi-Ariga, S.M.M. and Ariga, H. (2003) Mol. Cancer Res. 1, 247–261.
[35] Takahashi, K. et al. (2001) J. Biol. Chem. 276, 37556–37563.
[36] Kotaja, N., Aittomäki, S., Silvennoinen, O., Palvimo, J.J. and Jänne, O.A. (2000) Mol. Endocrinol. 14, 1986–2000.
[37] Tan, J.-A. et al. (2000) Mol. Endocrinol. 14, 14–26.